Fast Solid Phase Extraction of Polychlorobiphenyls and Chlorinated Pesticide Residues from Mussels Using Sep-Pak Cartridges

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Key Words

Gas chromatography
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Summary

A rapid method for the determination of chlorinated pesticides and polychlorinated biphenyls in mussels (Mytilus sp.) is reported. The mussel sample is homogenized and extracted with acetonitrile. The organic solution is concentrated and successively diluted with distilled water solution (12 g L^{-1} NaCl). The organic compounds from water solution are adsorbed onto a NH_2 Sep-Pak cartridge.

The clean-up step, in which the polychlorobiphenyls and chiorinated pesticides are separated in different eluates, is achieved by passing 25 mL of a 40 % methanol aqueous solution through the NH_2 Sep-Pak and the C_{18} Sep-Pak cartridges connected in series.

The polychloroblphenyls are desorbed from the NH_2 Sep-Pak cartridge whilst the chlorinated peslicides are recovered from the C_{18} Sep-Pak cartridge.

In the separation of polychlorobiphenyls from the chlorinated pesticides tested in this work, only aldrin, heptachlor and 4,4'-DDD are partially adsorbed with the polychlorobiphenyls onto the NH_2 Sep-Pak cartridge.

The average recovery is ≥ 95.0 % with a relative standard deviation ≤ 5.0 %. The limits of detection for different pesticides and polychlorobiphenyl congeners are 0.01 and 0.008 $\mu g\ Kg^{-1}$. The final determination is carried out by capillary gas chromatography with ECD.

Introduction

Polychlorinated biphenyls (PCBs) have been available as industrial chemicals since 1930, and their widespread application in the following 60 years has resulted in a universal distribution of these persistent and ubiquitous environmental contaminants [1]. Moreover, their carcinogenic and teratogenic effects [2, 3], as well as their high chemical stabillty and lipophilia with consequent ability to bioaccumulate have been demonstrated [4, 5]. Since mussels (Mytilus sp.), like other living aquatic organisms, can concentrate contaminants from their environment because of their ability to bioccumulate most pollutants they are thus very useful for monitoring contamination levels in coastal zones [6–14]. For this reason several methods have been set up to determine pollutants in fish and shellfish, with a special emphasis on organochlorine pesticides and PCBs because of their stability and of their persistence in the environment [15–22]. The critical step is the clean-up which is used to separate organochlorine pesticides and PCBs from co-extractives and to separate chlorinated pesticides from PCBs that might interfere in the analysis [23–24].

This paper describes a rapid method for the analysis of organochlorine pesticide residues and PCBs in mussels using an efficient clean-up step in which the PCBs and chlorinated pesticides are taken into different Sep-Pak cartridges.

Experimental

Materials

Acetone, n-hexane, acetonitrile, ethylacetate and methanol from Carlo Erba (Milano, Italy) were all of pesticide grade, sodium chloride and anhydrous sodium sulphate from Carlo Erba were of analytical grade. The standard polychlorophenols (2,4-dichlorophenol; 2,4,6-trichlorophenol; 2,3,5-trichlorophenol; pentachlorophenol), polychloroanilines (2,4-dichloroaniline; 2,4,6-trichloroaniline; 2,3,5-trichloroaniline), polychloronitrobenzenes (2-chloronitrobenzene; 2,4-dichloronitrobenzene; 2,3,4-trichloronitrobenzene) and poly-

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Table I. Paramiters of the calibration plots costructed from 5 points and estimated detection limits.

Sustances	Concentration range in standards used for calibration plots ($\mu g\,L^{-1}$)	Correlation coefficients of calibration plots	Detection limit ($\mu g\,kg^{-1}$)
Heptachlor	0.1-16	0.9998	0.03
Heptachlor epoxide	0.1-18	0.9998	0.02
Aldrin	0.1-17	0.9996	0.02
Endrin	0.1-20	0.9998	0.O2
4,4'-DDD	0.1-18	0.9997	0.03
α-Endosulfan	0.1-17	0.9996	0.05
Dieldrin	0.1-20	0,9998	0.02
PCB 1	0.21-15	0.9996	0.06
PCB 15	0.1-15	0.9997	0.05
PCB 36	0.1-15	0.9998	0.05
PCB 44	0.1-15	0.9998	0.03
PCB 126	0.1-18	0.0998	0.01
PCB 138	0.1-16	0.9998	0.01
PCB 180	0.1-16	0.9998	0.008
PCB 1260*	8.0-30	0.9966	0.18
PCB 1260**	8.0-30	0.9956	0.08

parameters for total area of Aroclor 1260
**parameters for the highest peak in the chromatogram (R_T = 29.3 min.)

chlorobenzenes (1,3-dichlorobenzene; 1,3,4-trichlorobenzene; 1,2,4,5-tetrachlorobenzene) were from Carlo Erba. The pesticide standards were from Riedel-de Haën (Germany) and Aroclor 1232 and 1260 plus eight individual PCB congeners from LabService Analytica S. r. l. (Anzola Emilia, Bologne, Italy).

Standard solutions were prepared by dissolving the chlorinated pesticides, Aroclor and PCB congeners in acetone (50 mg L^{-1}). These solutions then were diluted with acetone to prepare the final spikings (10 mg L^{-1}, 1 mg L^{-1} and 0.1 mg L^{-1}). The examined pesticides and PCB congeners were: lindane, aldrin, heptachlor, heptachlorepoxide, dieldrin, endrin, 4,4-DDD, α-endosulfan and 2-chlorobiphenyl (no. 1), 4,4'-dichlorobiphenyl (no. 15), 2,4',5-trichlorobiphenyl (no. 31), 2,2',3,3'-tetrachorobiphenyl (no. 44), 3,3',4,4',5-pentachlorobiphenyl (no. 126), 2,2',3,4,4',5-hexachlorobiphenyl (no. 138) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (no. 180) and 2,2',3,3',4,4',5,6'-octachlorobiphenyl (no. 195).

NH_2 Sep-Pak and C_{18} Sep-Pak cartridges, containing 100 mg adsorbent, were from Merck (Germany).

Apparatus

A gas chromatograph model 86.10 HT (DANI, Monza, Italy) equipped with Ni^{63} electron capture detector (ECD), programmed temperature vaporizer injector (PTV) and data processor (HP 3396A) was used.

A fused-silica capillary column with chemically bonded phase (SE–54) was prepared in this laboratory [25–26] with the following characteristics: 22 m x 200 μm i. d., *N* (theoretical plate number) = 118,000 for *n*-dodecane at 90 °C, k (capacity factor) = 5.2, d_f (thickness of liquid phase) = 0.2 μm, U_{opt} (optimum linear velocity of carrier gas) = 31.7 cm s^{-1} (hydrogen carrier) and UTE % (utilization of theoretical efficiency) = 89 %.

A Rotavapor (Buchi 461) and homogeniser Danamix TR 330 were used.

GC Analysis and Quantification

Carrier gas: hydrogen, u = 35 cm s^{-1}.

Oven temperature program: isothermal at 70 °C and then programmed at 5 °C min^{-1} to 270 °C.

PTV injection: total sample injection mode. After 2 s from injection the vaporizer was heated at 800 °C min^{-1} from 60 °C to 280 °C and cooled after 120 s; the splitter valve was closed for 60 s.

EC detector: temperature 280 °C; make up gas nitrogen at 55 mL min^{-1}.

The concentrations of the chlorinated pesticides, PCB congeners and PCBs were obtained from the calibration graph, which recorded the ratio $Area_{(for\ each\ pesticide)}$ / $Area_{(lindane,\ i.\ s.)}$, $Area_{(for\ each\ PCB\ congener)}$ / $Area_{(PCB\ 195,\ i.\ s.)}$ or $Area_{(total\ area\ of\ Aroclor)}$ / $Area_{(PCB\ 195,\ i.\ s.)}$ versus the concentration of each of the pesticides, PCB congeners or Aroclor in $\mu g\,L^{-1}$.

Quantitation of the chromatograms was based on peak areas using external standard calibration curves. Constrution of calibration graph: solutions of the standards were prepared at five concentration levels and chromatographed, plotting peak area versus concentration. Concentration ranges for each pesticide, for the PCB congeners and for Aroclor 1260 are shown in Table I.

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Procedures

Extraction.

Mussels free of valves were homogenized in a mixer.

5–10 g of the homogenised material were transferred to a beaker with a magnetic stirrer and extracted with three 10 or 20-mL portions of acetonitrile. The extracts were combined and filtered through a Gelman glass-fibre pad (pore size 5–10 μm) to remove any suspended particles. The Gelman glass-fibre pad was washed with two 5 mL-portions of acetonitrile, which were then added to the organic solution and evaporated to 1–1.5 mL in the Rotavapor with the water bath at 40 °C.

Clean-up.

The concentrated extract was diluted with distilled water solution (12 g L^{-1} NaCl) to 25 mL, shaken for 10 minutes and allowed to rest for 30 minutes. They were then put in a glass reservoir connected to the NH_2 Sep-Pak cartridge. Before use, the cartridge was washed with 3 mL pentane, 3 mL ethylacetate followed by 3 mL methanol and 250 mL distilled water. This amount of water is required to ensure hydration of the cartridge and to obtain secure sampling of pesticides, (as has been shown reference [27]), and PCBs.

The flow rate (3–5 mL min^{-1}) was regulated by a water pump. Experiments were carried out using commercial samples of mussels spiked with the standard solution of chlorinated pesticides, PCB congeners or Aroclor. The organics from water sample was adsorbed onto a cartridge by passing 25 mL sample at about 3–5 mL min^{-1}. The sample vessel was rinsed with 4–5 mL of distilled water which was passed through the cartridge and the residual water removed by applying a vacuum for 5 min.

The PCBs and chlorinated pesticides were desorbed by introducing into the cartridge 500 μL ethylacetate, which was collected in a glass vial with a conical bottom. Lindane and PCB 195 (i. s.) were added and the ethylacetate solution concentrated under a nitrogen flow down to 20–50 μL. The blank was analysed using the same experimental conditions. Finally, 1 μL was injected into the GC.

Separation of PCB's from Chlorinated Pesticides

The PCBs and chlorinated pesticides adsorbed on the NH_2 Sep-Pak cartridge were separated by passing 25 mL methanol aqueous solution (40 %) through the trap. The PCBs were recovered from the NH_2 Sep-Pak cartridge using 500 μL of ethylacetate while the clorinated pesticides were desorbed from a C_{18} Sep-Pak cartridge, connected in series with the NH_2 trap, with 500 μL of ethylacetate.

Results and Discussion

In Table I calibration data and detection limits obtained for each of the chlorinated pesticides, PCB congeners and Aroclor 1260 are reported. The linearity of the response to the pesticides, PCB congeners and Aroclor were good for the concentation ranges used. Detection limits for chlorinated pesticides, PCB congeners and Aroclor used in this study are adequate for estimating these compounds in mussel sample. These values were determining according to Knoll's definition [28], i. e., an analyte concentration that produces a chromatographic peak equal to three times the standard deviation of the baseline noise. For PCBs we used the largest peak to estimate the detection limit for this study.

In Table II the percentage recoveries of each of the investigated pesticides, PCB congeners and other

Table II. Recovery of PCB congeners and chlorinated pesticides from NH_2 Sep-Pak cartridge using 500 μL ethylacetate, after elution of 25 mL mussel water sample

Substances	Concentration level ($\mu g\ L^{-1}$)	Recovery %	RSD %
Heptachlor	0.5	102	5.6
	5	98	3.2
	15	96	2.5
Heptachlor epoxide	0.6	101	6.5
	5	98	2.6
	15	97	2.8
Aldrin	0.3	100	2.2
	5	98	2.1
	15	97	2.2
Endrin	0.4	101	3.6
	6	98	2.0
	18	98	2.1
Dieldrin	0.2	100	1.9
	5	99	2.0
	16	98	2.1
4,4'-DDD	0.6	98	2.3
	5	97	2.3
	15	98	2.2
α-Endosulfan	0.8	103	5.4
	6	99	2.0
	12	98	2.1
PCB 1	0.5	101	3.3
	4	96	1.9
	10	95	2.1
PCB 15	0.5	98	3.0
	5	96	2.7
	12	96	2.5
PCB 36	0.2	98	2.8
	5	96	2.3
	10	95	2.1
PCB 44	0.2	102	4.6
	4	98	3.0
	12	98	2.6
PCB 126	0.2	102	5.0
	4	98	3.1
	11	97	2.6
PCB 138	0.2	101	3.6
	5	98	3.1
	12	97	3.9
PCB 180	0.1	100	2.5
	3	98	2.3
	12	98	2.3
polychlorobenzenes*	5	–	–
polychloronitrobenzenes*	8	–	–
polychlorophenols*	8	–	–
polychloroanilines*	10	–	–

RSD % = relative standard deviation from three determinations.
*see text for the compounds used.

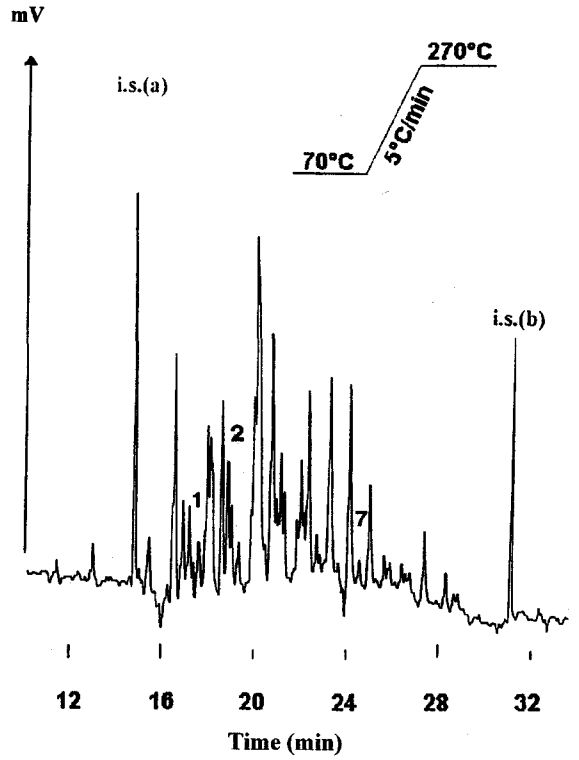

Figure 1

Gas chromatogram of Aroclor 1232 (12.35 ppb) and chlorinated pesticide residues desorbed from a NH_2 Sep-Pak cartridge. Peaks: **1** = heptachlor (0.012 ppb), **2** = aldrin (0.12 ppb), **7** = 4,4'-DDD (0.01 ppb). i. s. (**a**) = lindane and i. s. (**b**) = PCB 195. See text for experimental conditions.

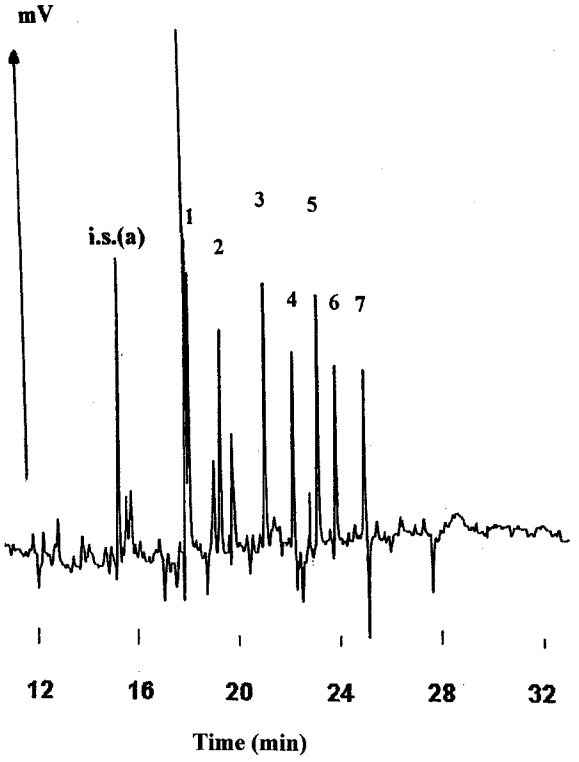

Figure 2

Gas chromatogram of chlorinated pesticides desorbed from a C_{18} Sep-Pak cartridge connected in series with the NH_2 trap. Peaks: **1** = heptachlor (0.46 ppb), **2** = aldrin (0.37 ppb), **3** = heptachlorepoxide (0.49 ppb), **4** = α-endosulfan (0.48 ppb), **5** = dieldrin (0.48 ppb), **6** = endrin (0.48 ppb), **7** = 4,4'-DDD (0.45 ppb). i. s. (**a**) = lindane. See text for experimental conditions.

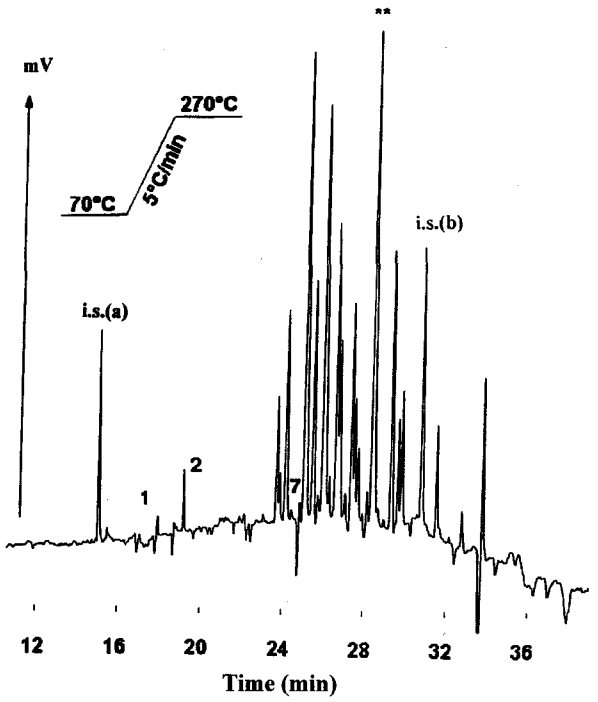

Figure 3

Gas chromatogram of Aroclor 1260 (17.28 ppb) and chlorinated pesticide residues desorbed from a NH_2 Sep-Pak cartridge. Peaks: **1** = heptachlor (0.022 ppb), **2** = aldrin (0.23 ppb), **7** = 4,4'-DDD (0.02 ppb). i. s. (**a**) = lindane and i. s. (**b**) = PCB 195. See text for experimental conditions.

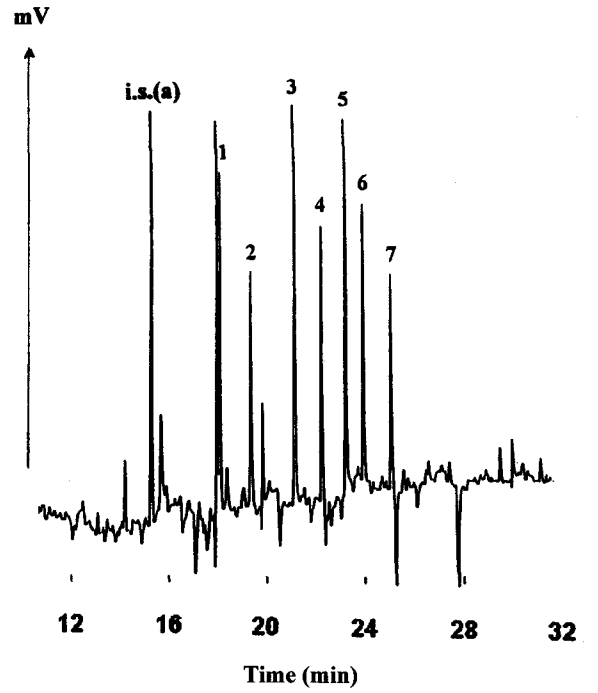

Figure 4

Gas chromatogram of chlorinated pesticides desorbed from a C_{18} Sep-Pak cartridge connected in series with the NH_2 trap. Peaks: **1** = heptachlor (0.96 ppb), **2** = Aldrin (0.64 ppb), **3** = heptachlorepoxide (0.98 ppb), **4** = α-endosulfan (0.97 ppb), **5** = dieldrin (0.98 ppb), **6** = endrin (0.97 ppb), **7** = 4,4'-DDD (0.93 ppb). i. s. (**a**) = lindane. See text for experimental conditions.

chlorinated compounds at different concentrations are reported. The values were obtained by passing 25 mL of the mussel sample spiked with the chlorinated pesticides, PCB congeners and other organochlorine compounds through the NH_2 Sep-Pak cartridge. Then this trap was eluted with 500 μL of ethylacetate to recover the pesticides and PCB congeners. Recoveries averaged 95.0 % with a relative standard deviation ≤ 5.6 % and were considered satisfactory. It was found that polychlorophenols, polychloroanilines, polychloronitrobenzenes and polychlorobenzenes are not adsorbed under these experimental conditions. Consequently, the NH_2 Sep-Pak cartridges make it possible to adsorb the chlorinated pesticides and PCBs and to separat them from polychlorobenzenes, polychloronitrobenzenes, polychloroanilines and polychlorophenols. In a previous work [29] the author has studied the isotherms and the breakthrough curves of these substances and the optimum experimental conditions to recover and to separate the PCBs from chlorinated pesticides using the NH_2 Sep-Pak cartridge.

Table III shows the pergentages of PCB congeners and chlorinated pesticides desorbed from the NH_2 Sep-Pak cartridge. The results refer to 25 mL mussel sample spiked with PCB congeners and pesticides passed through the trap. After washing the trap with 25 mL 40 % methanol aqueous solution, the PCB congeners and pesticides were recovered using 500 μL ethylacetate. The recovery was ≥ 95.0 % with a relative standard deviation ≤ 5.0 %; only the aldrin (25,2 %), 4,4'-DDD (2.4 %) and heptachlor (3.2 %) were desorbed from the NH_2 Sep-Pak cartridge.

Therefore, using 25 mL of an aqueous solution (40 % methanol), PCB congeners can be separated from chlorinated pesticides.

Figure 1 shows a gas chromatogram obtained from 25 mL of a mussel sample spiked with chlorinated pesticides (0.5 ppb each) and Aroclor 1232 (13 ppb) passed through the NH_2 Sep-Pak cartridge. This chromatogram shows Aroclor 1232 (95 %), aldrin (24 % – 0.12 ppb), heptachlor (2.4 % – 0.012 ppb) and 4,4'-DDD (2.0 % – 0.01 ppb) desorbed from the trap with 500 μL ethylacetate, after elution of 25 mL of a 40 % methanol aqueous solution.

Figure 2 shows the chlorinated pesticides (heptachlor: 0.46 ppb, heptachlorepoxide: 0.49 ppb, aldrin: 0.37 ppb, dieldrin: 0.48 ppb, endrin: 0.48 ppb, α-endosulfan: 0.48 ppb, 4,4'-DDD: 0.45 ppb) adsorbed on the C_{18} Sep-Pak cartridge connected in series with the NH_2 cartridge after elution of methanolic aqueous solution.

Figure 3 reports a gas chromatogram obtained from 25 mL of a mussel sample, spiked with chlorinated pesticides (1 ppb each) and Aroclor 1260 (18 ppb) passed through the NH_2 trap. This chromatogram shows Aroclor 1260 (96 %), aldrin (23 % – 0.23 ppb), heptachlor (2.2 % – 0.022 ppb) and 4,4'-DDD (2.0 % – 0.02 ppb) desorbed from the NH_2 Sep-Pak cartridge with 500 μL ethylacetate after elution of 25 mL of a 40 % methanol aqueous solution. Figure 4 shows the chlorinated pesticides (heptachlor: 0.96 ppb, heptachlorepoxide: 0.98 ppb, aldrin: 0.64 ppb, endrin: 0.97 ppb, α-endosulfan: 0.97 ppb, dieldrin: 0.98 ppb and 4,4'-DDD: 0.93 ppb) desorbed from the C_{18} Sep-Pak cartridge connected in series with the NH_2 trap after elution of methanolic aqueous solution.

Table III. Recovery of PCB congeners and pesticide residues from the NH_2 Sep-Pak cartridge, after washing the trap with 25 mL 40 % methanol aqueous solution.

Substances	Concentration level ($\mu g\,kg^{-1}$)	Recovery %	RSD %
PCB 1	0.3	95.1	5.0
PCB 15	0.2	95.0	4.6
PCB 36	0.2	96.2	4.1
PCB 44	0.06	96.1	4.1
PCB 126	0.05	97.4	3.0
PCB 138	0.05	97.5	2.8
PCB 180	0.05	97.3	2.9
Aldrin	0.2	25.2	3.2
heptachlor	0.2	3.2	3.8
4,4'-DDD	0.3	2.4	4.3

RSD % = relative standard deviation from three determinations

Conclusions

The use of the NH_2 Sep-Pak cartridges give excellent recoveries of chlorinated pesticides and PCB congeners from mussei samples. The cartridge allows it to adsorb pesticides and PCBs and separates them from polychlorophenols, polychloroanilines, polychloronitrobenzenes and polychlorobenzenes.

Moreover, with a simple clean-up step, the PCBs can be separated from the chlorinated pesticides. The recovery of PCB congeners is ≥ 95.0 % with a relative standard deviation ≤ 5.0 %. The method proposed is simple, rapid and reproducible.

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