EJAC
Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 9(2): 66-78, 2014

Development and Validation of New Clean-Up Approach for 21 Organo-Chlorinated Pesticides (OCPs)

Amal Al-Rashdan [*] **, Murad I.H. Helaleh**

Kuwait Institute for Scientific Research (KISR), Deputy Director General Office for Research, 13109 Safat, P.O. Box 24885, Kuwait.

Abstract

A validated procedure was developed for the separation and clean-up of Organo-Chlorinated Pesticides **(**OCPs) extracted from fish samples, using different adsorbent materials and different solvents composition. Pressurized liquid extraction (PLE) has been utilized for the extraction of OCPs. PLE provides one of the best alternative to conventional extraction methods. The different adsorbent materials as well as solvents composition were evaluated for the best clean-up steps of OCPs extracted from fish samples. Among the eight examined adsorbent materials, florisil and alumina were selected with hexane: ethylacetate (8:2, v/v) solvent mixture throughout the experiment. The highest recoveries were obtained (75-99.8 %). Detection was performed by GC-MS in Negative Chemical Ionization (NCI) mode, due to its high sensitivity and selectivity to chlorinated compounds. Bio-Beads SX-3 Based on Gel permeation Chromatography (GPC) was utilized for the removal of fat. The accuracy of the method (n= 5) is expressed as recovery (%) that was calculated to be between 56% and 98%, and the precision of the method is expressed as Relative Standard Deviation (RSD), that was obtained to be between 5% and 28 %.

Keywords:

Organo-Chlorinated Pesticides (OCPs); Pressurized Liquid Extraction (PLE); adsorbent materials; Gel Permeation Chromatography (GPC); Negative Chemical Ionization (NCI); marine sample

1. Introduction

Persistent Organic Pollutants (POPs) consist of heterogeneous groups including, Organo-Chlorinated Pesticides (OCPs), Polychlorinated Biphenyls (PCBs) and other organic pollutants [1]. POPs are characterized by high chemical, biological stability and lipophilicity, which makes POPs persist in the environment and bio-accumulate within the food chain [2]. POPs are toxic chemicals that have the capability to stay stable in the environment over long period of time. In the year 2001 Stockholm Convention, the Organochlorinated Pesticides (OCPs) and the Polychlorinated Biphenyls (PCBs) were given special attention and thus were included in the so called "dirty dozen"[3].

Organochlorinated Pesticides (OCPs) such as 1,2,3,4,5,6-hexachlorocyclohexane (HCH) and 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) are considered as ubiquitous environmental contaminants [4]. They are persistent toxicants that tend to accumulate in the food web. They have the capability to effect the ecosystem and the human health. The complex nature of the matrix requires to develop a trace analysis for OCPs

[*] **Corresponding Author**
E-mail: dramel_alrashdan@yahoo.com
ISSN: 1306-3057

compounds. Sample treatment consists of two steps: one step is to destroy the lipids by using SX3-300 Bio-beads (GPC) and the second step is the clean-up step that is performed by using different adsorbents.

The extracts of tobacco containing low polar OCPs was cleaned by using florisil followed by silica gel [5]. OCPs extract has been purified with 5% deactivated alumina with florisil in different samples [6-7]. The extracts of quillemotes rivers and guillemot eggs were cleaned for OCPs, PCBs and polybrominated diphenyl ethers (PBDEs) on a multilayer column packed with deactivated alumina, activated silica and activated silica impregnated with sulfuric acid [8], while the extracts of bald eagle tissue for OCPs, PCBs and Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/DFs) were subjected to silica gel, alumina, acidic silica and activated carbon column for clean-up and fractionation [9]. Activated silica was used for the fractionation containing PCBs, OCPs, and Poly Aromatic Hydrocarbons (PAHs) from the mussel extract [10-11]. Nerin et al. [12] tested several adsorbents such as 3% activated silica and 5% deactivated florisil with different solvents to clean-up the fog extracts for OCPs. The best results were found to be with 3% deactivated silica and hexane. Silica is known to retain polar compounds (e.g. fatty acid, phospo-lipids, ionized compounds). Alumina is used to retain some semi-polar interferences.

The analytical methods used for the determination of OCPs in environmental samples (fish) consist of several steps for sampling, sample treatment, fractionation and detection of targeted compounds. The sample treatment is multi-steps procedure that its basic concept is to convert a complex matrix into a sample that is suitable for the analysis.

Regardless, the technique used for extraction, different types of components such as, lipids, pigments are mostly present in the extract and must be removed in order to identify and to quantify lower levels of analytes and to reduce deterioration of chromatograms. Several methods of clean-up have been developed in order to remove the co-extracted matrix and minimize their negative effects. The necessity of the clean-up step is to completely remove the bulk of the co-extracted material, as well as those compounds that behave same as to the analytes that could potentially interfere in the final quantification.

Therefore, the main target of this study is to achieve the followings: (1) to extract the 21 OCPs group analytes from the matrix; (2) to remove the un-wanted organic compounds, which may interfere with the compounds of interest; (3) to provide a suitable adsorbent material free from interferences (clean-up), (4) finally to convert the extracted analytes from the matrix into a more suitable concentration level. A comparative study using different types of adsorbents was conducted in order to improve the sample preparations without sacrificing the quality of measurements (sensitivity, accuracy, precision, repeatability, reproducibility and recovery) in fish samples for the analyses of organochlorinated pesticides (OCPs). Moreover, a GC-MS (NCI) method was applied for the analysis of OCPs.

2. Experimental

2.1 Standards, Chemicals and Samples

Standard solution of OCPs containing: α-HCH; β-HCH; J-HCH; δ-HCH; heptachlor; aldrin; heptachlor epoxide; T-chlordane; endosulfan-I; cis-chlordane; T-nonachlor; dieldrin; p-p-DDE; endrin; endosulfan-II; cis-nonachlor; p-p-DDD; endrin aldehyde; p-p-DDT; endrin ketone and methoxychlore were prepared in hexane. A stock solution of mirex at 300 pg μL^{-1} was prepared in hexane and kept refrigerated until it is needed to be used as internal standard. The OCPs were obtained from AccuStandard (M-680P) in New York, US. A standard stock solution of 25 $\mu g\ mL^{-1}$ of each OCP compounds was used to prepare the standard working calibration solutions.

All solvents were pesticide-grade. Hexane (H) and dichloromethane (DCM) were supplied by Merck (Darmstadt, Germany). Nitrogen gas was used to concentrate the extract. The evaporator (Heidolph-Verwenden, Germany) and SX-3 Bio-Beads (200-400 mesh) were purchased from Bio-Rad Laboratories GmbH, in Munich, Germany. Anhydrous sodium sulfate (EMD-Chemical, Darmstadt, Germany) was purchased from Sigma Aldrich Chemie GmbH in Steinhein, Germany. Silica gel (100-200 mesh) was obtained from Aldrich (Steinhein, Germany). Aluminum oxide (70-230) and florisil were obtained from VWR-Baker. Fish samples were collected from the local market in Kuwait to validate the method on real samples.

2.2 Instrumentation

OCPs were quantified on an Agilent 5973 inert mass selective detector, and on an Agilent Technology 6890 network gas chromatography (GC) system coupled with mass spectrometry (MS) with a negative chemical ionization (NCI) ion source. The system was operated in selective ion monitoring (SIM) mode, and 1 µl of sample solution was injected into the GC in the auto-sampler's splitless mode. The capillary column used was a DB-5MS (30 m x 0.25 mm I.D., 0.25 µm film thickness). The initial oven temperature was 90^{o}C, which was held constant for 1.0 min. It was then increased to 160^{o}C at a rate of 15^{o}C/min, with no hold time, followed by increase in temperature to 250^{o}C at a rate of 2^{o}C/min, and finally increase in temperature to 270^{o}C at a rate of 20^{o}C/min, where it was maintained for 5 min. The helium carrier gas flow rate was maintained at 1.1 ml/min. The transfer line temperature of the GC-MS interface and the ion source temperature were held at 265^{o}C and 285^{o}C, respectively. The MS was conducted in the NCI mode with methane as the reagent gas (40 ml/min).

2.3 Sample Preparation and Purification

The edible portion of the fish sample were homogenized. The wet fish sample (5 g) was mixed with anhydrous sodium sulfate to reduce the amount of water and then it is extracted using PLE system. The wet fish samples were grinded to small sizes, which might facilitate the analyte transport to the solvent particle surface. The grinded samples were filled into the cell and the dead volume of the cell was filled with intermatrices, such as hydromatrix. The extraction was performed with 10% (H:DCM). The extract was concentrated by a rotary evaporator to 10 ml. The samples were cleaned with gel permeation chromatography (GPC), GPC column was packed with 12 g SX-3 bio-beads and filled with hexane: dichloromethane (1:1), when it is not used. 100 ml [H: DCM (1:1)] was used to elute the extract , and GPC column was used to remove less than 1.0 % fat. The first 45 ml was discarded and the remaining solvent was collected. The extract was concentrated to appropriate volume (1 ml) using rotary-vapor concentrator and finally the 1 ml goes under gentle stream of clean dry nitrogen. The extract then was subjected to different layer of adsorbents (eight adsorbents) as described in Table1. The column was plugged with glass wool. A slurry method was used to fill the column. The multilayer column (glass column with 15 mm inner diameter and 30 cm long) was packed from top to bottom. Each column was prepared freshly before its use as part of quality control procedures. Elution of samples was carried out at a rate of about 1 ml/min, using different solvent mixtures as shown in Table1.

2.4 Pressurized Liquid Extraction (PLE)

Automated PLE extraction was used (FMS, Waltham, Massachusetts, USA). A stainless- steel extraction cell was supported with Teflon end-caps and filters. The PLE system was controlled by means of a PC using DMS 6000 software that shows the real time, temperature and pressure. The pump, flow rate, solvent time, valves status and cooling were adjusted during the extraction run by the software. Extraction was carried out at a temperature

above the solvent's boiling point and under its pressure to maintain the liquid state of the organic solvent, which keeps the solvent below its critical condition, as well as maintaining its viscosity and its salvation power. Under the selected conditions, the extraction efficiency was enhanced, and the amount of solvent required was minimized. Following is the PLE program that was utilized in the effective extraction of the OCPs:

1- Filling cells with solvent (input: open; output: open); time= 2 min.
2- Pressurizing cell (input: open; output: close); time= 1.0 min.
3- Heating and maintaining pressure (input: close; output: close); temp.= 120^{o}C; time= 30 min.
4- Cooling (pump: off; input: close; output: close); time= 15 min; fan= on
5- Depressurizing (input: close; output: open); time= 0.02 min.
6- Rinsing sample (input: open; output: open); time= 2.5 min.
7- Purging with N_2 (N_2: 35 psi) (input: close; output: open); time= 1.0 min.
8- Opening All valves; Time= 0.02 min.

The PLE system was washed after 2 sets of extraction run using the following program:

1- Filling column (input: open; output: open); time= 2.5 min.
2- Flushing bypass (input: open; output: close); time= 1.0 min.
3- Depressurizing (input: close; output: open); time= 0.02 min.
4- Purging with N_2 (input: close; output: open);time=0.02 min.
5- Ending stage (all are closed).

3. Results and Discussion

3.1 Separation of OCPs using Different Adsorbents Column

J. Hong et al. [13], used different PLE extraction solvents to extract chlorinated pesticides from spiked fish tissue. No significant differences were observed in all solvents used in the extraction, which may be due to their good dissolving capabilities for the chlorinated compounds. However, lipids with large amount, were extracted when using dichloromethane or hexane as extraction solvents.

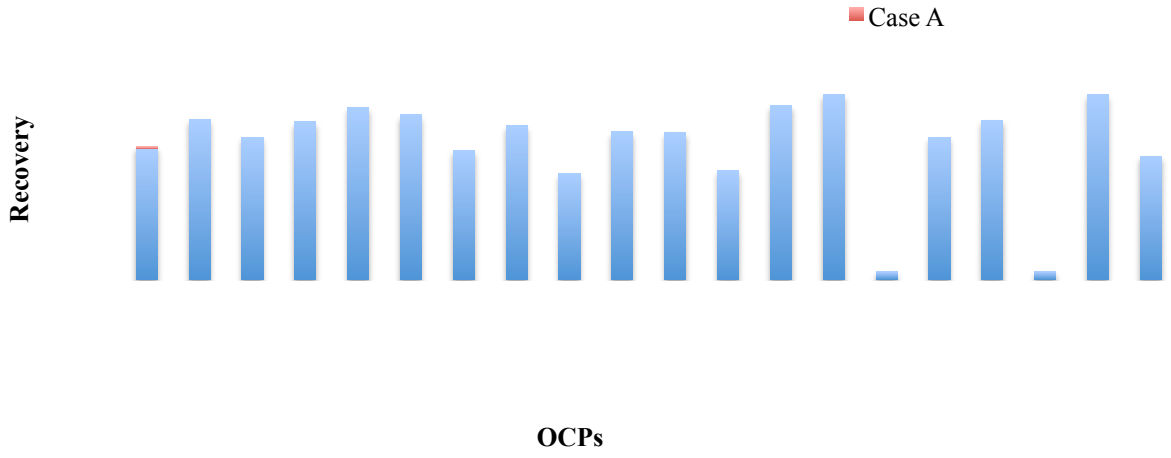
It's possible to isolate OCPs by using column chromatography. Different chromatographic columns with different solvent mixtures were studied, in order to find the best adsorbent for the elution of the 21 OCPs group. The adsorbent materials, the solvent composition which were used in the experimental test for OCPs and their negative effect on the elution of OCPs were presented in Table 1. Using 17 g florisil and 70 mL hexane + 50 mL (H:DCM; 70:30, v/v)+ 40 ml DCM, the recoveries of the spiked sample were varied from 56.12 to 97.89%. However, it showed that the average recoveries range did not exceeded 5% for End-II and endrin aldehyde [Fig. 1, case A]. Using acidic and basic silica column showed negative effect on most of the 21 OCPs group, such as: dieldrin, aldrin, End-I, End-II, endrin and p-p-DDD [Fig. 1, case B]. Previously reported in clean-up methods that some chlorinated pesticide were degraded when using acid or base. End-I and End-II were decomposed and lost their sulfate group when acidic/ basic conditions were applied. Dieldrin was degraded into its chlorinated products and also diol derivatives were degraded when acidic conditions were applied. Some chlorinated pesticides were slowly hydrolyzed in aqueous basic conditions. Therefore, acidic or basic treatment must be avoided for the pesticides analysis [14].

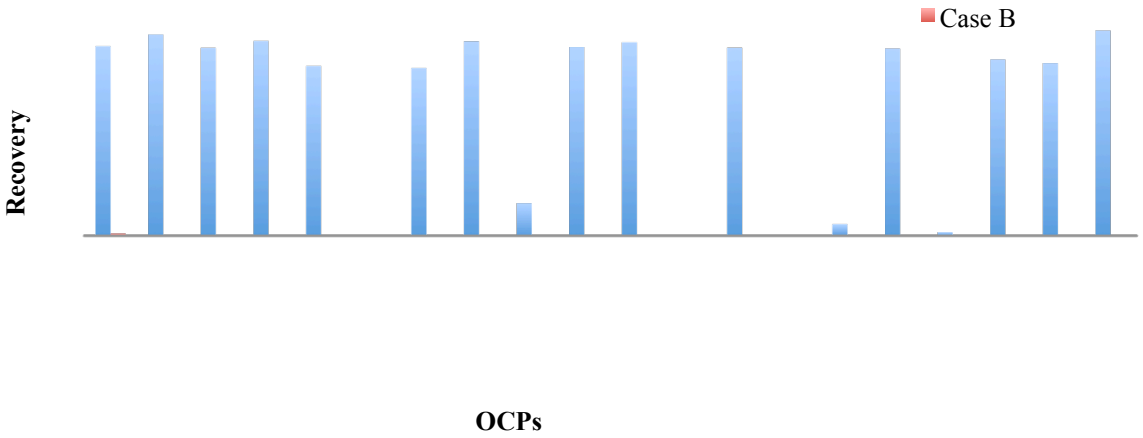
When using florisil (17 g), with different eluant compositions: (1) 70 mL hexane and 50 mL [DCM:H (50%)]; (2) 70 mL hexane+ 50 mL [10% (H:DCM)], a low recovery was obtained for most of OCPs as shown in [Fig. 1, case C and D]. When using florisil (1g) with

13 mL [acetone: hexane (1:9; v/v)], the average recoveries for most of the OCPs were improved and they were varied from 65.8 to 104.9% as shown in [Fig. 1, case E]. However, the same conclusion was driven when using florisil (5g) + 2 g alumina with 35 ml [hexane: ethylacetate (8:2; v/v)]. The recoveries varied from 74.9 to 99.8% as shown in [Fig. 1, case F]. The 3 g silica with 135 ml Hexane + 15 ml DCM, showed good recoveries, except for few OCPs as shown in [Fig. 1, case G].

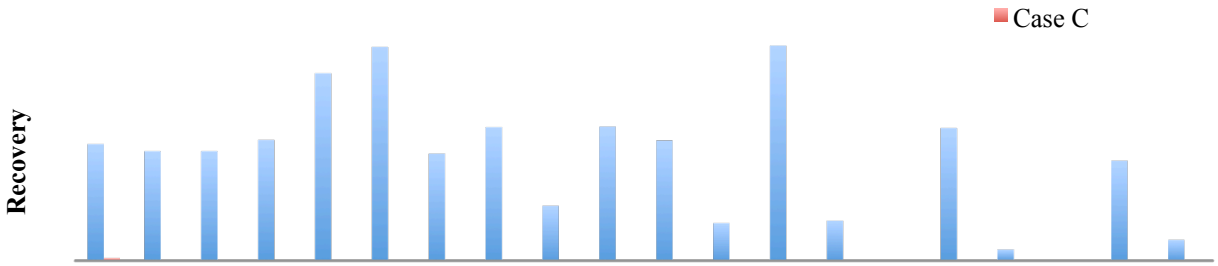
Table 1. Types of adsorbents; eluant solvent mixtures and negative effect on elution of OCPs.

Case No.	Adsorbent	Solvent mixture	Spiked OCPs (ng/g)	Negative effect on elution of OCPs
A.	17g florisil+ 2g Na_2SO_4	70 ml H + 50 ml [H: DCM; [(70:30, v/v)]+ 40 ml DCM	1000	End-II and endrin aldehyde
B.	10g acidic silica (24%)+ 10g basic silica (1N)+ 5g Al_2O_3+ 2g Na_2SO_4	60 ml [H: DCM (1:1; v/v)]	750	Aldrin, End-I, dieldrin, endrin, Endo-II, p-p^{-} DDD,
C.	17g florisil+ 2g Na_2SO_4	70 ml H + 50 ml [H: DCM; (1:1; v/v)]	1000	Low recoveries on most of the OCPs, except on hept., aldrin and p-p^{-}DDE
D.	17g florisil+ 2g Na_2SO_4	70 ml H + 50 ml [(10%H:DCM)]	1000	Low recoveries on most of the OCPs, except on δ-BHC, hept., aldrin and p-p^{-}DDE
E.	1g florisil+ 2g Na_2SO_4	13 ml [(Acetone: H; 1:9, v/v)]	500	High recoveries for most of the OCPs
F.	5g florisil+ 2g Al_2O_3+ 2g Na_2SO_4	35 ml [(H: ethylacetate; 8:2, v/v)]	500	High recoveries for most of the OCPs
G.	3g silica gel+ 2 g Na_2SO_4	135 ml H+ 15 ml DCM	1000	High recoveries for most of the OCPs, except for End-I, p-p^{-} DDD, endrin aldehy, endrin ketone.

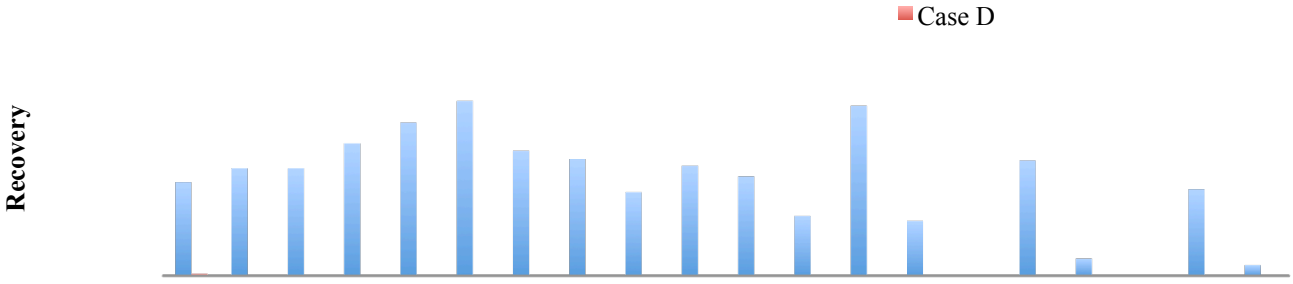




OCPs



OCPs



OCPs

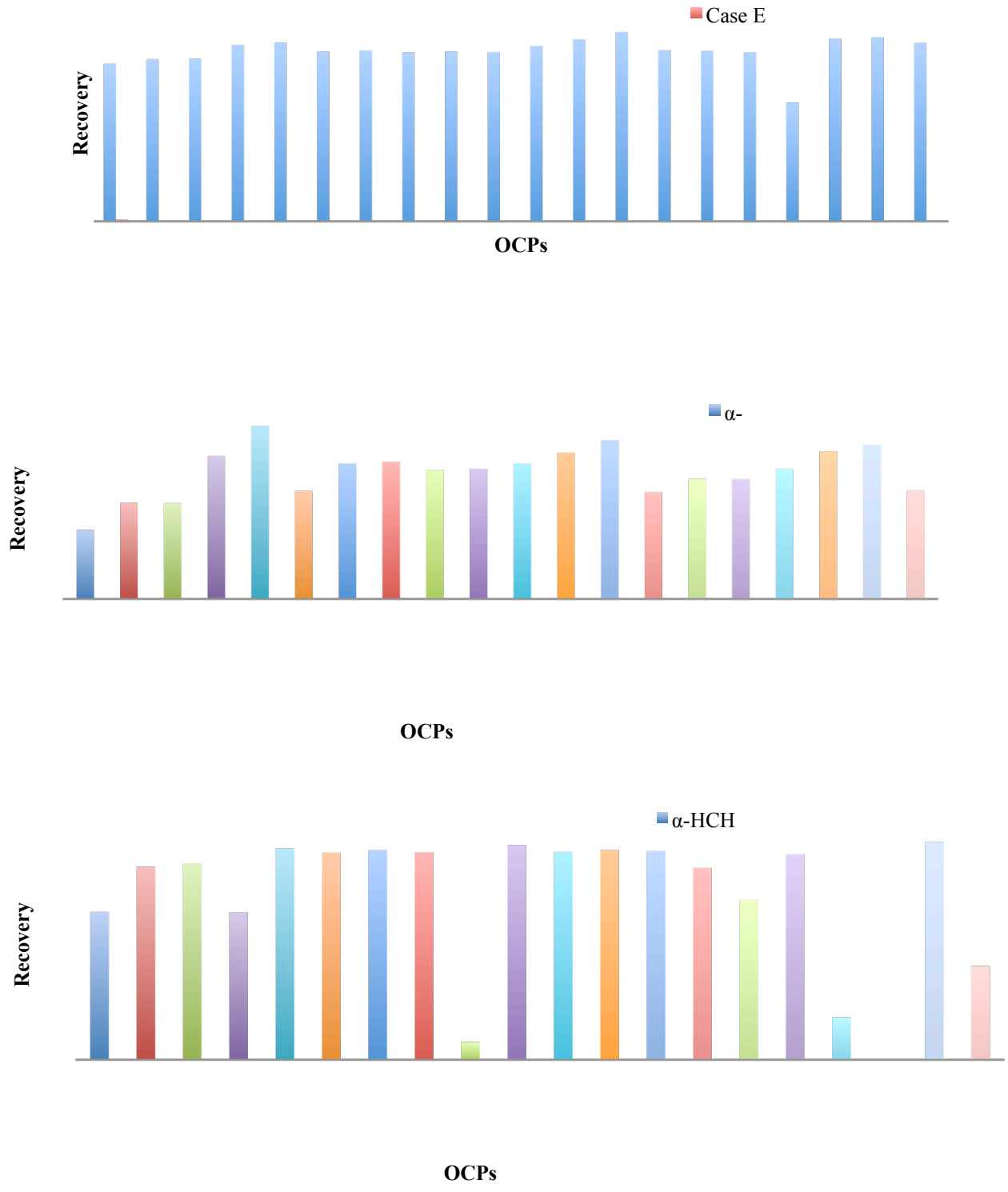


Fig. 1. Recoveries percentages of the selected OCPs were obtained by spiking the standards before the extraction, using different adsorbents and solvent mixtures. The detail of each case is described in Table 1.

Based on the results obtained, the most efficient separation of OCPs was achieved, when using a florisil and alumina with hexane: ethylacetate (8:2; v/v), and a florisil with acetone: hexane (1:9; v/v). Florisil with a composite solvent of 70 mL hexane + 50 mL

[H:DCM (70:30; v/v)] + 40 mL DCM, also showed good recoveries, except for End-II and endrin aldehyde.

Therefore, using one of the previously mentioned adsorbent with solvent composition could be acceptable to obtain better recoveries for the 21 OCPs group. Florisil with eluant [H:ethylacetae (8:2; v/v)] was used throughout the experiment. Official EPA method 3630C, 3610B and 3620B (using silica gel, alumina and florisil clean-up) have been successfully applied for the purification of organic extracts obtained from solid samples [15].

Some pesticides showed poor elution efficiency such as: β, γ and δ-HCH isomers; heptachlore, methoxychlore, dieldrin, endrin, endo-I & II, when florisil/ silica was used with hexane solvent. This could be due to the fact that these compounds are strongly retained on the silica and florisil adsorbent surface. However, in order to improve elution when using hexane, the polarity of elution solvent was increased by using acetone or ethylacetate. Most of the chlorinated pesticides were eluted with H:ethylacetae (8:2; v/v) and acetone: hexane (1:9; v/v), on both adsorbent surface (florisil + alumina or florisil alone). For all chlorinated pesticides, elution recoveries were found to be more than 95%. Therefore, the purification method of the extract was selected to be florisil/ alumina with elution of H:ethylacetae (8:2; v/v) mixture, throughout the experiment.

3.2 Effect of PLE Operating Parameters

Temperature is an important factor in PLE to enhance recoveries. Increasing temperature has positive effects on increasing the extraction efficiency of the analytes, leading to improve mass transfer of the analytes and consequently cause it to increase the recoveries. Moreover, increasing temperature leads to reduce the surface tension and the viscosity of the solvent, which facilitate and improve the sample wetting and matrix penetration [16]. PLE is a solid-liquid extraction step, taking place in close-vessels at elevated temperature and pressure. The pressure is applied to maintain the organic solvents in its liquid state. The Temperature should be above the solvents' atmospheric boiling points. These conditions allow the solvents to be below their critical conditions, but also enhance their salvation power and lower their viscosities, which lead to a higher diffusion rate for the extraction of the analytes. Applying all these conditions, the extraction efficiency is increased and the solvent needed is minimized.

Time is one of the important parameters to be considered and therefore, the time devoted to the optimization and development of the extraction procedure can be reduced. However, PLE is an attractive alternative method compared with conventional soxhlet method, due to the following reasons; its fast, less solvent consumption, and sequentially it allows to extract 6 samples with the use of different sample vessels sizes.

In general, increasing temperature (> 140^{o}C) causes serious disruption in the solute-matrix interactions resulting from Van der Waals forces, hydrogen bonding and or dipole attractions. These interactions could affect the recovery percentage obtained [17].

The extraction recoveries (50-78%) were decreased at temperatures above 140^{o}C for most of the compounds . Temperatures above 140^{o}C could result in the co-extraction of contaminants, that would affect the GC/MS (NCI) analysis. At a higher temperatures (> 140^{o}C), the chromatograms showed more background noise that lead to the identification of the peaks was difficult . This was assumed to be due to the presence of co-extracted material at higher temperatures. The highest extraction efficiencies were obtained at temperatures ranging from 100 to 140^{o}C. Based on these results, 120^{o}C was selected for verification and optimization of the PLE method. Pressure produced no significant effect on the extraction process. A pressure of 1500 psi has been used in several studies to extract analytes from

environmental matrices [18], as higher pressures are generally applied to keep solvents in their liquid states [19-20].

Therefore, a default pressure of 1500 psi was selected for this experiments. A 10% [hexane: DCM] mixture produced the best extraction efficiencies for OCPs (75-99.8%), as compared with solvent mixtures of 20% [hexane: DCM] and 40% [hexane: DCM]. Increasing the percentage of DCM, leads to darken the extracts, indicating co-elution of materials. The same observation has been reported in several studies [21-22.]. Therefore, to minimize the amount of co-extracted material, which may be due to the presence of fat in the marine tissue samples [21-22], 10% [hexane: DCM] was chosen for the extraction steps in this experiment.

3.3. Characterization of the Gel Permeation Chromatography (GPC) Column

The technique is based on molecular size separation and is primarily used to fractionate and remove lipids (>500^{o}A), which elute first from the column. A standard solution of OCPs was transferred into a gel permeation chromatography (GPC) column packed with 12 g of SX-3 Bio-Beads gel (200-400 mesh). The column was washed with 25 mL [hexane: DCM (1:1, v/v)] mixture. Then 100 mL [hexane: DCM (1:1, v/v)]solvent mixture was used to elute OCPs. The first 45 mL was discarded, since all of the lipids were eluted out. The next fraction (45-100 mL) was collected, since all of the OCPs was completely recovered in this elution. The recoveries of all targeted compounds were in the range of 85.2-102.6%. The advantages of GPC over concentrated sulfuric acid or saponification are its nondestructive nature, which allows large amounts of lipids to be handled, and it has greater applicability for unknown contaminants.

3.4 Method Validation

3.4.1 Quality Control

Set of experiments were conducted to obtain acceptable and reliable data by using mirex as internal standard (I.SD) . The extracted fresh and blank samples were spiked with I.SD. (300 pg/µl) before extraction. All analytical data were assessed for compliance with acceptable criteria for method validation; the average of recoveries was required to be within 70-125%. However recoveries obtained for this experiment were generally over 95% for most of the OCPs compounds. Thus, the recoveries were considered to be satisfactory, and no interference or serious co-elution was encountered during the evaluation process.

3.4.2 Matrix Effect

Aliquot (5 g) of wet sample was spiked with a known concentrations of OCPs (250 ng/g). The spiked and non-spiked samples were both extracted at the same time, along with a procedural blank (Na_2SO_4). The matrix effect was evaluated in order to determine any adverse effects on the sample concentration. The obtained chromatograms of the spiked samples were matched with those of the non-spiked samples and the blank. This showed no matrix effect for any OCP compounds.

3.4.3 Method Linearity

Triplicate injection of 1 µL of a set of four standard solutions containing different concentrations of OCPs (250, 500, 750, 1000 pg μL^{-1}) were checked under the optimization conditions of GC/MS (NCI). Internal standard (mirex= 300 pg μL^{-1}) was added to the standard solutions. The calibration graphs were obtained in the range 250-1000 pg μL^{-1} with a correlation coefficients (r^2) exceeding 0.996 for most of the OCPs compounds. The correlation coefficients (r^2) were satisfactory in the concentration range assayed.

The results obtained for the retention times were acceptable over sets of standards and samples that were studied throughout the experiment. Table 2, represents the calibration data, correlation coefficient (r^2), regression equations, calibration range, retention times, limit of detections and limit of quantitation for the group of OCPs were analyzed by NCI mode.

Table 2. Calibration data, correlation coefficient (r^2), regression equations, calibration range, retention times, LOD and LOQ, for the different OCPs analyzed by NCI mode.

PCBs	Correl. Coeff. (r^2)	Regress. Equation	Calib. range (pg/ µl)	Retention time, min ± S.D. (*n*= 3)			LOD (ng/g)	LOQ (ng/g)	m/z
				250 (pg/ µl)	500 (pg/ µl)	1000 (pg/ µl)	------	-------	------
α-BHC	0.999	7.65e-0001	250-1000	10.49 ±0.02	10.49 ±0.01	10.48 ±0.01	0.08	0.26	255/257
β-BHC	1.000	5.02e-001	250-1000	11.66 ±0.06	11.63 ±0.01	11.62 ±0.01	0.20	0.66	255/257
J-BHC	0.999	1.39e+000	250-1000	11.82 ±0.02	11.81 ±0.01	11.81 ±0.01	0.05	0.17	255/257
δ-BHC	0.999	5.45e-001	250-1000	12.99 ±0.02	12.98 ±0.01	12.98 ±0.02	0.11	0.36	255/257
Heptachlore	0.999	9.24e-001	250-1000	15.25 ±0.02	15.24 ±0.01	15.24 ±0.02	0.07	0.23	264/266
Aldrin	0.998	8.66e-001	250-1000	17.15 ±0.03	17.13 ±0.02	17.14 ±0.02	0.08	0.26	235/237
heptachlore epoxide	0.998	7.95e-000	250-1000	19.71 ±0.03	19.69 ±0.02	19.69 ±0.02	0.02	0.66	235/237
T-chlordane	0.998	3.4e+000	250-1000	21.30 ±0.03	21.28 ±0.02	21.29 ±0.02	0.01	0.03	408/410
Endosulfan-I	0.999	6.94e+000	250-1000	22.06 ±0.03	22.04 ±0.02	22.04 ±0.03	0.03	0.10	406/408
Cis-Chlordane	0.997	1.93e+000	250-1000	22.35 ±0.03	22.33 ±0.02	22.34 ±0.02	0.03	0.10	408/410
T-Nonachlore	0.998	2.31e+000	250-1000	22.72 ±0.03	22.69 ±0.01	22.70 ±0.03	0.15	0.50	442/444
Dieldrin	0.997	4.26e-001	250-1000	23.88 ±0.03	23.86 ±0.02	23.87 ±0.03	0.16	0.53	316/318
p-p-DDE	0.997	3.87e-001	250-1000	24.29 ±0.03	24.28 ±0.01	24.29 ±0.02	0.16	0.49	235/237
Endrin	1.000	6.18e-002	250-1000	25.37 ±0.03	25.34 ±0.01	25.36 ±0.03	0.01	0.03	380/382
Endosulfan-II	0.999	7.27e+000	250-1000	26.18 ±0.03	26.15 ±0.02	26.15 ±0.02	0.02	0.66	406/408
Cis-Nonachlore	0.998	3.28e+000	250-1000	27.25 ±0.03	27.24 ±0.02	27.24 ±0.03	0.05	0.17	442/444
p-p-DDD	0.996	1.55e+000	250-1000	27.66 ±0.03	27.64 ±0.02	27.64 ±0.03	0.04	0.13	380/382
Endrin Aldehyde	0.999	1.89e+000	250-1000	29.51 ±0.07	29.48 ±0.08	29.45 ±0.03	0.75	2.48	380/382
p-p-DDT	0.999	7.41e-002	250-1000	30.22 ±0.03	30.21 ±0.02	30.21 ±0.03	0.46	1.52	318/320
Endrin ketone	0.999	1.22e-001	250-1000	32.84 ±0.03	32.83 ±0.02	32.83 ±0.03	0.15	0.50	306/308
Mirex	--	--	300	35.59	---	--	--	--	402/404

3.4.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detections (LODs) were obtained as the compound concentration that cause a peak height equal to three times the baseline noise, whereas the limit of quantitations (LOQs) were obtained as the compound concentration that cause a peak height equal to ten times the baseline noise. Thus LODs and LOQs obtained for OCPs using these conditions are presented in Table 2, and the values were found to be between 0.01-0.75 ng g^{-1} and 0.03-2.48 ng g^{-1} for LODs and LOQs, respectively.

3.4.5 Precision and Accuracy

The precision of the proposed method is expressed in terms of relative standard deviation (RSD). Wet fish sample was spiked at three different levels of OCPs standards (250, 500, 1000 ng/g). Repeatability was performed five times (n= 5) in the same days under the same conditions. The calculated values showed that the relative standard deviations (RSDs) for most of OCPs compounds were ranging between 5% to 28%. The accuracy was evaluated by analyzing one sample on two different days for OCPs. Reproducibility for OCPs were ranging from 70.3% to 100.3 %, with RSDs between 0.16% and 9.8 %.

3.5 Concentrations of OCPs in fish samples

The fish samples were collected from Kuwait's fish market and tested by the presented method in order to evaluate the levels of OCPs compounds. The OCPs levels in fish samples were ranging from 0.03 to 6.93 ng g^{-1} (w/w) that is in agreement with the levels evaluated by the two methods [23-24]. The levels obtained from the two methods were as follows: 0.02-6.37 ng g^{-1} (w/w) and 0.03-5.77 ng g^{-1} (w/w), for method [23] and method [24], respectively. Moreover, comparison of the levels obtained in this study with those obtained by Fang and Yang from China are equal to 5.77 ng g^{-1} (w/w) in fish [25-26], indicating that the levels obtained in this study are comparative to the levels obtained by other methods. Based on this study and other related studies, total ∑OCP concentrations in Kuwait's seafood are deemed to be generally low. Higher pesticide levels are mainly due to the presence of HCH (α, β, γ and δ) and dieldrin.

4. Conclusion

The comprehensive method was established, based on PLE extraction, clean-up of OCPs extracts from fish samples with different adsorbent materials. The detected OCPs group showed good accuracy, precision, and linearity range in the method studied. The advantage derived from using NCI source along with GC/MS detection is being capable of obtaining high selective method, resulting in eliminating the interferences of substances from sample matrices. Furthermore, allowing detection of OCPs in fish tissue at low ng/g levels. This method provides better recoveries when using florisil/alumina with H: ehtylacetate. The relative standard deviation of the method was acceptable and within the required range. PLE offers the advantage of reducing the amount of organic solvent consumed and it can be easily automated.

References

1. Wania F, Mackay D (1996) Tracking the Distribution of Persistent Organic Pollutants. Environ. Sci. Technol. 30: 390A-396A.
2. Bjorklund E, Nilson T, Bowadt S (2000) Pressurised liquid extraction of persistent organic pollutants in environmental analysis. Trends Anal. Chem. 19(7): 434-445.

3. Giergielewicz-Mozajska H, Dabrowski L, Namiesnik J (2001) Accelerated Solvent Extraction (ASE) in the Analysis of Environmental Solid Samples — Some Aspects of Theory and Practice. Crit. Rev. Anal. Chem. 31(3): 149-165.

4. Willett KL, Ulrich EM, Hites RA (1998) Differential toxicity and environmental fates of hexachlorocyclohexane isomers. Environ Sci Technol 32: 2197–2207

5. Haib J, Hofer I, Renand JM (2003) *J. Chromatogr. A*., 1020: 173-187.

6. Barriada-Pereira M, Concha-Grana E, Gonzalez-Castro MJ, Muniategui-Lorenzo S, Lopez-Mahia P, Prada-Rodriguez D, Fernandez-Fernandez E (2003) *J. Chromatogr. A,* 1008: 115-122.

7. M. Barriada-Pereira, M.J. Gonzalez-Castro, S. Muniategui-Lorenzo, P. Lopez-Mahia, D. Prada-Rodriguez, E. Fernandez-Fernandez. 2005. *Chemosphere*, 58: 571-1578.

8. K. Vorkamp, J.H. Christensen, M. Glasius, F.F. Riget. 2004. *Marine Pollut. Bull.*, 48: 111-121.

9. K.S. Kumar, K. Kannan, J.P. Giesy, S. Masunaga. 2002. *Environ. Sci. Technol.*, 36: 2789-2796.

10. M.K. So, X. Zhaug, J.P. Giesy, C.N. Fung, H.W. Fong, J. Zheng, M.J. Kramer, H. Yoo, P.K.S. Lam. 2005. *Mar. Pollut. Bull*., 51: 677-687.

11. S. Jayaraman, R.J. Pruell, R. Mc Kinney. 2001. *Chemosphere* 44: 181-191.

12. C. Nerin, R. Batle, M. Sartaguda, C. Pedrocchi. 2002. *Anal. Chim. Acta*, 464: 303-312.

13. J. Hong, H.-Y. Kim, D.-G. Kim, J. Seo, K.-J. Kim. 2004. *J. Chromatogr. A*, 1038: 27-35.

14. J. Meadows, D. Tillitt, J. Huckins, D. Schroeder. 1993. *Chemosphere* 11: 1993-2006.

15. Internet Website: http://www.epa.gov/

16. B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl. 1996. *Anal. Chem.* 68: 1033.

17. J. Saulo, M. Abalos, E. Abad, J. Perera, M.G. Martrat, M.A. Adrados, J.R. Austrui, K. Martinez, J. Revera. 2005. Mass Spectromerty Lab., Dept of Ecotechnologies, IIQAB-CSIC C/Jordi Girona, 18: 08034, Spain.

18. M. Numata, T. Yarita, Y. Aoyagi, Y. Tsuda, M. Yamazaki, A. Takatsu, K. Ishikawa, K. Chiba, K. Okamaoto. 2007. *Anal. Bioanal. Chem.* 387: 2313-2323.

19. J.J. Ramos, C. Dietz, M.J. Gonzalez, L. Ramos. 2007. *J. Chromatogr. A*. 1152: 254-261..

20. U.N. Fidalgo, G.E. Blanco, M.A. Sanz. 2007. *Anal Chim Acta* 590: 1-16.

21. Z. Zhang, E. Ohiozebau, S.M. Rhind. 2011. *J. Chromatogr. A*, 1218: 1203-1209.

22. P. Suchan, J. Pulkrabova, J. Hajslova, V. Kocourek. 2004. *Anal. Chim. Acta*. 520: 193-200.

23. M.I.H. Helaleh, A. Al-Rashdan. 2012. *J. Environ. Protect.* accepted for publication.

24. Helaleh MIH, Al-Rashdan A, Ibtisam A (2012) Simultaneous analysis of organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) from

marine samples using automated pressurized liquid extraction (PLE) and Power Prep™ clean-up. Talanta, 94: 44-49.

25. Yang N, Matsuda M, Kawano M, Wakimoto T (2006) PCBs and organochlorine pesticides (OCPs) in edible fish and shellfish from China. Chemosphere 63(8): 1342-1352.
26. Jie F, Kai-Xiong W (2007) Multiresidual Analysis of Organochlorine Pesticides, Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons in Marine Shellfishes by Gas Chromatography-Ion Trap Mass Spectrometry. Chin. J. Anal. Chem. 35(11): 1607-1613.