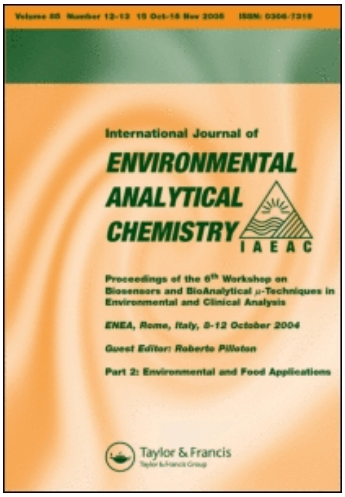

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Simultaneous determination of pyrethroid, organophosphate, and organochlorine pesticides in fish tissue using tandem solid-phase extraction clean-up

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SIMULTANEOUS DETERMINATION OF PYRETHROID, ORGANOPHOSPHATE, AND ORGANOCHLORINE PESTICIDES IN FISH TISSUE USING TANDEM SOLID-PHASE EXTRACTION CLEAN-UP

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A method was developed for the simultaneous analysis of pyrethroid, organophosphate, and organochlorine pesticides in fish tissue. Different extraction solvents and solid-phase extraction clean-up procedures were tested. The best approach was to extract by sonication with acetonitrile and 10% methanol, followed by clean-up of extracts using C_{18}, Florisil and Na_2SO_4 tandem solid-phase extraction cartridges. Gas chromatography with an electron-capture detector was used for analyte determination. All 26 target pesticides were detected using the new method in a single analytical run. The method detection limits ranged from 0.13 to 1.40 μg/kg, while recoveries of the analytes ranged from 86.1 to 133.8% with relative standard deviations ≤12.1% at a spiked concentration of 5 μg/kg. The method was developed to assess possible pesticide contamination in fish collected from lakes at a proposed Illinois National Guard Armory site.

Keywords: Pyrethroids; Organochlorine pesticides; Organophosphate pesticides; Tandem solid-phase extraction; Fish tissue

INTRODUCTION

Although banned for several decades, organochlorine (OC) pesticides are still detectable in fish tissue today because of their high lipid solubility and chemical stability [1]. In addition to the long-lived OCs, current-use pesticides like pyrethroid and organophosphate insecticides are also a concern because of their extensive use and high toxicity to nontarget species, and because they often accumulate in fish tissue [2]. Therefore, an effective and simple method to analyze pyrethroid, OP, and OC insecticides simultaneously in fish needs to be established.

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Successful quantitative analysis of pesticide residues in fish tissue relies on an effective clean-up step after extraction to remove the interfering co-extracts. Various techniques have been developed to isolate target pesticides from fish tissues. Liquid–liquid partitioning [3] is a well-established method to separate pesticides from fish tissue, but it is laborious and requires large solvent volumes. To overcome the disadvantages of this technique, an on-column liquid–liquid partitioning method was developed using a diatomaceous earth column [4]. Adsorption chromatography is another proven technique to isolate pesticides from fish lipids. The most commonly used absorbents include Florisil [5–7], alumina [8,9], and silica gel [9,10]. The major drawbacks of this technique are the high solvent usage, low potential for automation and poor batch-to-batch reproducibility. Because of the difference in molecular weight of pesticides and lipids, gel permeation chromatography (GPC), in which the separation is based on molecular size rather than on polarity, is also a recommended method by the Association of Analytical Communities (AOAC International) [11]. GPC has been widely used to isolate pesticides from lipids, though it requires expensive equipment, uses a large volume of solvents and cannot be used for fractionation [7,10,12–14]. Owing to its automation and relatively high separation potential, normal phase liquid chromatography (NPLC) has recently become an alternative to the conventional adsorption chromatography for the purification of animal tissue extracts [15–17]. However, the need for an expensive instrument and complex operation limits its application. Sweep co-distillation and dialysis were also used as clean-up techniques for trace analysis of pesticides in fish tissue [7,18,19]. Other lipid-removal methods, including saponification and treatment with sulfuric acid, have also been used, but these methods are known to lead to a possible loss of some analytes.

Solid-phase extraction (SPE) is widely used for sample clean-up and concentration because it requires small solvent volumes, requires no specialized equipment, is easy to operate and has a rapid sample throughput. Doong and Lee [20] compared the clean-up efficiencies of different SPE cartridges for analyzing 14 OCs in a Standard Reference Material (SRM1945, Whale Blubber), and they found that Florisil clean-up provided the best recoveries. Schenck *et al.* [21] also used Florisil SPE cartridges for the clean-up of OCs in foods and found that 2% ether in hexane was the best elution solvent to isolate 24 OCs from the lipid fraction. In addition to the use of polar SPE cartridges, nonpolar SPE (C_{18}) cartridges also have been employed to clean-up acetonitrile extracts of fish tissue for OCs analysis [22]. To broaden the polarity range of target pesticides detected, both polar and nonpolar SPE cartridges are often introduced in tandem. Schenck *et al.* [23–25] established a tandem SPE clean-up method for OCs in both nonfatty and fatty fish. Volz and Johnston [26] also used the tandem SPE technique for the clean-up of 10 OCs in wildlife tissues. Recently, Dabrowska *et al.* [27] investigated several types of SPE cartridges, and a combination of phenyl bonded silica, C_{18} and alumina was found to provide the best result for clean-up of soil and sediment extracts.

The objective of this study was to develop a single method that would permit the determination of five pyrethroid, one OP and 20 OC pesticides in fish tissue. This method was required to assess possible pesticide contamination in fish samples collected from lakes located in the proposed Illinois National Guard Armory site in Sparta, Illinois. The newly developed method was then compared with a sulfuric acid clean-up method.

EXPERIMENTAL

Chemicals

The pyrethroids analyzed in this study included: *cis*-permethrin, *trans*-permethrin, esfenvalerate, bifenthrin, and lambda-cyhalothrin. The organophosphate pesticide tested was chlorpyrifos, while the organochlorine pesticides included alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, gamma-chlordane, alpha-chlordane, endosulfan I, *p*,*p*′-DDE, diedrin, endrin, *p*,*p*′-DDD, endosulfan II, *p*,*p*′-DDT, endrin aldehyde, endosulfan sulfate, methoxychlor and endrin ketone. Pesticide standards were purchased from Protocol (Middlesex, NJ).

Acetonitrile, methanol, hexane, ethyl ether, toluene, anhydrous Na_2SO_4, and anhydrous $MgSO_4$ were purchased from Fisher Scientific (Pittsburgh, PA). Solvents used in this study were all pesticide grade. The SPE cartridges, C_{18} (octadecyl), were purchased from Agilent Technologies (Palo Alto, CA), while the Florisil-PR, alumina-N, and silica cartridges (1000 mg) were all purchased from Alltech Associates Inc. (Deerfield, IL). 4,4′-Dibromooctafluoro-biphenyl (DBOFB) (Supelco, Bellefonte, PA) was used as a surrogate to verify the extraction and clean-up efficiency of the newly developed method.

Instrumentation and Calibration

Analysis of the final extracts was performed on an Agilent 6890 series GC equipped with an Agilent 7683 autosampler and an electron capture detector (Agilent Technologies, Palo Alto, CA). Two columns, an HP-5MS (30 m × 0.25 mm × 0.25 μm film thickness; Agilent Technologies) and a DB-608 (30 m × 0.25 mm × 0.25 μm film thickness; Agilent Technologies) were used to confirm the analytical results. Helium and nitrogen were employed as the carrier and makeup gas, respectively. A 2-μL sample was injected into the GC using a pulsed split-less mode. The oven was set at 100°C, heated to 250°C at 10°C/min, then heated to 280°C at 3°C/min and held at 280°C for 15 min. The flow rates of carrier gas were 3.2 and 1.8 mL/min for the HP-5MS and DB-608 columns, respectively. Calibration was based on area using three external standards. The standard solutions contained 10, 50, and 100 ng/mL each of pesticide and surrogate standard. When the samples spiked with 1 μg/kg of each analyte were analyzed, however, a standard series of 1, 10, and 50 ng/mL was used for the quantitative measurement. These solutions were analyzed using the GC-ECD methods detailed above. The calibration curves were linear within this concentration range. Qualitative identity was established using a retention window of 1% with confirmation on a second column.

Sample Fortification

Channel catfish tissue (*Ictalurus punctatus*) was used for the method development and method validation, and these control fish were obtained from the Little Grassy Fish Hatchery (Carbondale, IL). No target pesticides were detected in the control fish tissue, and the lipid content of the tissue was 12%. Fish fillets were cut into small pieces and homogenized using a blender. The homogenized samples were fortified

with a target analyte mixture solution at four different concentration levels (1, 5, 25, and 50 μg/kg). The blank was spiked with 20 μg/kg of the surrogate 4,4′-dibromooctafluoro-biphenyl (DBOFB) only.

Collection of Fish from the Armory Site

Fish samples were collected from 15 lakes located in the proposed Illinois National Guard Armory site (240–280 ha in total) in Sparta, IL. This area consisted of a reclaimed mining site surrounded by agriculture fields. Fish samples were collected from this site from November 2002 to August 2003. Sixty-four fish including five species, yellow bullhead (*Ameiurus catus*), common carp (*Cyprinus carpio*), spotfin shiner (*Cyprinella spiloptera*), smallmouth buffalo (*Ictiobus bubalus*), and channel catfish (*I. punctatus*), were collected and analyzed during this sampling.

Extraction Procedures

Frozen fish fillets were initially homogenized in a blender. Approximately 2 g of ground fish tissue was removed and mixed with anhydrous $MgSO_4$ until dry in a 50-mL centrifuge tube that was cooled on ice. A 25-mL aliquot of extraction solvent (either acetonitrile, acetone, methanol or 10% methanol in acetonitrile) was added as extraction solvent and the mixture sonicated for 3 min in pulse mode (3 s on, 1 s off) using a high-intensity ultrasonic processor (Model VCX 400, Sonics and Materials Inc., Newtown, CT). The extract was centrifuged for 5 min with an IEC Clinical Centrifuge (International Equipment Company, Needham Heights, MA).

Clean-up Procedures

The supernatant was then decanted into a beaker and diluted with distilled water to a final volume of 200 mL (Fig. 1). Water is the weakest elution solvent for a C_{18} SPE cartridge, which is a reversed phase adsorbent, so an aqueous solution is beneficial for retaining the analytes on the C_{18} cartridge during the sample loading step. This thoroughly mixed aqueous solution was loaded on a C_{18} SPE cartridge that was previously conditioned with 3 mL of hexane, 3 mL of acetone, 3 mL of methanol, and 6 mL of distilled water, sequentially, at a flow rate of approximately 1 drop/s. After the extract was loaded on the cartridge, the glassware used for the extractions was rinsed with 6 mL of water and this wash added to the C_{18} cartridge. The cartridge was dried for 10 min with vacuum and the eluate discarded.

In a separate step, an anhydrous Na_2SO_4 cartridge was connected on top of a normal phase adsorbent cartridge (e.g. Florisil, silica or alumina) and the cartridges conditioned with 6 mL of hexane. These cartridges were then attached below the dried C_{18} cartridge (Fig. 1). The purpose of the anhydrous Na_2SO_4 cartridge was to remove any residual water from the system, while the normal phase absorbent was used to separate the pesticides from the lipid interferences. The following solvent systems were tested for each adsorbent. Six milliliters of a 3% toluene in hexane solution was added to the tandem cartridges and eluted at a rate of approximately 1 drop/s. The eluent was collected. To minimize the co-elution of unwanted compounds from the C_{18} cartridge, the C_{18} cartridge was removed and the bottom two cartridges

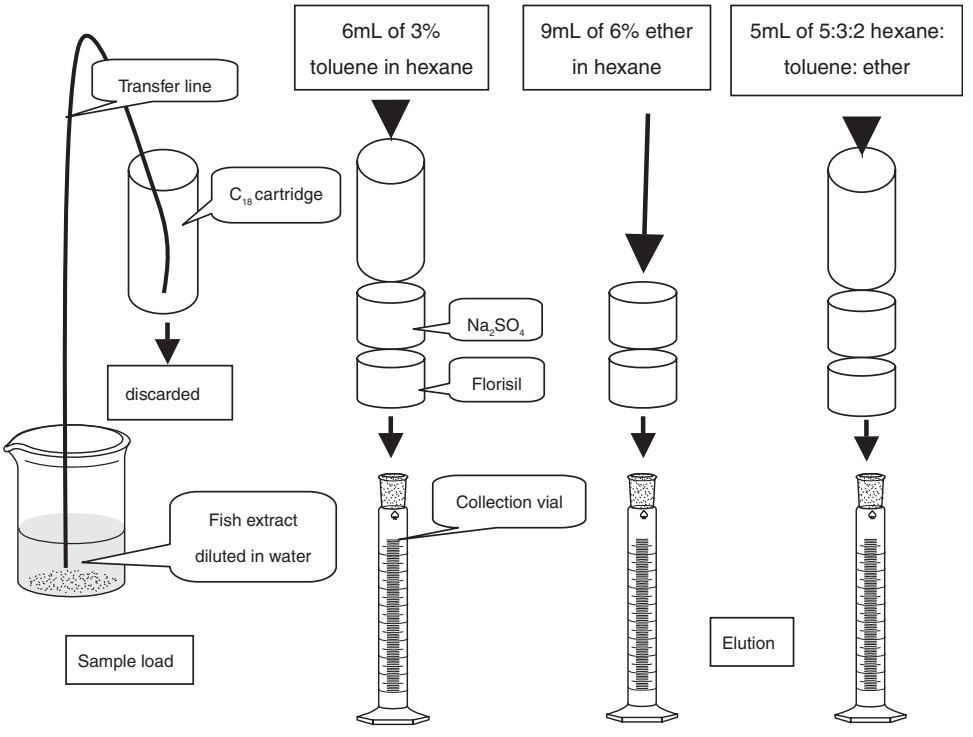

FIGURE 1 Schematic diagram of the tandem SPE clean-up procedure.

eluted with 9 mL of a 6% ethyl ether in hexane solution. The eluent again was collected. After the solvent was eluted from the bottom two cartridges, the C_{18} cartridge was reconnected on top of the other cartridges, and 5 mL of a hexane : toluene : ethyl ether (5 : 3 : 2, v/v/v) solution was used to elute the more polar pesticides from the cartridges (Fig. 1). The eluent was collected, combined with earlier eluents and evaporated to less than 1 mL using a gentle flow of nitrogen. The final residue was solvent-exchanged to hexane prior to GC-ECD analysis.

For the clean-up with the Florisil cartridge alone, the extract was evaporated with a TurboVap II evaporator (Zymark, Hopkinton, MA) to approximately 5 mL and then evaporated to dryness under a gentle stream of nitrogen using a Pierce Model 18780 Reactivap^{TM} (Rockford, IL). The residue was redissolved in 1 mL of hexane and loaded on a Florisil column that was preconditioned with 6 mL hexane. Nine milliliters of a 6% ethyl ether in hexane solution was used to elute the target pesticides, and the eluent was evaporated to 1 mL under a gentle flow of nitrogen prior to analysis by GC-ECD.

For the sulfuric acid treatment, the extract was solvent-exchanged to hexane (1 mL) with the same evaporation method described above, and 5 mL of a 50% sulfuric acid solution was added. The solution was vortexed for 1 min and the hexane layer removed to another vial after the separation of phases. An additional 2 mL of hexane was added to the sulfuric acid layer, the mixture was shaken, the hexane layer was combined with the previous extract, and the total extract was concentrated to 1 mL under a gentle flow of nitrogen prior to analysis by GC-ECD.

RESULTS AND DISCUSSION

Method Development

Extraction Solvent Selection

Water-miscible solvents easily break up fish tissue during an extraction procedure because of the high moisture and fat content of fish tissue. Therefore, acetonitrile (ACN), acetone, methanol, and a 10% methanol in ACN solution were tested as solvents to extract pesticides from fish tissues. As shown in Fig. 2, the mixture of methanol and acetonitrile (1 : 9, v/v) provided the highest recoveries for most of the target pesticides (>75% with the exception of esfenvalerate, which had a lower recovery, <50%). The good recoveries noted for the ACN and methanol solution can be attributed to the extraction capacity of acetonitrile, which easily dissolves a large range of pesticides but does not dissolve lipids to an appreciable level coupled with methanol's great solvolytic ability that further releases bound residues from the matrix [28]. However, at the same time, co-extraction of lipids from the fish tissues was still problematic using these extraction solvents, so clean-up was necessary for all of the samples.

Choices of Absorbents

A number of SPE absorbents including silica, alumina, Florisil, and C_{18} were tested to separate the pesticides from the extracted fish tissue, and these results are detailed in Fig. 3. Because it is not easy to deactivate SPE cartridges, they were used without

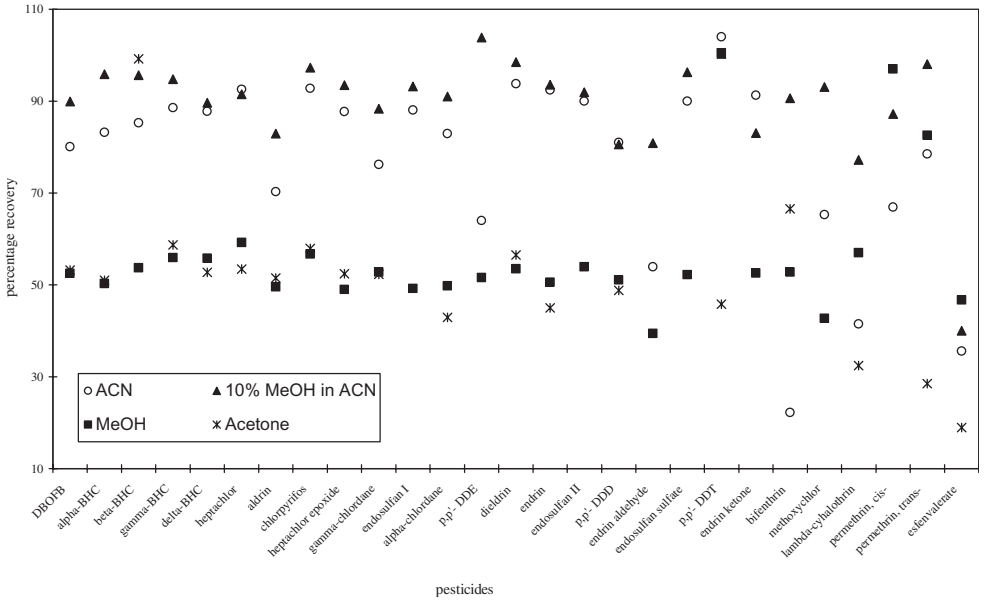

FIGURE 2 Influence of extraction solvents, acetonitrile (ACN), methanol (MeOH), acetone, and 10% MeOH in ACN, on recoveries of the target pesticides. Extracts were cleaned using tandem C_{18}, Florisil, and Na_2SO_4 cartridges, and elution solvents included 6 mL of 3% toluene in hexane, 9 mL of 6% ether in hexane and 5 mL of 5 : 3 : 2 hexane : toluene : ether. Each data point represents the mean of two replicates.

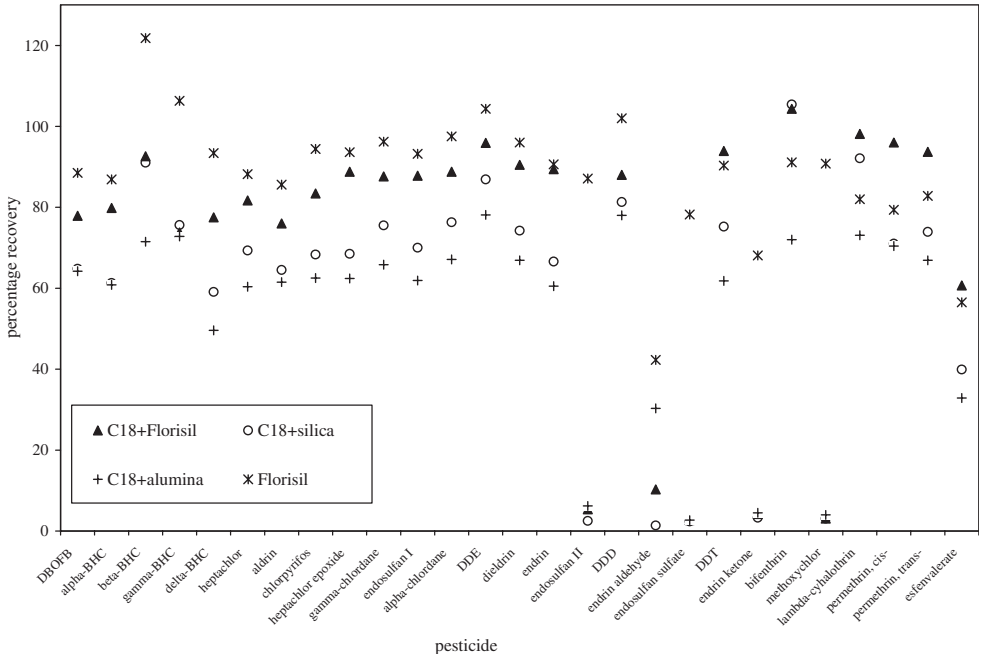

FIGURE 3 Comparison of different absorbents (C_{18} and Florisil cartridges, C_{18} and silica cartridges, C_{18} and alumina cartridges, and Florisil cartridge). A Na_2SO_4 cartridge was included with all of the different absorbents tested. Fish tissue was extracted with acetonitrile, and the elution solvents included 6 mL of 3% toluene in hexane and 9 mL of 6% ether in hexane. Each data point represents the mean of two replicates.

treatment. A Na_2SO_4 cartridge was used with each treatment to remove excess water. The activation sites for silica and alumina absorbents proved to be too strong, thereby overly retaining the target pesticides and resulting in relatively low recoveries (typically $<75\%$). Florisil and a tandem setup with Florisil and C_{18} worked better as absorbents with recovery of most analytes being $>80\%$ (Fig. 3). Further evaluation of these absorbents showed that the tandem setup with Florisil and C_{18} provided cleaner chromatograms than the Florisil alone (Fig. 4). At the same time, the tandem clean-up method obviated the time-consuming extraction solvent-exchange step. Therefore, the tandem setup with Florisil and C_{18} was chosen for further study. This is in agreement with the results of Dabrowska *et al*. [27], who found that the separation of cholesterol, its derivations, and other interfering substances from the analytes was improved with the use of C_{18} cartridges within the clean-up setup.

Elution Solvents Selection

Table I shows the recoveries of pesticides with different elution solvent mixtures using the tandem Florisil and C_{18} SPE setup. The majority of the pesticides were eluted from the cartridges when 6 mL of a 3% toluene in hexane solvent mixture was used (Table I, M1), but recoveries were not uniformly good. A further elution of the cartridges with 9 mL of 6% ether in hexane (Table I, M2) provided better recoveries for most of the pesticides with the exception of some of the relatively polar OCs (e.g. endosulfan II, endrin aldehyde, endosulfan sulfate, endrin ketone, methoxychlor). A solvent mixture

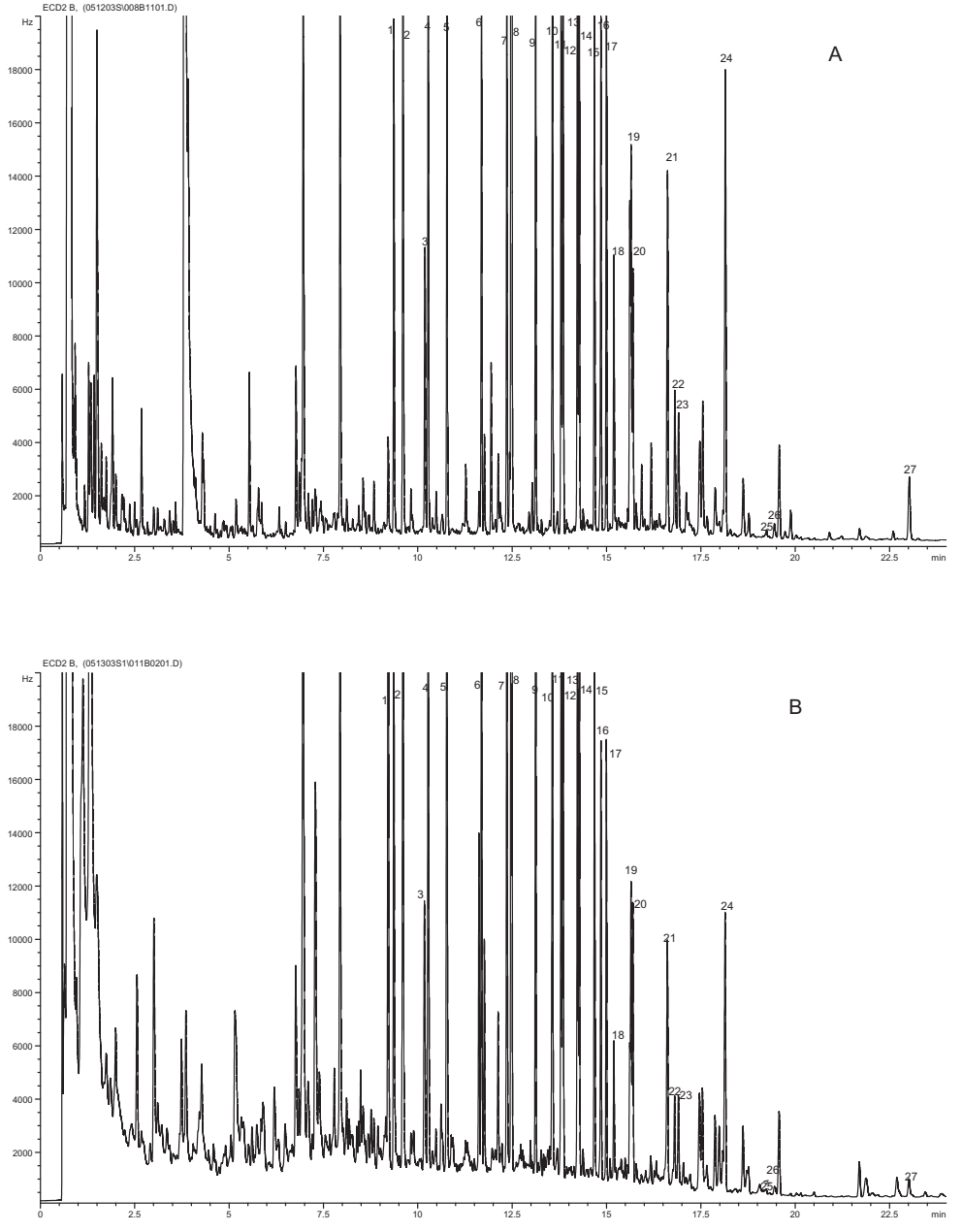

FIGURE 4 Sample chromatograms comparing sample clean-up using tandem C_{18}, Florisil, and Na_2SO_4 cartridges (A) or Florisil and Na_2SO_4 cartridges (B). An HP-5-ms GC column was used with a pesticide concentration of 25 µg/kg. Peaks: 1: DBOFB; 2: alpha-BHC; 3: beta-BHC; 4: gamma-BHC; 5: delta-BHC; 6: heptachlor; 7: aldrin; 8: chloropyrifos; 9: hetachlor epoxide; 10: gamma-chlordane; 11: endosulfan I; 12: alpha-chlordane; 13: dieldrin; 14: *p,p′*-DDE.; 15: endrin; 16: endosulfan II; 17: *p,p′*-DDD; 18: endrin aldehyde; 19: endosulfan sulfate; 20: *p,p′*-DDT; 21: endrin ketone; 22: bifenthrin; 23: methoxychlor; 24: lambda-cyhalothrin; 25: *cis*-permethrin; 26: *trans*-permethrin; 27: esfenvalerate.

TABLE I Percent recoveries of pesticides using different elution solvent mixtures

Compound	*Recovery (%)*			
	M1[a]	*M2*[b]	*M3*[c]	*M2 + M3*
DBOFB (surrogate)	73.1	75.7	44.4	89.9
Alpha-BHC	85.1	81.9	75.4	95.9
Beta-BHC	74.4	101.6	97.9	95.6
Gamma-BHC	71.0	83.3	89.1	94.8
Delta-BHC	18.4	72.4	50.6	89.7
Heptachlor	91.9	76.0	45.1	91.5
Aldrin	68.6	71.4	37.0	82.9
Chlorpyrifos	57.6	90.2	76.0	97.3
Heptachlor epoxide	64.2	86.5	69.8	93.5
Gamma-chlordane	72.2	80.4	44.8	88.4
Endosulfan I	52.3	87.7	61.3	93.2
Alpha-chlordane	73.6	80.8	48.8	91.0
p,p′-DDE	75.1	85.8	37.2	103.8
Dieldrin	40.5	89.6	68.2	98.5
Endrin	39.3	83.3	66.6	93.6
Endosulfan II	1.0	7.8	72.8	91.9
p,p′-DDD	95.2	88.4	40.7	80.6
Endrin aldehyde	2.6	1.8	58.1	80.9
Endosulfan sulfate	4.0	0.1	89.9	96.3
p,p′-DDT	73.9	82.3	82.4	100.3
Endrin ketone	1.5	3.4	70.8	83.0
Bifenthrin	39.9	88.8	31.9	90.6
Methoxychlor	5.8	2.8	82.3	93.0
Lambda-cyhalothrin	13.0	75.2	27.2	77.2
Cis-permethrin	42.4	81.8	26.0	87.2
Trans-permethrin	33.9	77.3	24.9	98.1
Esfenvalerate	4.0	64.3	24.8	40.0

[a]M1: Elution with 6 mL of 3% toluene in hexane. [c]M2: elution with 6 mL of 3% toluene in hexane and 9 mL of 6% ether in hexane. [b]M3: Elution with 5 mL of hexane : toluene : hexane (5 : 3 : 2, v/v/v), C_{18}, Florisil, and Na_2SO_4 tandem cartridges were used ($n = 2$). Extraction solvent: 25 mL of acetonitrile. Spiked concentration: 50 µg/kg.

of hexane : toluene : ether (5 : 3 : 2, v/v/v) was found to elute these relatively polar OCs reasonably well, though the average recovery of all the pesticides using this solvent mixture was lower (Table I, M3). Therefore, a combination of elution solvents (Table I, M2 + M3) was chosen to recover the target pesticides.

Comparison of Tandem SPE Clean-up and Sulfuric Acid Methods

Treatment with concentrated sulfuric acid is a very effective clean-up method for lipid removal and has been used in a number of studies [29,30]. However, this method can destroy some of the target OC pesticides [31]. A comparison of the newly developed tandem SPE clean-up method with sulfuric acid treatment clean-up is provided in Table II.

The two methods provided comparable recoveries for most of target pesticides with the exception of endrin, endrin aldehyde, and esfenvalerate that were lost during the sulfuric acid treatment. Similarly, the recovery of endrin ketone significantly increased as a result of the sulfuric acid treatment. This may be due to endrin and endrin aldehyde being transformed into endrin ketone during the sulfuric acid treatment.

TABLE II Percent recovery of analytes using tandem SPE clean-up (C1) *vs*. sulfuric acid treatment (C2)

Compounds	*Recovery* (%)	
	C1[a]	*C2*[a]
DBOFB	93.8	82.6
Alpha-BHC	101.0	81.1
Beta-BHC	116.1	107.1
Gamma-BHC	110.7	94.1
Delta-BHC	99.7	91.5
Heptachlor	97.3	86.0
Aldrin	90.1	75.8
Chlorpyrifos	95.3	84.2
Heptachlor epoxide	101.5	91.9
Gamma-chlordane	100.2	85.4
Endosulfan I	99.9	84.8
Alpha-chlordane	106.5	96.4
p,p′-DDE	133.8	124.5
Dieldrin	98.8	89.2
Endrin	97.2	18.0
Endosulfan II	96.4	101.7
p,p′-DDD	111.4	79.6
Endrin aldehyde	88.2	20.6
Endosulfan sulfate	101.5	114.7
p,p′-DDT	117.2	178.6
Endrin ketone	104.3	152.9
Bifenthrin	100.8	80.9
Methoxychlor	106.4	94.8
Lambda-cyhalothrin	86.1	75.6
Cis-permethrin	107.9	104.0
Trans-permethrin	103.0	111.6
Esfenvalerate	100.5	40.2

[a]Fish tissue (control fish) was spiked with 5 μg/kg and extracted with 25 mL of acetonitrile–methanol (9 : 1, v/v) ($n = 2$).

Method Validation

The newly developed method was validated using control catfish spiked at 1, 5, and 25 μg/kg (Table I) and 50 μg/kg (Table III). Analyte recoveries ranged from 87.7 to 126.0%, from 86.1 to 117.2%, from 78.6 to 97.7%, and from 77.2 to 103.8% for the control fish spiked at the four concentration levels, respectively, with the exception of *p,p′*-DDE and esfenvalerate. The recovery of *p,p′*-DDE was extremely high (230.1%) at the spiked concentration of 1 μg/kg. This may be due to the co-elution of matrix interferences, and this co-elution effect decreased with increasing analyte concentration. The recovery of esfenvalerate was reduced when the spiked concentration increased. In our previous study [32], we found that pyrethroids, especially esfenvalerate, were retained onto the Florisil absorbent more strongly than the OCs [32]. Therefore, the low recovery of esfenvalerate may be the result of the relatively poor ability of the chosen elution solvents to elute these compounds from the cartridges. This was especially true at the higher concentration and when the Florisil cartridge was not deactivated.

The reproducibility of an analytical method is characterized by the relative standard deviations (RSD). The percent RSDs were $< 10\%$ for most pesticides at the spiked concentrations of 5 and 25 μg/kg ($n = 4$) and $< 20\%$ at the low concentration of

TABLE III Recovery and relative standard deviations (RSD) of the selected surrogate, organochlorine, organophosphate, and pyrethroid insecticides at different spiked concentrations

Compounds	*MDL* (μg/kg)	1 μg/kg ($n=7$)		5 μg/kg ($n=4$)		25 μg/kg ($n=4$)	
		Recovery (%)	*RSD* (%)	*Recovery* (%)	*RSD* (%)	*Recovery* (%)	*RSD* (%)
DBOFB	0.44	103.7	13.4	93.8	2.1	82.4	6.8
Alpha-BHC	0.22	105.8	6.8	101.0	1.9	89.4	5.0
Beta-BHC	0.45	112.7	12.7	116.1	2.7	92.6	2.3
Gamma-BHC	0.28	119.8	7.4	110.7	2.4	92.4	2.0
Delta-BHC	0.19	103.1	5.9	99.7	5.2	90.4	1.0
Heptachlor	0.33	106.5	9.8	97.3	2.8	89.5	3.4
Aldrin	0.19	91.9	6.7	90.1	3.2	78.1	4.6
Chloropyrifos	0.21	95.1	6.9	95.3	3.0	93.4	3.5
Heptachlor epoxide	0.47	117.3	12.8	101.5	5.1	94.1	3.8
Gamma-chlordane	0.17	101.7	5.4	100.2	3.3	87.7	0.7
Endosulfan I	0.25	106.9	7.5	99.9	4.3	89.1	3.1
Alpha-chlordane	0.14	106.1	4.2	106.5	2.3	88.9	3.0
p,p′-DDE	1.40	230.1	19.4	133.8	5.8	95.7	7.4
Dieldrin	0.19	110.8	5.5	98.8	1.9	97.7	2.6
Endrin	0.28	118.1	7.6	97.2	0.6	93.2	1.1
Endosulfan II	0.29	101.2	9.0	96.4	3.1	93.8	2.2
p,p′-DDD	0.28	124.0	7.1	111.4	5.2	84.3	5.2
Endrin aldehyde	0.13	99.6	3.9	88.2	3.3	82.7	2.6
Endosulfan sulfate	0.60	113.5	16.9	101.5	4.4	93.6	3.1
p,p′-DDT	0.55	126.0	13.8	117.2	3.2	88.5	8.9
Endrin ketone	0.29	103.1	9.1	104.3	4.5	88.7	5.1
Bifenthrin	0.28	87.7	10.0	100.8	4.7	82.7	9.5
Methoxychlor	0.32	111.2	9.2	106.4	3.0	96.4	6.9
Lambda-cyhalothrin	0.19	92.4	6.5	86.1	10.4	78.6	10.1
Cis-permethrin	0.66	120.6	17.3	107.9	12.1	92.8	5.3
Trans-permethrin	0.53	113.4	14.8	103.0	4.4	96.7	2.0
Esfenvalerate	0.24	93.8	8.1	100.5	9.3	56.6	25.8
Average		111.7	11.3	100.8	4.6	88.5	5.1

MDL: Method detection limits.

1 μg/kg ($n=7$). The MDL is an important parameter used to assess an analytical method and is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero [33]. The MDLs were computed from seven replicate fish samples spiked at 1 μg/kg and were calculated as follows: $\text{MDL} = st_{(0.99,\,n-1)}$, where s is a standard deviation of the seven replicate measurements and $t_{(0.99,\,n-1)} = 3.14$ is a t-distribution value taken at a confidence level of 0.99 and 6 degrees of freedom. As shown in Table III, the MDLs of the target pesticides ranged from 0.13 to 1.40 μg/kg. Taking into account the coelution problem of *p,p′*-DDE at a low concentration, we chose 5 μg/kg as the detection threshold for this newly developed method.

Method Application

Sixty-four fish from five species were collected from the 15 lakes located in the proposed Illinois National Guard Armory and analyzed by the method described above. The results are summarized in Table IV. Of the 26 target pesticides analyzed in fish tissue, 12 were detected above the detection threshold of 5 μg/kg. Of the fish sampled, 22 contained pesticide residues at detectable levels with an average total pesticide

TABLE IV Analytical results of fish (total of 64) collected from lakes in the proposed Illinois National Guard Armory

Pesticide	*No. of times detected*	*Average concentration, range* (μg/kg)
Beta-BHC	3	6.84, 5.17–8.02
Gamma-BHC	1	6.34
Heptachlor	3	11.21, 5.03–22.63
Chloropyrifos	1	14.98
Gamma-chlordane	1	9.09
p,*p*′-DDE	1	5.20
Dieldrin	2	6.69, 5.82–7.56
Endrin	1	6.40
p,*p*′-DDD	1	6.55
p,*p*′-DDT	13	6.22, 5.04–9.70
Cis-permethrin	4	8.13, 5.49–11.44
Trans-permethrin	4	7.76, 5.26–10.57

concentration of 10.4 μg/kg. Permethrin was the only pyrethroid detected, and chlorpyrifos, the only OP analyzed, was found in a single fish sample with a concentration of 15.0 μg/kg. With a concentration of 22.6 μg/kg, heptachlor was detected at the highest concentration in any sample, while *p*,*p*′-DDT was detected, most frequently being found in 13 of 64 fish samples with an average concentration of 6.2 μg/kg.

CONCLUSION

A tandem SPE with a reverse phase absorbent (C_{18}) and a normal phase absorbent (Florisil) was chosen as the preferred clean-up method after the fish sample was extracted by sonication with a solvent mixture of methanol and acetonitrile (1 : 9, v/v). Three solvent mixtures, including 3% toluene in hexane, 6% ether in hexane, and a mixture of hexane : toluene : ether (5 : 3 : 2, v/v/v), were found to work best as elution solvents to recover the target pesticides from the fish tissue. This newly developed method is simple, consumes only small amounts of solvent and does not require highly expensive equipment.

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