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Simultaneous analysis of organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) from marine samples using automated pressurized liquid extraction (PLE) and Power Prep™ clean-up

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ABSTRACT

An automated pressurized liquid extraction (PLE) method followed by Power Prep™ clean-up was developed for organochlorinated pesticide (OCP) and polychlorinated biphenyl (PCB) analysis in environmental marine samples of fish, squid, bivalves, shells, octopus and shrimp. OCPs and PCBs were simultaneously determined in a single chromatographic run using gas chromatography–mass spectrometry-negative chemical ionization (GC–MS-NCI). About 5 g of each biological marine sample was mixed with anhydrous sodium sulphate and placed in the extraction cell of the PLE system. PLE is controlled by means of a PC using DMS 6000 software. Purification of the extract was accomplished using automated Power Prep™ clean-up with a pre-packed disposable silica column (6 g) supplied by Fluid Management Systems (FMS). All OCPs and PCBs were eluted from the silica column using two types of solvent: 80 mL of hexane and a 50 mL mixture of hexane and dichloromethane (1:1). A wide variety of fish and shellfish were collected from the fish market and analyzed using this method. The total PCB concentrations were 2.53, 0.25, 0.24, 0.24, 0.17 and 1.38 ng g^{-1} (w/w) for fish, squid, bivalves, shells, octopus and shrimp, respectively, and the corresponding total OCP concentrations were 30.47, 2.86, 0.92, 10.72, 5.13 and 18.39 ng g^{-1} (w/w). Lipids were removed using an SX-3 Bio-Beads gel permeation chromatography (GPC) column. Analytical criteria such as recovery, reproducibility and repeatability were evaluated through a range of biological matrices.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent contaminants that have high thermal stability and high dielectric constants. They are used in many industrial fields due to their high stability under different conditions, high thermal conductivity, and low electric conductivity [1]. PCB production was banned in the early 1970s in the United States (US) due to the serious effects PCBs have on health and the environment [2]. The US Environmental Protection Agency (EPA) subjects materials with more than 50 μg g^{-1} of total PCBs to strict regulations [3]. PCBs are characterized by lipophilicity, resistance to degradation, bioaccumulation and biomagnification in the food chain, resulting in concerns about the effect of exposures to these chemicals on human health [4].

Organochlorinated pesticides (OCPs) are ubiquitous environmental contaminants that tend to accumulate in the food chain and affect ecosystem and human health [5,6], and most organohalogenated compounds, such as PCBs and OCPs, are considered to be widespread, persistent environmental pollutants. Like PCBs, OCPs have been commercially produced for use in agricultural and industrial applications [7].

The United Nations Environment Programme (UNEP) has identified 12 persistent organic pollutants (POPs), all of which contain chlorine compounds, as top-priority pollutants due to their negative impact on the environment and human health. POPs have been found in fishponds, where they persist for long periods of time and are transferred into food chains, accumulate in marine organisms, and eventually are consumed by humans [8]. Although, humans are exposed to POPs via multiple sources, contaminated fish constitutes one of the major pathways [9]. Therefore, data on the presence of PCBs and OCPs in fish and other edible marine species are important from the ecological and human health points of view [10].

Marine species are used as bioindicators of POPs [11], and their presence in fish and shellfish can be used to assess the pollutants in marine environments [12]. The contaminants in biological samples need to be determined, since little information is available on the routes of exposure of PCBs and OCPs for humans [13]. Previous studies have determined the presence of PCBs and OCPs in marine environmental samples, applying separate extraction and clean-up methods [14–17]. However, few studies on the multi-residue

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analysis of PCBs and OCPs in marine organisms, including fish, squid, bivalves, shells, octopus and shrimp [18] have been reported.

Pressurized liquid extraction (PLE) is recognized as an official EPA method (3545) for the determination of POPs in solid samples [19] and biota [20–23]. Bjorklund et al. [24] used PLE to extract PCBs from fat-containing organisms such as fish, while Gomez-Ariza et al. [25] used PLE to extract PCBs from biota samples. Many other studies have applied PLE in sample preparation for environmental analysis [26,27]. Effective analytical methods that can quantitatively evaluate the levels of OCPs and PCBs at low concentrations ($ng\,g^{-1}$) are greatly needed.

New analytical extraction techniques that are less tedious and less time-consuming [10,28] have emerged. PLE, which depends on the application of high-pressure and temperature extraction to bring the sample to a temperature greater than the boiling point of the solvent, is one such technique. Liquid–liquid extraction (LLE) is labor-intensive and allows for the extraction of large-volume samples at a low extraction cost. However, the method does not allow for high throughput analysis. Microwave-assisted extraction (MAE) is less automated than SPE. Soxhlet extraction is both time-consuming and labor-intensive, and requires large amounts of solvent. Soxhlet extraction has been used for quantitative determination of POPs [29]. Sample clean-up is the most critical step, since the analytes of interest must be accurately separated from the fatty matrix material. The Power Prep™ system has been used for biotic environmental samples [30–32] and to clean-up samples with low fat contents [33]. Fluid Management Systems' (FMS) Power Prep™ was used to clean biological samples containing low fat contents of polychlorinated dibenzo-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and coplanar PCB compounds [34,35]. To the best of our knowledge, no procedure has been previously reported for simultaneous automated extraction and clean-up of these groups using PLE and Power Prep™. Moreover, simultaneous determination of OCPs and PCBs in a single chromatographic run has not been reported using GC–MS-NCI.

2. Experimental

2.1. Marine samples

Marine samples of fish, squid, bivalves, shells, octopus and shrimp collected from the local fish market in Kuwait were analyzed to evaluate the performance of automated PLE extraction and automated Power Prep™ clean-up. Fresh samples (5 g) of each species were mixed with anhydrous sodium sulphate. Aliquots of 25 $ng\,mL^{-1}$ of surrogate standard mixture ($^{13}C_{12}$-labeled PCB) and 50 $ng\,mL^{-1}$ of mirex internal standard (I.SD.) were added to each extracted sample.

2.2. Chemicals and standards

All solvents were pesticide-grade. Hexane and dichloromethane were supplied by Merck (Darmstadt, Germany). Nitrogen gas was used to concentrate the extract. The evaporator (Heidolph-Verwenden, Germany) and anhydrous sodium sulphate (EMD-Chemical, Darmstadt, Germany) were purchased from Sigma Aldrich Chemie GmbH in Steinheim, Germany. SX-3 Bio-Beads (200–400 mesh), purchased from Bio-Rad Laboratories GmbH, in Munich, Germany. The PCBs (EC-4133) contained the following congener numbers: 2,4,4′,5-tetraCB (74); 2,3′,4′,5-tetraCB (70); 2,2′,3,5′,6-pentaCB (95); 2,2′,4,5,5′-pentaCB (101); 2,2′,4,4′,5-pentaCB (99); 2,2′,3,4,5′-pentaCB (87); 2,3,3′,4′,6-pentaCB (110); 2,2′,3,5,5′,6-hexaCB (151); 2,2′,3,4′,5′,6-hexaCB (149); 2,3′,4,4′,5-pentaCB (118); 2,2′,4,4′,5,5′-hexaCB (153); 2,3,3′,4,4′-pentaCB (105); 2,2′,3,4,4′,5′-hexaCB (138); 2,2′,3,3′,4,6′-hexaCB (132); 2,3,3′,4,4′,6-hexaCB (158); 2,2′,3,4′,5,5′,6-heptaCB (187); 2,2′,3,4,4′,5′,6-heptaCB (183); 2,2′,3,3′,4,4′-hexaCB (128); 2,2′,3,3′,4′,5,6-heptaCB (177); 2,2′,3,3′,4,4′,6-heptaCB (171); 2,3,3′,4,4′,5-hexaCB (156); 2,2′,3,4,4′,5,5′-heptaCB (180); 2,3,3′,4,4′,5′,6-heptaCB (191); 3,3′,4,4′,5,5′-hexaCB (169); 2,2′,3,3′,4,4′,5-heptaCB (170); 2,2′,3,3′,4,5,5′,6,6′-nonaCB (208); 2,2′,3,3′,4,4′,5,6′-octaCB (195); 2,2′,3,3′,4,4′,5,5′-octaCB (194); 2,2′,3,3′,4,4′,5,5′,6-nonaCB (206) and decaCB (209). A $^{13}C_{12}$-labeled PCB mixture (EC-4058) was obtained from Cambridge Isotope Laboratories, and contained the following congener numbers: 2,2′,3,4,4′,5′-hexaCB ($^{13}C_{12}$, 99%) (138); 2,2′,4,4′,5,5′-hexaCB ($^{13}C_{12}$, 99%) (153); 2,2′,3,4,4′,5,5′-pentaCB ($^{13}C_{12}$, 99%) (180); and decaCB ($^{13}C_{12}$, 99%) (209). A standard stock solution of 128.8 $ng\,mL^{-1}$ was used to prepare the standard working calibration solutions for most of the PCB compounds.

The OCPs used for the analysis were 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. The OCPs were obtained from AccuStandard (M-680P) in New York, USA. A standard stock solution of 25 $\mu g\,mL^{-1}$ of each OCP compound was used to prepare the standard working calibration solutions.

2.3. PLE system

Automated PLE extraction was used (FMS, Waltham, MA, 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, valve state and cooling were adjusted during the extraction run by the software. Extraction was carried out under pressure at a temperature above the solvent's boiling point to maintain the liquid state of the organic solvent, which keeps the solvent below critical condition, as well as maintaining viscosity and salvation power. Under the selected conditions, the extraction efficiency was enhanced, and the amount of solvent required was minimized.

2.4. Automated Power Prep™ system

Automated clean-up was performed using the Power Prep™ system (FMS, Waltham, MA, USA). The system is controlled by software through a control module. The valves, pump, pressure modules, and flow were controlled automatically by the software. The internal pressure which did not exceed 35 psi and was monitored by pressure gauges. The system includes 3–6 way electrostatic valves driven by the PC's software. Valve modules (V1–V6) select the solvent and columns. PCB and OCP clean-up was conducted using a disposable silica column (6 g) packed with PTFE tubes sealed in Mylar packaging supplied by FMS. Two different solvent compositions were used to elute the analytes from the pre-packed silica column, i.e., (A) 80 mL hexane and (B) 50 mL of a 1:1 (v/v) hexane:DCM mixture.

2.5. GC–MS-NCI conditions

PCBs and OCPs were quantified on an Agilent 5973 inert mass selective detector, 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 was a DB-5MS (30 m × 0.25 mm I.D., 0.25 μm film thickness). The initial oven temperature was 50 °C, which was held constant for 5 min. It was then

increased to 160 °C at a rate of 5 °C/min, with no hold time and then to 260 °C at a rate of 3 °C/min, where it was maintained for 10 min. The helium carrier gas flow rate was maintained at 1.2 mL/min. The transfer line temperature of the GC–MS interface and the ion source temperature were held at 260 °C and 250 °C, respectively. The MS was conducted in the NCI mode with methane as the reagent gas.

3. Results and discussion

3.1. Effect of PLE operating parameters

Temperature plays a significant role in improving extraction efficiency by enhancing the solubility of the analyte in the solvent, thus improving mass transfer from the matrix to the solvent [36]. In general, increasing temperature 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 [37,38].

The extraction efficiency was tested at different temperatures (80, 100, 120, 140 and 160 °C). The extraction efficiency increased dramatically with increases in temperature from 80 °C (65–82%) to between 100 and 140 °C (90–102%). The extraction recoveries decreased at temperatures above 140 °C (50–78%) for most compounds. Temperatures above 140 °C could result in the co-extraction of contaminants, which would affect the GC/MS (NCI) analysis. However, at higher temperatures (>140 °C), the identification of the peaks was difficult and the chromatograms showed more background noise. This was assumed to be due to the presence of co-extracted material at the higher temperatures. The highest extraction efficiencies were obtained at temperatures ranging from 100 to 140 °C. Based on these results, 120 °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 [39,40], as higher pressures are generally applied to keep solvents in a liquid state [41,42]. Therefore, a default pressure of 1500 psi was selected for our experiments. A 10% DCM:hexane mixture produced the best extraction efficiencies for OCPs (79–104%) and PCBs, compared with solvent mixtures of 20% DCM:hexane (45–130%) and 40% DCM:hexane (46–88%). The extracts were very dark with DCM, indicating co-elution of materials. The same observation has been reported in several studies [43,44,41]. To minimize the amount of co-extracted material, which may be due to fat in the marine tissue samples [43,44,41], 10% DCM:hexane was chosen for the optimization tests.

3.2. GPC column

A combined standard solution of OCPs and PCBs (41.7 ng mL^{-1} OCPs and 9.08 ng mL^{-1} PCBs) was transferred into a gel permeation chromatography (GPC) column packed with 12 g of SX-3 Bio-Beads (200–400 mesh). The column was washed with 25 mL of the hexane:DCM (1:1, v/v) mixture. Then 100 mL of the solvent mixture was used to elute the OCPs and PCBs. The first 45 mL was discharged, since all of the lipids were eluted in it. The next fraction (45–100 mL) was collected, since all of the OCPs and PCBs were completely recovered in this elution. The recoveries of all target compounds were in the range of 85.2–102.6%. The advantages of GPC over concentrated sulphuric acid or saponification are its non-destructive nature, which allows large amounts of lipids to be handled, and its greater applicability for unknown contaminants.

3.3. Quality control

A set of experiments to obtain acceptable, reliable data, including surrogate ($^{13}C_{12}$-PCBs) and mirex (I.SD) were performed. $^{13}C_{12}$-PCBs were added to tested samples, and recoveries of the surrogate were determined for quality control purposes. The recoveries of the surrogate $^{13}C_{12}$-PCBs ranged from 90.8 to 97.6% with relative standard deviations (RSDs) of less than 25% (RSD = 4–22%). The values obtained met the criteria of acceptance for USEPA Methods 1668A and 1613B [45,46]. The extracted fresh and blank samples were spiked with I.SD. (50 ng mL^{-1}) before extraction. All analytical data were assessed for compliance with acceptable criteria. The average recoveries were required to be within 70–125%. Recoveries were generally over 80% for three replicates. Thus, the recoveries were considered to be satisfactory, and no interference or serious co-elution was encountered during the evaluation process. The background contaminations for some PCBs and OCPs in blanks were as follows: heptachlore = 0.8 ng g^{-1}, T-chlor = 0.04 ng g^{-1}, End-I = 0.05 ng g^{-1}, PCB-183 = 0.02 ng g^{-1}, PCB-128 = 0.04 ng g^{-1}, PCB-177 = 0.03 ng g^{-1}, PCB-171 = 0.03 ng g^{-1} and PCB-156 = 0.02 ng g^{-1}.

3.4. Matrix effect

A 5 g aliquot of fresh sample was spiked with known concentrations of OCPs (41.7 ng g^{-1}) and PCBs (9.08 ng g^{-1}). 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 sample and the blank, and showed no matrix effect for any OCP or PCB.

3.5. Linearity of the method

The linearity of the method for OCP and PCB spiking of standard solutions was evaluated over a range of concentrations (3.63–18.17 ng mL^{-1} for PCBs and 41.67–166.67 ng mL^{-1} for OCPs). The response was linear, with a correlation coefficient (r^2) >0.990 for most of the compounds (Table 1). The standard deviations (SDs) were calculated for the OCP and PCB retention times, and the results indicate no clear deviation between the standard chromatograms, the samples and the reference materials. Chromatograms of the real shrimp sample analysis for OCPs and PCBs are shown in Fig. 1, while chromatograms of shell samples spiked with standard OCPs and PCBs are shown in Fig. 2.

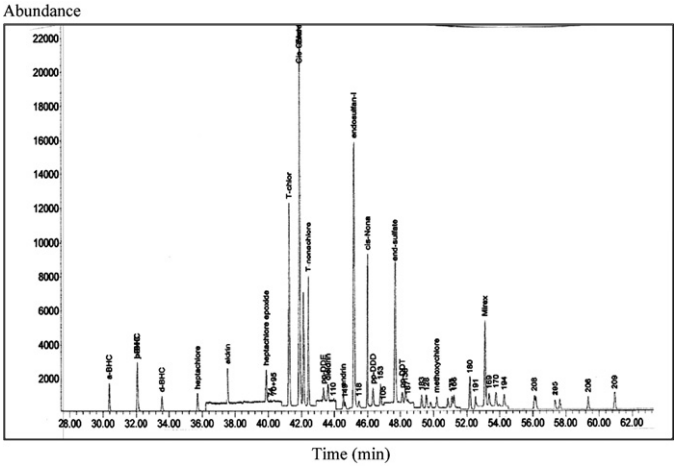

Fig. 1. GC–MS-NCI chromatogram for the simultaneous analysis of OCPs and PCBs extracted from shrimp sample.

Table 1
Calibration data, linear range, correlation coefficient and LOD for the simultaneous analysis of OCPs and PCBs.

OCPs + PCBs	Calibration range (ng mL^{-1})	Correlation coefficient (r^2)	Limit of detection (LOD) (ng g^{-1})
α-BHC	41.67–166.67	0.995	0.31
B-BHC	41.67–166.67	0.989	0.18
γ-BHC	41.67–166.67	0.989	0.85
δ-BHC	41.67–166.67	0.990	0.85
Heptachlore	41.67–166.67	1.000	1.77
Aldrin	41.67–166.67	0.996	3.66
Hept.epoxide	41.67–166.67	0.994	4.09
PCB-74	3.63–18.17	0.956	4.86
PCB-70 + 95	3.63–18.17	0.971	4.86
T-chlor	41.67–166.67	0.989	0.08
End-I	41.67–166.67	0.990	0.04
cis-Chlord	41.67–166.67	0.995	0.58
T-nonachlore	41.67–166.67	0.990	0.06
pp-DDE	41.67–166.67	0.992	1.85
Dieldrin	41.67–166.67	0.990	0.57
PCB-110	3.63–18.17	0.989	1.84
Endrin	41.67–166.67	1.000	0.80
PCB-149	3.63–18.17	0.955	0.74
Endo-II	41.67–166.67	0.993	0.29
PCB-118	3.63–18.17	0.973	0.74
cis-Nona	41.67–166.67	0.989	0.03
pp-DDD	41.67–166.67	0.987	2.58
PCB-153	3.63–18.17	0.958	1.51
PCB-105	3.63–18.17	0.985	6.29
Endo-sulfate	41.67–166.67	0.995	0.22
pp-DDT	41.67–166.67	0.995	3.33
PCB-138	3.63–18.17	0.966	1.53
PCB-187	3.63–18.17	0.972	2.49
PCB-183	3.63–18.17	0.952	0.51
PCB-128	3.63–18.17	0.992	0.15
Methoxychlore	41.67–166.67	0.995	7.48
PCB-177	3.63–18.17	0.960	0.46
PCB-171	3.63–18.17	0.965	0.31
PCB-156	3.63–18.17	0.992	0.34
PCB-180	3.63–18.17	0.972	0.25
PCB-191	3.63–18.17	0.996	0.31
PCB-169	3.63–18.17	0.996	0.83
PCB-170	3.63–18.17	0.971	0.29
PCB-194	3.63–18.17	0.961	0.18
PCB-208	3.63–18.17	0.963	0.33
PCB-195	3.63–18.17	0.969	0.33
PCB-205	3.63–18.17	0.963	0.33
PCB-206	3.63–18.17	0.952	0.329
PCB-209	3.63–18.17	0.995	0.06

3.6. Repeatability and reproducibility

Fresh marine samples (w/w) were spiked with different concentrations of OCPs and PCBs (3.63 and 9.08 ng g^{-1} PCBs, and 83.3, 41.67 and 166.67 ng g^{-1} OCPs). Repeatability was evaluated using six replicates each for the OCPs and for PCBs, all analyzed on the same day, under the same conditions. The reproducibility was evaluated by analyzing one sample on three and four different days for OCPs and PCBs, respectively. Reproducibility recovery ranges ± RSDs were as follows: 88.3–111 ± 0.23–27.1% and 67.3–102 ± 0.04–8.15% for PCBs and OCPs, respectively. The repeatability recovery ranges ± RSD were as follows: 69.2–119 ± 0.48–23.2%, and 79.2–108 ± 0.96–24.6% for PCBs and OCPs, respectively (Tables 2 and 3).

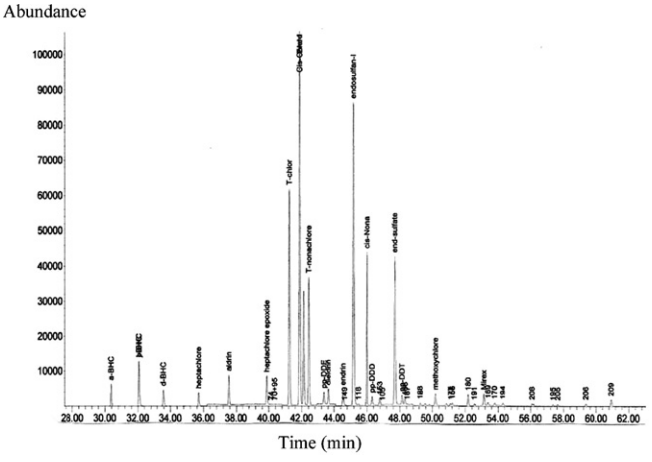

Fig. 2. GC–MS-NCI chromatogram for the simultaneous analysis of OCPs and PCBs extracted from spiked shell sample. OCP and PCB spiking concentrations 41.67 ng g^{-1} and 3.63 ng g^{-1}, respectively.

3.7. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined at the residual levels (ng g^{-1}, w/w). The signal-to-noise ratios were LOD = 3 and LOQ = 10. The method's detection limits ranged from 0.15 to 4.86 ng g^{-1} (w/w) for PCBs and from 0.04 to 7.48 ng g^{-1} (w/w) for OCPs (Table 1).

3.8. Concentrations of PCBs in fish and shellfish samples

The levels of PCBs in marine samples collected from Kuwait's fish market were as follows: 0.17, 0.24, 0.25, 0.24 and 1.38 ng g^{-1} (w/w) for octopus, bivalves, squid, shells and shrimp, respectively, and for fish, the PCB levels ranged from 0.44 to 2.53 ng g^{-1} (w/w).

Table 2
PCB recoveries from different marine samples using the recommended procedure.

PCB	Repeatability	Repeatability	Reproducibility
	Recovery (%) (RSD%)[a]	Recovery (%) (RSD%)[b]	Recovery (%) (RSD%)[c]
PCB-74	101 ± 12	845 ± 25	95 ± 0.5
PCB-70 + 95	119 ± 23	88 ± 23	95 ± 9
PCB-110	82 ± 17	89 ± 21	101 ± 7
PCB-149	77 ± 14	96 ± 18	98 ± 23
PCB-118	79 ± 15	97 ± 15	94 ± 7
PCB-153	86 ± 16	98 ± 22	111 ± 9
PCB-105	79 ± 23	88 ± 23	109 ± 13
PCB-138	76 ± 7	76 ± 7	101 ± 3
PCB-187	77 ± 10	89 ± 11	90 ± 10
PCB-183	76 ± 15	76 ± 8	102 ± 12
PCB-128	87 ± 5	84 ± 3	113 ± 13
PCB-177	77 ± 11	82 ± 3	95 ± 19
PCB-171	76 ± 20	75 ± 16	101 ± 23
PCB-156	69 ± 11	77 ± 5	101 ± 12
PCB-180	85 ± 12	79 ± 17	104 ± 4
PCB-191	81 ± 17	77 ± 21	99 ± 25
PCB-169	92 ± 13	80 ± 7	99 ± 0.2
PCB-170	83 ± 5	74 ± 6	99 ± 3
PCB-194	79 ± 15	83 ± 6	95 ± 16
PCB-208	72 ± 13	80 ± 0.5	94 ± 23
PCB-195	77 ± 14	81 ± 6	88 ± 0.9
PCB-205	77 ± 10	84 ± 0.7	95 ± 12
PCB-206	76 ± 13	80 ± 8	96 ± 6
PCB-209	88 ± 19	85 ± 22	111 ± 12

[a] Shrimp, shells, octopus, sponge (spiking concentration 3.63 ng mL^{-1}). $n = 4$.
[b] Octopus, sponge (spiking concentration 3.63 ng mL^{-1}). $n = 2$.
[c] Fish hamour (spiking concentration 9.08 ng mL^{-1}). $n = 2$.

The results obtained in the current study were lower than those detected in fish, i.e., 3.77 ng g^{-1} (w/w), and shellfish, i.e., 2.92, 5.20 and 1.26 ng g^{-1} (w/w) for octopus, squid and clam, respectively, collected from Dalian, Tianjin and Shanghai [47], and were lower than those in shellfish species = 0.18–1.34 ng g^{-1} (w/w) from Zhejiang, China [48]. The highest PCB levels, which were found in shrimp (1.38 ng g^{-1}, w/w), were higher than levels reported in shrimp in various cities of Catalonia (i.e., 0.46 pg g^{-1} (w/w) [49], and in shrimp and mussels (i.e., 0.20 ng g^{-1}) [50]. Moreover, the results obtained in this study suggest that the levels of PCBs in Kuwait's fish are not as high as those in European fish, and the values do not exceed the levels set by the French Food Standards of 2 mg kg^{-1} [50].

3.9. Concentrations of OCPs in fish and shellfish samples

The levels of OCPs in marine samples collected from Kuwait's fish market were as follows: 10.72, 0.92, 18.39, 5.13 and 2.86 ng g^{-1} (w/w) for shells, bivalves, shrimp, octopus and squid, respectively. Levels in fish ranged from 5.53 to 30.4 ng g^{-1} (w/w). Comparison of the levels obtained in this study with those obtained from Dalian, Tianjin and Shanghai, i.e., 12.46 ng g^{-1} (w/w) in squid, 3.98 ng g^{-1} (w/w) in octopus, 39.68 ng g^{-1} (w/w) in clams, and 5.77 ng g^{-1} (w/w) in fish [48], indicates that the levels obtained in Kuwait were lower in some samples and higher in others. The predominant pesticides detected in species obtained from Zhejiang, China,

Table 3
OCP recoveries from different marine samples.

OCP	Repeatability	Repeatability	Reproducibility
	Recovery (%) (RSD%)[a]	Recovery (%) (RSD%)[b]	Recovery (%) (RSD%)[c]
α-BHC	98 ± 19	89 ± 17	96 ± 8
β-BHC	98 ± 18	104 ± 8	97 ± 0.3
γ-BHC	97 ± 18	99 ± 9	98 ± 2
δ-BHC	97 ± 20	96 ± 7	99 ± 0.3
Heptachlore	95 ± 19	97 ± 9	99 ± 6
Aldrin	103 ± 21	97 ± 7	98 ± 2
Heptachlore epoxide	108 ± 25	98 ± 8	101 ± 0.04
T-chlordane	103 ± 19	97 ± 7	98 ± 3
Endosulfan-I	106 ± 21	89 ± 19	100 ± 2
cis-Chlordane	83 ± 19	94 ± 12	88 ± 2
T-nonachlore	104 ± 23	95 ± 16	101 ± 0.7
pp-DDE	103 ± 19	107 ± 9	93 ± 2
Dieldrin	104 ± 21	93 ± 2	99 ± 2
Endrin	101 ± 20	99 ± 0.9	97 ± 2
Endosulfan-II	99 ± 21	101 ± 2	99 ± 5
cis-Nonachlore	104 ± 15	92 ± 17	93 ± 4
pp-DDD	99 ± 3	79 ± 8	67 ± 4
Endosulfan-sulfate	98 ± 21	101 ± 3	99 ± 6
pp-DDT	96 ± 20	93 ± 6	101 ± 3
Methoxychlore	94 ± 21	91 ± 7	99 ± 4

[a] Shrimp, shells, octopus, sponge (spiking concentration 83.3 ng mL^{-1}). $n = 5$.
[b] Octopus, sponge (spiking concentration 41.67 ng mL^{-1}). $n = 2$.
[c] Fish hamour (spiking concentration 166.67 ng mL^{-1}). $n = 2$.

ranged from 1.36 to 22.5 ng g^{-1} (w/w) p′-DDE, 1.02 to 26.5 ng g^{-1} (w/w) p-p′-DDD and 68 to 50.7 ng g^{-1} (w/w) p-p′-DDT [47]. Based on this study and other related studies, total $\sum$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. Conclusions

A comprehensive simultaneous method was established for the determination of OCPs and PCBs based on automated extraction and automated clean-up in fish and shellfish samples, which were collected from Kuwait's local fish market. The separation of OCPs and PCBs showed good accuracy, precision and linearity in the ranges studied. The advantage derived from the use of NCI along with GC–MS detection is the selectivity of the method, which results in efficient elimination of interfering substances from the sample matrix, allowing the detection of low levels of OCPs and PCBs in fish and shellfish tissues. The method was verified using a mixture of two groups of standards, and applied successfully for the analysis of fish and shellfish samples. It can be used to analyze both PCBs and OCPs simultaneously in a single chromatogram run, Thus reducing the cost and time required for the pretreatment of fish and shellfish samples. The effectiveness of the combined PLE and Power Prep™ clean-up system makes this method a powerful tool for the analysis of biota samples.

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