*ScienceAsia* 29 (2003): **127-134**

# Determination of Organochlorine Pesticides in Commercial Fish by Gas Chromatography with Electron Capture Detector and Confirmation by Gas Chromatography–Mass Spectrometry

**Arunee Therdteppitak* and Kittima Yammeng**
Department of Chemistry, Faculty of Science, Burapha University, Bangsean, Chonburee 20131, Thailand.
*Correspondence author.

*Received 29 Apr 2002*
*Accepted 20 Dec 2002*

**ABSTRACT** A method has been studied for the analysis of 16 organochlorine pesticides (OCPs) in Fish. The method was based on ultrasonic extraction using *n*-hexane-acetone (90:10, v/v) for 30 min, three times. The extracts were fractionated and cleaned up with a 5 g Florisil column. Diethylether:*n*-hexane (6:94,v/v) and (50:50,v/v) were used as elute solvents. Two fractions were collected separately. The mean recoveries of the 15 compounds were in the range of 82% to 96% with 3% to 11% RSD, except for endosulfan II only 76% recovery with 6% RSD was obtained. The concentrations of analytes were determined by gas chromatography with electron capture detection. Positive results were confirmed by gas chromatography-mass spectrometry in selected ion mode (SIM). The limit of detection and quantitation were in the range 0.02 to 0.34 ng/mL and 0.10 to 1.0 ng/mL, respectively. The method has been applied for the analysis of organochlorine pesticides in the edible portion of 10 different kinds of fish, namely, striped snaked-head (*channa striats*), common silver barb (*Barbodes gonionotus*), tubtim (*Oreochromis niloticus*), nile tilapia (*Oreochromis niloticus*), sand goby (*Oxyeleotris marmoratus*), common carp (*Cyprinus carpio*), grey feather (*Notopterus notopterus*), common climbing perch (*Anabas testudineus*), snake skin gourami (*Trichogaster pectoralis*) and moonlight gourami (*Trichogaster microlepis*). Fat contents ranged from 2% to 9%. The OCPs alpha-BHC, gamma-BHC, delta-BHC, aldrin, endosulfan I, p,p′-DDE and dieldrin were detected in 9 of 10 species. The highest concentration was delta-BHC, 35±1 ng/g wet weights in *Channa striats*.

KEYWORDS: Organochlorine pesticides, fish, ultrasonic extraction, GC-ECD, GC-MS

## INTRODUCTION

Many persistent, bioaccumulated organochlorine pesticides (OCPs) have been extensively used in many countries. Compounds such as DDT′s and diedrin persist in the environment for long periods and continue to contaminate aquatic food webs, often at levels thought to be hazardous to both human and ecosystem health.[1,2]

Various techniques of isolation, preconcentration and clean-up of pesticide residue extracts from lipid-containing tissue have been carried out. Soxhlet extraction with a variety of organic solvents, for example, hexane, ether, acetone, alcohol and their combinations, was among the most common method for extracting OCPs from lipid-containing tissue. Other methodologies include centrifugation and ultrasonic and supercritical fluid extraction. The extracts obtained from the methods mentioned generally required clean up and fractionation before analysis. Commonly used methods for clean-up of raw extracts of samples are chromatographic columns filled with adsorbent such as Florisil, alumina, silica gel, mixtures of alumina and silica gel, gel permeation on Bio-Beads SX3, XAD-2 resin GPC, etc. Some of these methods are expensive, as the cost of the adsorbent constitutes a majority of the entire cost of analysis. The elution solvents used also varied.[3-10]

The objective of this study was to investigate the analytical methods to determine 16 OCPs in fish tissue, using a column containing Florisil for clean-up and fractionation with different solvents. The developed method has been applied to determine OCPs in commercial fish collected from major breeding areas in Thailand. Gas chromatography with electron capture detection (GC-ECD) produced positive results which were confirmed with GC-mass spectrometric (GC-MS) detection.

## Materials and Methods

### Reagents

Solvents were used without further purification. Acetone, *n*-hexane and dichloromethane (DCM) were HPLC grade, from Fisher Scientific Ltd., USA. Diethylether and Florisil (60-100 mesh) were PR grade, from Carlo Erba, Italy. Florisil was activated overnight (12 h) at 130 °C before use. Petroleum ether was AR grade, from Mallinckrodt. The 16 pesticide standard mixtures of hexachloro-cyclohexane(HCH) ($\alpha$, $\beta$, $\gamma$, $\delta$ isomers), heptachlor, aldrin, heptachlor epoxide, endosulfan (I, II ), endosulfane sulfate, diedrin, endrin, endrin aldehyde and the diphenyl trichloroethane group ( 4,4′-DDD, 4,4′-DDE, 4,4′-DDT) were 2000 ppm in hexane-toluene(50:50), and the internal standard 2,4,5,6-tetrachloro-m-xylene was 200 ppm in methanol. All were from Supelco, USA. Anhydrous sodium sulphate [granulated for residue analysis (Merck)] was activated at 200°C for 2 h before use. All glassware was washed with detergent, rinsed with purified water and acetone, and heated to 180-200 °C for 2 h.

### Sample Selection and Preparation

Fish species samples were purchased from a commercial fishery market, Sapan Pra, Ampae Bangprakong, Bangkok and from the fishery farm in Ampae Bang Boa, Samut Prakarn Province. The sizes and weights are shown in Table 1. After removal of the skin, the muscle tissue was dissected as much as possible from each fish. The tissue was homogenized with a commercial meat grinder. The mixing was repeated until the composite sample appeared to be homogeneous, then it was kept frozen at 4 °C until extraction.

Fish tissue of nile tilapia (*Oreochromis niloticus*) was used to validate the methodology.

### Chromatographic Fractionation Test

Initial tests were made to assess the ability of different solvents to fractionate the 16 OCPs from the Florisil column. The standard solutions of 16 pesticides (100 ppb, 0.25 mL) were applied to 5 g of activated Florisil topped with 1 cm height of anhydrous sodium sulfate, contain in a glass column 20.0 cm in length with 1.0 cm I.D. And the column is pre-washed with *n*-hexane for the experiment scheme A to F, and with petroleum ether for scheme G, prior to the addition of the standard solutions. The column flow rate was also adjusted to 2-5 mL/min. The first 2 mL of eluent was discarded. Then, 100 mL of each fraction (F1, F2, and F3) was collected. The following schemes (A to G) were attemped.

**A:** F1: diethyl ether:*n*-hexane (6:94, v/v), F2: diethyl ether:*n*-hexane (15:85, v/v)

**B:** F1: diethyl ether:*n*-hexane (6:94, v/v), F2: diethyl ether:*n*-hexane (15:85, v/v),
F3: diethyl ether:*n*-hexane (50:50, v/v)

**C:** F1: diethyl ether:*n*-hexane (6:94, v/v), F2: diethyl ether:*n*-hexane (50:50, v/v)

**D:** F1: *n*-hexane, F2: diethyl ether:*n*-hexane (50:50, v/v)

**E:** F1: DCM:*n*-hexane (20:80, v/v), F2: DCM:*n*-hexane (50:50, v/v)

**F:** F1: DCM:*n*-hexane (20:80, v/v), F2: DCM: *n*-hexane:acetonitrile (25:74.65:0.35, v/v)

**G:** F1: diethyl ether:petroleum ether (6:94, v/v), F2: diethyl ether:petroleum ether (15:85,v/v), F3: diethyl ether petroleum ether(50:50, v/v).

Each fraction was concentrated to approximately 5 mL or less by vacuum rotary evaporator, and the volume reduced to less than 1 mL using a Kuderna-Danish (KD) concentrator. The concentrated aliquot was blown down with nitrogen, the internal standard was added, and the final volume was adjusted to 1 mL for GC-ECD quantification.

### Soxhlet Extraction

A 10 g amount of fish tissue was weighed into a beaker containing 50 g anhydrous sodium sulfate and mixed. The sample mixture was transferred to an extraction thimble, and placed in a Soxhlet extractor. The mixture was extracted for 4h with 150 mL of acetone-*n*-hexane sovents. The ratios of acetone:*n*-hexane, 20:80 and 10:90 (v/v), were used. The extracts were filtered, concentrated by vacuum rotary evaporator, and the volume reduced to 1 mL using the KD concentrator.

The 0.25 mL of the concentrated extract was applied to 5 g of activated Florisil column topped with 1 cm of anhydrous sodium sulfate, which was pre-washed with *n*-hexane. The column was eluted with diethyl ether:*n*-hexane (6:94, v/v), and diethyl ether:*n*-hexane (50:50, v/v) respectively. Two fractions were collected, F1: 60 mL of diethyl ether:*n*-hexane (6:94, v/v) and F2: 80 mL of diethyl ether:*n*-hexane (50:50, v/v). Each fraction was concentrated and analyzed by the procedure described in chromatographic fractionation test section.

A spiked standard mixture was added to 40 ng/g wet weight of fish tissue sample before extraction was performed to evaluate the recovery of compounds.

### Ultrasonic Extraction

A 10 g amount of fish tissue was weighed into a flask containing 50 g anhydrous sodium sulfate and mixed. Then, 50 mL of acetone:*n*-hexane (10:90 v/v) was added and the distillation column was connected to the flask. Extraction was done for 30 min, filtering off the

**Table 1.** Organochlorine pesticide concentrations (ng/g wet weight), fat content (%wt/wt), length and weight of commercial fishes collected from Saparn Pra, Bangkok and Bang Boa, Samutprakarn.

| Samples | Concentration( ng/ g wet weights) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | length (cm) | weight (g) | %fat | alpha-BHC | Gamma-BHC | delta-BHC | aldrin | endo sulfan I | p,p′-DDE | dieldrin |
| Striped snaked-head*(channa striats)* | 30 - 35 | 700 - 1000 | 6.74 | nd | nd | 35.0±1.0 | nd | nd | nd | 7.5±2.1 |
| Common silver barb(*Barbodes gonionotus*) | 20 - 25 | 400 - 600 | 5.37 | nd | nd | 14.2±0.5 | nd | nd | nd | 2.7±0.3 |
| tubtim(*Oreochromis niloticus*) | 25 –3 0 | 500 - 600 | 7.25 | nd | nd | nd | nd | 13.0±0.3 | 8.2±0.8 | nd |
| Nile tilapia(*Oreochromis niloticus*) | 20 - 25 | 400 - 500 | 2.55 | nd | nd | nd | nd | nd | 1.4±0.1 | nd |
| | | | | [a]2.1±0.1 | | | | | [a]1.2±0.2 | [a]1.0±0.1 |
| Sand goby(*Oxyeleotris marmoratus*) | 22 - 25 | 400 - 500 | 4.94 | 2.0±0.1 | nd | nd | nd | nd | nd | nd |
| Common carp(*Cyprinus carpio*) | 18 - 22 | 200 - 300 | 2.19 | nd | nd | nd | nd | nd | 1.1±0.2 | 0.6±0.1 |
| grey feather (*Notopterus notopterus*) | 20 - 25 | 180 - 250 | 9.32 | 1.0±0.1 | nd | nd | 0.5±0.1 | nd | 1.9±0.1 | 2.3±0.3 |
| Common climbing perch(*Anabas testudineus*) | 14 - 17 | 150 - 200 | 5.96 | 9.9±0.6 | nd | nd | nd | nd | nd | 13.0±0.2 |
| snake skin gourami(*Trichogaster pectoralis*) | 15 - 18 | 100 - 150 | 2.33 | nd | 1.1±0.2 | nd | nd | nd | nd | nd |
| | | | | | | | [a]0.7±0.1 | | [a]1.9±0.1 | [a]1.1±0.1 |
| moonlight gourami (*Trichogaster microlepis*) | 8 - 10 | 10 - 15 | 1.89 | nd | nd | nd | nd | nd | nd | nd |

a:- from Bang Boa, Samut Prakarn: nd=nondetected

supernatant. The extraction was repeated two more times and all the supernatants combined. The concentration, fractionation and clean up steps were then done, as described in the soxhlet extraction section.

### Instrumental Analysis

A HP5890 gas chromatograph equipped with an electron capture detector (ECD) was used for GC analysis. The separation was performed on an Ultra 2 capillary column, 25 m x 0.32 mm I.D., 0.52 μm film thickness 5% phenyl methyl silicone (HP). The injector and detector temperatures were 200°C and 300°C, respectively. Helium was used as the carrier gas with flow rate 1.5 mL/min, nitrogen makes up gas at 40 psi. The temperature program was at 85°C (0 min) followed by a 35°C/min ramping to 210°C, held at 210°C for 2 min, and thereafter ramped by 2°C/min to 220°C, and then held there for 15 min.

GC-ECD positive results were confirmed with GC-mass spectrometric (GC/MS) detection. The HP 6890 gas chromatography interfaced to a HP5972 mass-selective detector, and a HP-5 MS column, 30 m x 0.25 mm ID, 0.25 μm film thickness 5% phenyl methyl siloxane was used. The temperature program used was the same as the temperature program of the GC-ECD. The injector temperature was 200°C. Helium was used as carrier gas with flow rate 1.2 min/mL, splitless injection volume of 1 μL, and purge time 0.5 min. Mass spectrometer settings: ionization energy, 70eV and ion source temperature 300°C. The analyses were operated in SIM mode. Identification was made on the basis of matching the mass spectrum and the retention time of the compound to that of a known standard.

## Results and Discussion

### Chromatographic Fractionation and Clean-Up

The column chromatography method allowed the separation of the contaminants of interest in fractions. For the schemes A to G, the recoveries of the 16 OCPs were in the ranges of 10% to 103%, 71% to 102%, 87% to 105%, 67% to 115%, 61% to 105%, 63% to 102% and 59% to 102%, respectively. The recovery results in Fig 1 showed that the polarity index of the solvents influenced how complete the elution of the target compounds were. For the first three solvent systems (A to C), endosulfan II, endrin aldehyde and endosulfan sulfate were observed in the latter fraction, since the elution solvent of diethyl ether: *n*-hexane (15:85,v/v) was not polar enough for those three compounds. The recoveries were improved significantly in changing the polarity of the eluting solvent by increasing the concentration of diethyl ether in *n*-hexane from 15% to 50% (B, C). From the overall recovery results for the 16 OCPs, the suitable solvent systems are schemes B and C. However, for solvent system B, endrin aldehyde was detected in three fractions, endosulfan II and ensosulfan sulfate were detected in two fractions, so the uncertainty of the experiment would then be increased, but only endrin aldehyde was detected in both fractions for C. Therefore scheme C would be the most efficient of the solvent systems studied, and was threrfore chosen. Representative gas chromatograms of the two fractions using C were shown in Fig 2.

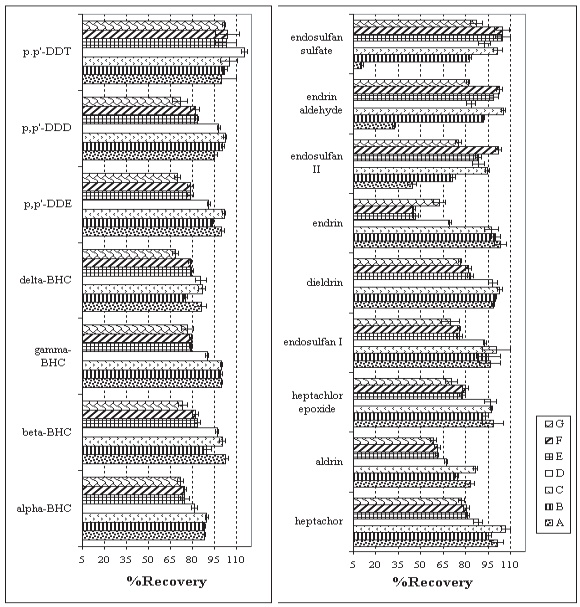


**Fig 1.** Recoveries of 16 organochlorine pesticides after fractionation on 5 g Florisil column elute with different solvent systems (n=3): **A:** F1: diethyl ether:*n*-hexane(6:94, v/v), F2: diethyl ether:*n*-hexane (15:85, v/v) **B:** F1: diethyl ether:*n*-hexane (6:94, v/v), F2: diethyl ether:*n*-hexane (15:85, v/v), F3: diethyl ether:*n*-hexane (50:50, v/v) **C:** F1: diethyl ether:*n*-hexane (6:94, v/v), F2: diethyl ether:*n*-hexane (50:50, v/v) **D:** F1: *n*-hexane, F2: diethyl ether:*n*-hexane(50:50, v/v) **E:** F1: DCM:*n*-hexane (20:80, v/v), F2: DCM:*n*-hexane(50:50, v/v) **F:** F1: DCM:*n*-hexane (20:80, v/v), F2: DCM:*n*-hexane:acetonitrile (25:74.65:0.35, v/v) **G:** F1: diethyl ether:petroleum ether (6:94, v/v), F2: diethyl ether:petroleum ether (15:85, v/v), F3: diethyl ether:petroleum ether (50:50, v/v).

The small dimensions of the column selected for clean up afford an easier and lower cost analysis. The 5 g amount of Florisil can tolerate up to 9% fat tissue for the 10 g of sample used in this study.

### Extraction Procedure and Recovery Study

The recoveries of the 4-h soxhlet extraction of spiked standards in 40 ng/g wet fish tissue in using two different ratios of acetone: *n*-hexane, 20:80 and 10:90, (v/v) are shown in Fig 3. The recoveries were in the range 72% to 92% with 1% to 4% RSD and 78% to 98%, with 1% to 9% RSD respectively. The paired sample *t*-test applied to the data showed that the differences were significant

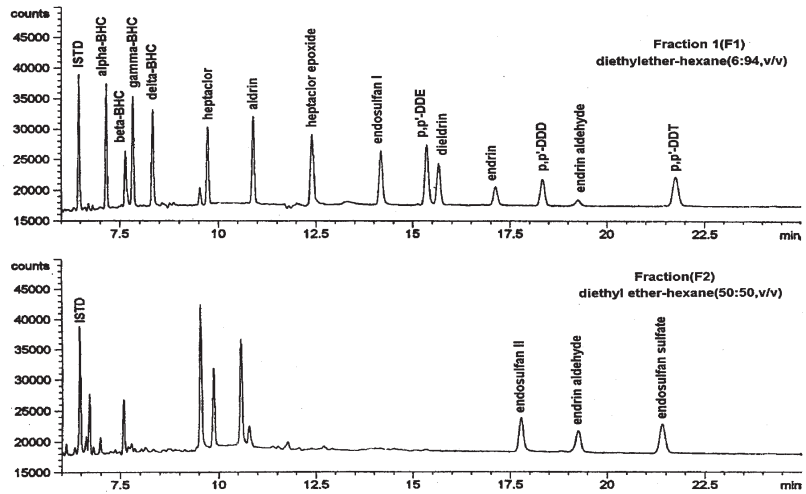


**Fig 2.** Gas chromatogram on Ultra II column of the two fractions (F1, F2) from the Florisil column. ISTD = internal standard

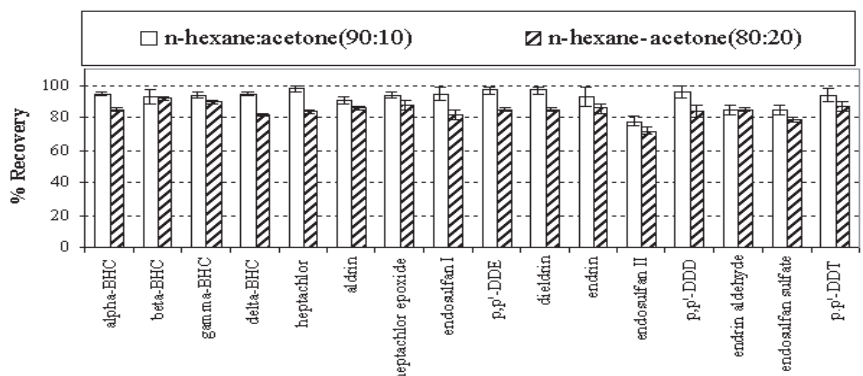


**Fig 3.** Recoveries of organochlorine pesticides from fish tissue (@ 40 ng/g wet wt, n=3), using 4-h soxhlet extraction with two different ratios of *n*-hexane-acetone mixtures

for alpha-BHC, delta-BHC p, p′-DDE and dieldrin at the 0.05 level. Better recoveries are observed using acetone:*n*-hexane 10:90 (v/v) overall. The recoveries of ultrasonic and soxhlet extraction were then compared using the extracted solvent, acetone:*n*-hexane 10:90 (v/v). The fortified standards were at three concentration levels of 10, 24 and 40 ng/g wet fish tissue, respectively. The results are presented in Table 2 and Fig 4. The mean recoveries were in the ranges of 76% to 96% with 3% to 11% RSD and 78% to 98%, with 3% to 4% RSD respectively. The recoveries of the two methods were not significantly difference at the 0.05 level. Since ultrasonic extraction requires shorter time (1.5 h) and no consumable thimble, we suggest that ultrasonic extraction should be the method of choice.

## Validation of the Method

### Repeatability

Three dilution standard mixtures (10, 50, and 100 μg/L of internal standard) were injected for five times of each concentration. Relative standard deviations (RSD) of the 16 pesticides ranged from 0.5% to 6.0%, 1.3% to 3.0% and 1.1% to 2.7% at 10, 50 and 100 μg/L concentrations, respectively.

### Calibration and Linearity of the Instrumental Response

The linearity of the detector response to the analytes was examined by the injection of standards at 6 concentrations, 1, 10, 25, 100, 500 and 1,000 μg/L. The regression coefficients ($r^2$) of all compounds were higher than 0.99 and for beta-BHC, aldrin, endrin, p, p′-DDD, they were higher than 0.999. Within these linearity ranges, calibration curves plotted from 6 concentrations (1, 10, 25, 50, 100 and 120 μg/L) were obtained for all compounds by regression analysis of peak areas versus injection concentrations. In all compounds, regression coefficients ($r^2$) were higher than 0.999. The calibration technique was the internal standard method.

## Limit of Detection (LOD) and Limit of Quantitative (LOQ)

Three-dilution standards (1, 2 and 4 μg/L) were injected for seven times at each concentration. For each standard solution, a standard deviation was obtained and the means of standard deviations were determined for each analyte. The instrumental LOD[11], expressed in ng $L^{-1}$, was obtained from standard deviation of the y-intercepts of regression lines multiplied by a factor of 3.3, and the LOQ by the same means but using a factor of 10. The LOD and LOQ were in the range 0.02 to 0.34 ng/mL and 0.10 to 1.0 ng/mL, respectively.

## Accuracy

Recovery tests were performed in order to study accuracy. The edible fish tissue sample of nile tilapia (*Oreochromis niloticus*) were fortified with a mixture of 16 OCPs to reach the final concentrations of 10, 24 and 40 ng/g wet weight, respectively. Table 2 shows the recovery of these compounds through the method, following the procedure described above.

## Analysis of Real Samples

The proposed method was applied to the analysis of fish samples collected from Saparn Pra commercial fish market in Bangkok and from a breeding farm at

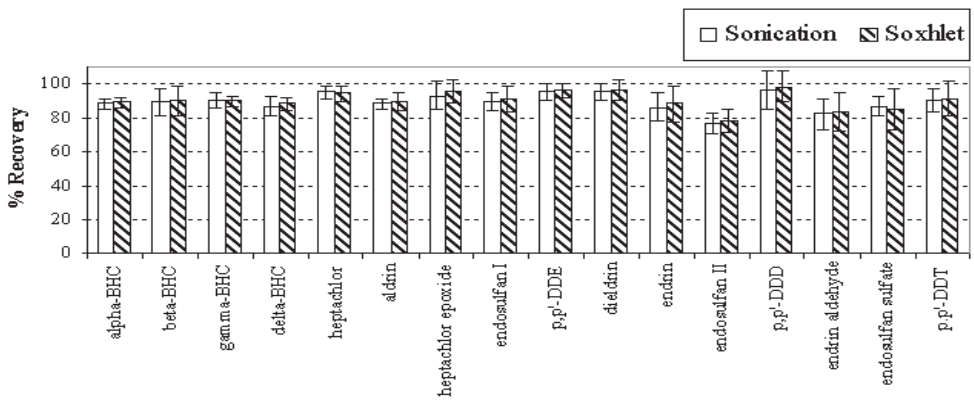


**Fig 4.** Mean recovery values of ultrasonic and soxhlet extraction. (n=5)

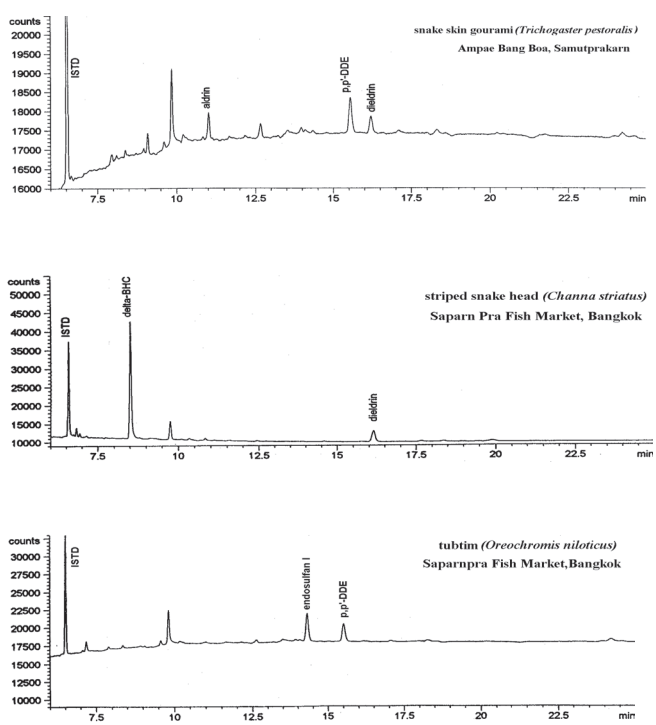


**Fig 5.** Gas chromatogram of different fish species from two different sources

**Table 2.** Recoveries at different fortification levels (10, 24 and 40 ng/g wet weight) using ultrasonic and soxhlet extraction followed by clean-up with 5 g florisil.

| Pesticides | Recovery (%)±SD(%) at given fortification level (ng/g wet weight) (n=5) | | | | |
|---|---|---|---|---|---|
| | [a]10 | [a]24 | [a]40 | Overall ultrasonic | [b]Overall soxhlet |
| Alpha-BHC | 79±2 | 91±2 | 94±1 | 88±3 | 89±3 |
| Beta-BHC | 82±7 | 93±2 | 91±3 | 89±8 | 90±9 |
| Gamma-BHC | 83±3 | 93±2 | 93±2 | 90±4 | 90±3 |
| Delta-BHC | 81±5 | 87±2 | 93±2 | 87±6 | 88±4 |
| Heptachlor | 92±3 | 93±2 | 99±2 | 95±4 | 94±5 |
| Aldrin | 84±2 | 90±1 | 90±2 | 88±3 | 89±5 |
| Heptachlor epoxide | 93±7 | 94±2 | 93±2 | 93±8 | 95±7 |
| Endosulfan I | 88±3 | 86±3 | 93±3 | 89±5 | 91±8 |
| p,p′-DDE | 95±2 | 94±3 | 95±3 | 95±5 | 96±4 |
| Dieldrin | 96±4 | 95±2 | 95±2 | 95±5 | 96±6 |
| Endrin | 85±5 | 86±2 | 88±6 | 86±8 | 88±11 |
| Endosulfan II | 70±4 | 79±2 | 78±4 | 76±6 | 78±7 |
| p,p′-DDD | 98±10 | 96±3 | 93±3 | 96±11 | 98±9 |
| Endrin aldehyde | 72±5 | 87±6 | 86±4 | 82±9 | 83±11 |
| Endosulfan sulfate | 85±4 | 90±3 | 85±4 | 87±6 | 85±12 |
| p,p′-DDT | 87±4 | 90±2 | 92±5 | 90±7 | 91±10 |

[a] ultrasonic extraction, [b]individual fortification levels of % recovery of soxhlet extraction not shown

Ampae Bang Boa, Samut Prakarn province. OCPs that were detected include alpha-BHC, gamma-BHC, delta-BHC, aldrin, endosulfan I, p, p′-DDE and dieldrin (Table 1), and representative chromatograms of fish samples are presented in Fig 5 Diedrin and p,p′-DDE had the highest frequency of occurrence in the fish species analyze, since these compounds are likely to persist in the environment. The highest concentration of delta-BHC was also detected in the largest size of fish.

For GC-MS confirmations, the eluents were concentrated further to meet the higher detection limits of the MS detector. The retention times of the compounds and the selected ions for confirmation residue identity of positive results of GC-ECD are summarized in Table 3.

**Table 3.** Retention times and ion masses of positive OCPs

| Compound | $t_R$(min) | m/z |
|---|---|---|
| alpha-BHC | 4.84 | 181,219 |
| gamma-BHC | 5.22 | 181,219 |
| delta-BHC | 5.52 | 109, 183, 219 |
| aldrin | 6.99 | 66, 263 |
| endosulfan I | 8.87 | 170, 195, 241 |
| p,p′-DDE | 9.57 | 246, 318 |
| dieldrin | 9.66 | 79 |

## Conclusions

The method of determination of 16 OCPs in edible part of fish tissue is based on 30 min ultrasonic extraction three times with acetone:*n*-hexane 10:90, v/v, fractionation and clean-up using 5 g Florisil. The eluting solvents were diethyl ether:*n*-hexane, 6:94 (v/v) and 50:50 (v/v). The concentrated eluents were analyzed by GC-ECD and GC-MS confirmation. The mean recoveries of the method with spiking at 10, 24 and 40 ng/g wet weight, respectively, were in the range 76% to 96% with 3% to 11% RSD. This finding suggests that the one-step clean-up procedure used is capable of monitoring the OCPs in muscle tissue with fat content up to 9%.

## Acknowledgements

The authors deeply appreciate the partial financial support from the Graduate College, Burapha University.

## References

1. Durham WB (1974) Pesticides in the Environment. *Industrial Pollution.* The van Nostrand Reinhold Company, New York, 387p.
2. Duke TW (1977) *Pesticide in aquatic environments and overview*. The Plenum Press, New York, 257p.
3. Mukherjee I and Gopal M (1996) Chromatographic techniques in the analysis of organochlorine pesticide residues, Review. *J Chromatogr A* **754**, 33-42.
4. Holstege DM , Scharberg DL, Tor ER, Hart LC and Galey, FD (1994) A rapid Multiresidue Screenfor Organophosphorus, Organochlorine, and *N*-Methyl Carbamate Insecticides in Plant and Animal Tissues. *J AOAC Int.* **77(5)**, 1263-74.
5. Long AR, Michaeld D and Steven AB (1991) Multiresidue Matrix Solid Phase Dispersion (MSPD) Extraction and Gas Chromatography Screening of Nine Chlorinated Pesticides in Catfish (*Ictalurus punctatus*) Muscle Tissue. *J AOAC Int.* **74(3)**, 663-8.

6. Lopez JE, Aguilar RL and Diez LM (1992) Comparative Study of Clean-up and Fractionation Methods for the Determination of Organochlorine Pesticides in Lipid by Gas Chromatography. *J Chromatogr* **591**, 303-11.
7. Porter ML, Yong, SJV and Burk JA (1970). A Method for the Analysis of Fish, Animal and Poultry Tissue for Chlorinated Pesticide Residues. *J AOAC Int* **53**(**6**), 1300-3.
8. Schenck FJ (1996) Screening of Non fatty Fish for of Organochlorine Pesticide Residues by Solid Phase Extraction Clean-up: Inter laboratory Study. *J AOAC Int* **79**(**5**), 1215-9.
9. Schenck FJ, Calderon L and Podhorniak LV (1996) Determination of Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Fatty Fish by Tandem Solid-Phase Extraction Clean-up. *J AOAC Int* **79**(**5**), 1209-14.
10. Snyder JS, Grob LR, Mary E and Timothy SO (1992) Comparison of Supercritical Fluid Extraction with Classical Sonication and Soxhlet Extractions for Selected Pesticides. *Anal Chem* **64**, 1940-6.
11. Krull I, Swartz M (1998) Determining Limits of Detection and Quantitation. *LC•GC* **16**(**5**), 464