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Supercritical fluid extraction and clean-up of organochlorine pesticides and polychlorinated biphenyls in mussels

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Abstract

The simultaneous extraction and clean-up of mussel samples followed by gas chromatography with electron-capture detection and mass spectrometric confirmation of 15 organochlorine pesticides (OCPs) and 11 polychlorinated biphenyls (PCBs) is developed using Florisil sorbent in the supercritical fluid extraction cell. The method detection limits vary from 1 to 10 ng/g for OCPs and from 2 to 15 ng/g for PCBs. Mean reproducibilities of 11% and 10% and mean recoveries of 80% and 53%, respectively, for OCPs and PCBs are obtained. The feasibility of the proposed supercritical fluid extraction method was confirmed by analyzing a certified reference material and mussels collected from Taiwan region. The method is simple, rapid and requires only small amounts of samples and solvents. It may serve as a screening protocol for the determination of OCPs and PCBs in mussels on a routine basis. © 1997 Elsevier Science B.V.

Keywords: Environmental analysis; Sample handling; Pesticides; Organochlorine compounds; Polychlorinated biphenyls

1. Introduction

Increasing population, agricultural activities and industrial development have made chemical pollution an issue of global concern [1]. The chemical wastes from the society will eventually sink in the world ocean. Among these wastes, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) have received increasing attention because of their extended use, long half-life time, high accumulation potential, harmful biological effects and inevitable impacts on the environment [2–4]. The assessment of current oceanic status to predict the pollution trends is indispensable to the integrity of the marine system and human health [5]. These critical issues were addressed by the program of global marine monitoring using bivalves, i.e., mussels, as sentinel organisms for monitoring the concentration of selected pollutants in coastal environments [6,7].

The most common method for extracting OCPs and PCBs from lipid-containing mussels is Soxhlet extraction, which uses large amounts of toxic solvents and is time-consuming. Co-extraction of OCPs, PCBs and lipids is generally encountered and requires extensive clean-up. The combination of lengthy extraction time and laborious clean-up procedures makes the pretreatment step become the limiting factor when analyzing large numbers of samples with advanced instrumental techniques [8–10]. Matrix solid-phase dispersion (MSPD) [11–14] and supercritical fluid extraction (SFE) [15–18]

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techniques were developed to overcome these drawbacks.

The objective of the present study was to investigate the use of SFE with on-line clean-up of lipids for the analysis of mussels contaminated with 15 OCPs [α-, β-, γ- and δ-benzene hexachloride (BHC), heptachlor, aldrin, heptachlor epoxide, endosulfan I, 4,4′-DDE, dieldrin, endrin, 4,4′-DDD, endrin aldehyde, endosulfan sulfate and 4,4′-DDT] and 11 PCBs (IUPAC numbers 18, 28, 44, 66, 101, 118, 126, 128, 156, 180 and 169) followed by gas chromatography with electron-capture detection (GC–ECD) at ng/g levels. The effect of combining MSDP to the SFE technique (MSPD-plus-SFE method) was discussed and compared to the SFE-only method. The proposed SFE method has been applied to determine OCPs and PCBs in mussels collected from major fishing and oyster breeding areas in the Taiwan region. GC–CED positive results were confirmed with GC–mass spectrometric (GC–MS) detection.

2. Experimental

2.1. Chemicals

The 15 OCPs, 11 PCB congeners, dibromooctafluorobiphenyl (DBOFB) and mirex were obtained from AccuStandard (New Haven, CT, USA). The DBOFB was added to the sample at the beginning of the extraction to check the possible loss of analytes during extraction. Standard calibration mixtures containing 100 ng/g mirex as the recovery standard were prepared by diluting the OCPs and PCBs mixtures in *n*-hexane by volume. The solvents of *n*-hexane and acetone were Optima grade from Tedia (Fairfield, OH, USA). The CO_2 extraction fluid was SFE grade from Scott Specialty Gases (Plumsteadville, PA, USA). The ODS (C_{18}, octadecylsilyl derivatized silica, 40 μm) was obtained from Nacalia (Tokyo, Japan). Florisil (PR grade 147–225 μm) was obtained from Janssen (Geel, Belgium). Silica gel (74–147 μm, ASTM 923 grade) was obtained from Aldrich (Milwaukee, WI, USA). Neutral alumina (64–193 μm, activity I) was obtained from Merck (Darmstadt, Germany). C_{18} was prewashed by continuously refluxing it in *n*-hexane–acetone (1:1) for 24 h in a Soxhlet apparatus followed by oven drying at 60°C for 1 h. Silica gel, Florisil and alumina were activated by drying at 150°C for 12 h before use.

2.2. Mussel samples and spiking methods

Live mussels of *Meretrix lusoria*, *Crassostrea gigas* and *Corbicula maximum* were purchased from the market. After splitting the shells, the mussel tissues were removed from the lower shell and lyophilized at −50°C for 2 days in an Alpha 1-4 freeze-drier (Christ, Germany). The lyophilized tissues were ground mechanically to obtain a homogeneous powder, which was later found to be free from OCPs and PCBs. An equal aliquot of powder from each mussel was blended to prepare the mussel blanks for fortified and control studies. Aliquots of lyophilized mussel blanks (~0.3 g) were loaded into 10-ml stainless-steel extraction vessels and followed by adding a known amount of OCPs (50 μl, 2 μg/ml), PCBs (50 μl, 1.75 μg/ml) and DBOFB (50 μl, 1 μg/ml) to prepare 100 ng/ml OCP and 87.5 ng/ml PCB fortified samples. The solvent was evaporated to dryness at ambient temperature for ~5 min [19]. Aliquots of 2.0 g Florisil (or C_{18}, silica gel, neutral alumina) sorbent were then added on top of the spiked mussel tissues. In the MSPD-plus-SFE method, aliquots of lyophilized mussel tissues (~0.3 g) were ground together with a 1.0 g C_{18}. The homogeneous C_{18}–mussel mixtures were then treated similarly to the SFE-only method.

The Certified Reference Material (CRM) MA-A-1/OC: a chlorinated hydrocarbon-containing copepod homogenate was obtained from International Atomic Energy Agency (Vienna, Austria) and used as received. A total of nine mussels (seven *Crassostrea gigas*, two *Mytilus smarrangdinus*) collected from west southern Taiwan (*Crassostrea gigas* T1, T2, T3), Machu island (*Crassostrea gigas* M1, *Mytilus smarrangdinus* M2, M3) and Kinmen island (*Crassostrea gigas* K1, K2, K3) coastal areas were used to further check the feasibility of the proposed SFE method.

2.3. Supercritical fluid extraction

All extractions were performed on a Suprex (Pittsburgh, PA, USA) PrepMaster equipped with an

AccuTrap collecting device. The CO_2 extracts containing the analytes were trapped by the deactivated fused-silica beads (55–105 μm, Sigma, MO, USA) in the AccuTrap first at −30°C. They were then eluted out at 30°C using an appropriate volume of *n*-hexane. The restrictor temperature was fixed at 80°C. Working extraction conditions were obtained using the sequential optimization approach [20]. The working extraction condition obtained was: pure CO_2, 250 atm (1 atm=101 325 Pa) extraction pressure, 50°C extraction temperature, 5 min static extraction time, 20 min dynamic extraction time, 2.0 g Florisil sorbent, 12 ml *n*-hexane eluting at 1 ml/min and 10-ml extraction vessel. This condition was used throughout the study, unless otherwise specified.

The *n*-hexane eluate in the collection vial was then transferred into a concentration tube and purged with nitrogen to a volume of ~0.8 ml. A 100 μl volume of mirex quantitation standard was added. The final volume of the eluate was adjusted to 1.0 ml and subjected to GC–ECD analyses. For GC–MS confirmations, the eluates were concentrated further to meet the higher detection limit of the MS detector.

2.4. Gas chromatography

GC–ECD analyses were carried out using a Fison 800 Series GC system equipped with a J&W DB-5 capillary column (60 m×0.25 mm I.D., 0.25 μm film thickness) and a ^{63}Ni ECD system. Samples were introduced into the GC column via a split injector system. The injector was operated in the split mode and at 250°C. Nitrogen was used as the carrier gas at a constant flow-rate of 1.0 ml/min. The column temperature was initially held at 90°C for 2 min, then programmed at 20°C/min to 200°C and held for 25 min, then at 4°C/min to 290°C and held for 10 min. The ECD temperature was at 300°C and used nitrogen as the make-up gas at a constant flow pressure of 180 kPa.

GC–MS confirmations were carried out using a HP 5890 Series II 5972 mass-selective detector (MSD) equipped with a J&W DB-5MS (30 m×0.25 mm I.D., 0.25 μm film thickness). The injector was operated in the split mode and at 250°C. Helium was used as the carrier gas at a flow-rate of 1.0 ml/min. The column temperature was initially held at 70°C for 2 min, then programmed at 20°C/min to 180°C and held for 2 min, then at 10°C/min to 300°C and held for 4 min. Effluents from the GC column were transferred via a transfer line held at 250°C and fed into a 70 eV electron impact ionization source held at 250°C. OCPs and PCBs were analyzed in two individual GC–MS runs operated in selected ion monitoring (SIM) mode as previously reported [14].

3. Results and discussion

3.1. Sample pretreatment and clean-up

Two methods, SFE-only and MSPD-plus-SFE, were evaluated in this study for on-line clean-up of lipids. Trapping sorbents were loaded on top of the mussel sample in the extraction vessels by both methods to retain the lipids. Trapping sorbents such as Florisil, C_{18}, silica gel and neutral alumina which have been successfully used in fish matrices [21,22] were evaluated. All sorbents, except Florisil, failed to produce clean chromatograms (results not shown). The cleanest chromatogram was obtained using Florisil sorbent (Fig. 1B). The disappearance of the matrix peaks in the chromatogram of samples without using trapping sorbents (Fig. 1A) evidenced the clean-up efficiency of Florisil sorbent. The chromatogram of samples subjected to additional MSPD treatments (Fig. 1C) appeared to be slightly better than that of samples subjected to Florisil clean-up only. The recoveries associated with the MSPD-plus-SFE method were generally lower, presumably due to the additional grounding step. Subsequent analyses were therefore conducted with SFE-only method.

3.2. Quantitation

The fortified levels used in this study were designed to encounter the trace contaminants usually found in biota samples used for environmental pollution monitoring. These values were much lower than the tolerable ranges of US Food and Drug Administration (FDA) Action Levels (e.g., 0.3 μg/g for heptachlor, aldrin, heptachlor epoxide and endrin; 5.0 μg/g for total DDT, 5.0 μg/g for total PCBs) [23]. The results obtained from this study would therefore be useful for monitoring OCPs and PCBs

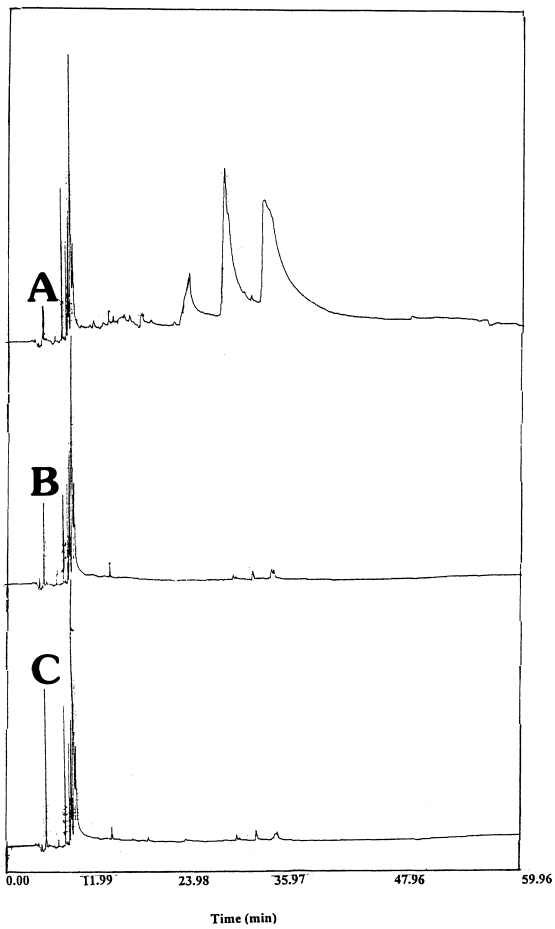

Fig. 1. Representative GC–ECD chromatograms of SFE extracts of mussel blanks: (A) no trapping sorbents in the extraction vessel; (B) using Florisil trapping sorbent in the extraction vessel, and (C) as for (B) but extraction vessel is loaded with a mixture of lyophilized mussel tissues and C_{18}.

in food supplies of similar matrix. The average recoveries, method detection limits (MDLs) and correlation coefficients for 15 OCPs and 11 PCBs in fortified mussels are summarized in Table 1.

For OCPs, the average recoveries ($n=3$) were greater than 70%, except for heptachlor epoxide, endosulfan I, endrin aldehyde and endosulfan sulfate. The mean recovery was 80%. The reproducibilities expressed as standard deviation (S.D.) were less than than 15%, except for endosulfan I and 4,4′-DDE. The mean reproducibility for the 15 OCPs expressed as average S.D. was 11%. The MDLs varied from 1 to 10 ng/g, which was below the US FDA Action Levels [23]. The correlation coefficients were better than 0.990, except for endrin. In conjunction with the unusually high recovery of 118% and a detailed inspection of the chromatogram of the blank indicated that the low correlation coefficient associated with endrine was due to a system interference. The results indicate that the proposed SFE method yields satisfactory extraction and determination of OCPs in mussel samples at the ng/g levels.

For PCBs, only PCBs 18 and 118 had average recoveries ($n=3$) greater than 70%. The recoveries of the other 9 PCBs ranged from 33% to 62%. The mean recovery was 53%. High-chlorine number containing PCBs with their hydrophobic properties would be favorably retained by the lipid matrix. We therefore attributed the low recoveries of PCBs to the trapping by the Florisil sorbent as well as the retaining by lipid matrix. These trapped and retained PCBs were not fully desorbed by the CO_2 fluid under the SFE conditions used. The reproducibilities expressed as S.D. were less than 15%, except for PCBs 126, 128 and 169. The mean reproducibility for the 11 PCBs expressed as average S.D. was 10%. The MDLs varied from 2 to 15 ng/g and decreased with the degree of analyte chlorination, presumably due to the ECD's increasing capture of electrons with increasing number of chlorine atoms in the PCBs. The MDLs were below the US FDA Action Levels [23]. The correlation coefficients were better than 0.990. The results indicate that the proposed SFE method yields acceptable extraction and determination of PCBs in mussel samples at the ng/g levels. The overall lower recoveries of PCBs is an inevitable consequence of using compromised SFE conditions for simultaneous extraction of two different classes of compounds, i.e., OCPs and PCBs.

3.3. Analysis of real samples

Real samples of a CRM and nine locally collected mussels were analyzed. A MA-A-1/OC CRM from IAEA was analyzed to further check the feasibility of using Florisil sorbent for direct on-line clean-up of lipids in mussels. The interferences from lipids were eliminated by treatment with Florisil sorbent as evidenced by the absence of background peaks in the chromatogram (Fig. 2). The measured values were in the acceptable range for 5 determined OCPs of

Table 1
Average percent recoveries, standard deviations, method detection limits and correlation coefficients for 15 OCPs and 11 PCBs in fortified mussels

Analyte	Recovery[a] average±standard deviation (%)	Method detection limit[b] (ng/g)	Correlation coefficient[c]
α-BHC	97±10	4	1.000
β-BHC	77±14	2	0.996
γ-BHC	85±10	3	0.999
δ-BHC	76±12	1	0.996
Heptachlor	89±9	3	0.996
Aldrin	70±8	1	0.996
Heptachlor epoxide	60±7	1	0.995
Endosulfan I	57±33	3	0.994
4,4′-DDE	90±18	2	0.996
Dieldrin	76±6	2	0.996
Endrin	118±10	3	0.893
4,4′-DDD	76±6	8	0.999
Endrin aldehyde	48±6	5	0.994
Endosulfan sulfate	44±7	10	1.000
4,4′-DDT	106±9	7	1.000
PCB18	73±8	14	0.998
PCB28	57±10	15	0.994
PCB44	62±5	6	0.994
PCB66	48±7	11	0.994
PCB101	46±4	12	0.994
PCB118	90±1	12	0.998
PCB126	33±21	8	0.998
PCB128	37±17	5	0.997
PCB156	47±14	2	0.999
PCB180	53±8	2	0.999
PCB169	40±19	4	0.999

[a] OCPs spiked at 100 ng/ml; PCBs spiked at 87.5 ng/ml; $n=3$.
[b] 3.14×standard deviation of seven replicate analyses of mussel blanks fortified at 5 ng/ml OCPs and 17.5 ng/ml PCBs levels.
[c] Five data points, $n=3$.

α-BHC, γ-BHC, aldrin, 4,4′-DDE and 4,4′-DDD and 4,4′-DDT (Table 2). The discrepancies in dieldrin and 4,4′-DDT results might be attributed to the difference in matrix. Nevertheless, the efficiency of Florisil sorbent for on-line clean-up of lipids in real biota samples is evident.

The SFE extracts of the nine mussels (seven *Crassostrea gigas*, two *Mytilus smarrangdinus*) analyzed in this study did not show visible color, indicating that the proposed SFE method is applicable to analyze real mussel samples. Representative chromatograms of SFE extracts of one mussel from each area were shown in Fig. 3. The absence of complicated background peaks further verified the feasibility of the proposed SFE method. The analytical results expressed as mean (range) were α-BHC 4 ng/g (2–6 ng/g), γ-BHC 3 ng/g (0–6 ng/g), δ-BHC 3 ng/g (0–7 ng/g), 4,4′-DDE 29 ng/g (0–43 ng/g), 4,4′-DDD 12 ng/g (0–31 ng/g), endosulfan sulfate 3 ng/g (0–23 ng/g), 4,4′-DDT 29 ng/g (0–131 ng/g), PCB18 22 ng/g (5–38 ng/g), PCB28 7 ng/g (0–18 ng/g) and PCB101 42 ng/g (0–69 ng/g). The contamination levels were far below the US FDA Action Levels [23]. Considering that chlorinated pesticides were banned in Taiwan since the early 1970s, the detected OCPs were presumably residues from previous usage. However, the unusually high levels of DDT-series pesticides found in Kinmen and Machu mussels are surprised. More research is needed to search for the origin of these DDT-series pesticides. Nevertheless, GC–MS-SIM analyses confirmed the presence of these contaminants.

A representative GC–MS-SIM chromatogram of

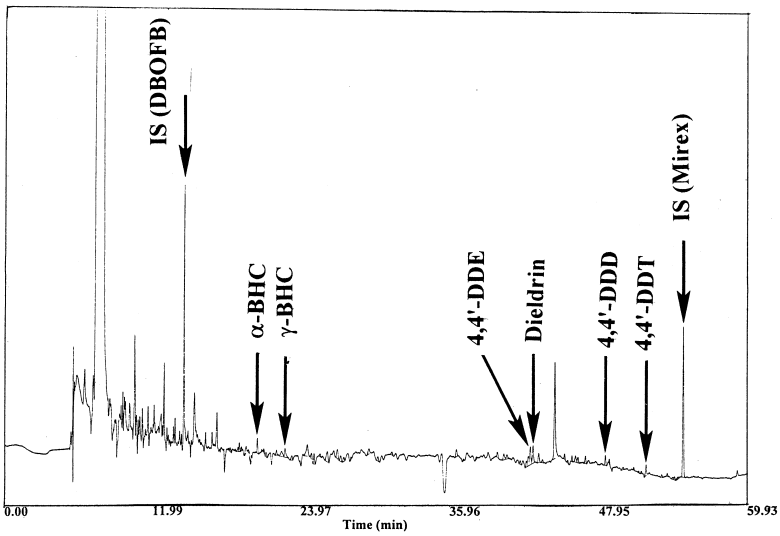

Fig. 2. GC–ECD chromatogram of SFE extracts of the IAEA CRM MA-A-1/OC, a copepod homogenate.

PCBs from a Machu mussel M2 is shown in Fig. 4. The dominant PCBs found were PCBs 18, 28 and 101. PCBs 18 and 101 were the major components found in most PCBs technical mixture such as Aroclor [24], which were among the major PCBs previously used in Taiwan before their ban at 1988. PCB 28 was found in many marine mammal tissue and mussel samples [25,26]. The presence of other m/z 256 and 258 peak pairs in Fig. 4 indicates that this mussel contained other monochlorinated biphenyls. The PCBs analytes studied in this study were selected based on their reported occurrence in the mussels and the resolution of the GC column used. A comprehensive analysis of PCBs in mussel samples might require the use of advanced instrumental methods such as the multidimensional GC [27,28]. Nevertheless, the proposed SFE method is simple, rapid and requires only small amount of samples and solvents. Useful results from mussel monitoring can still be obtained.

4. Conclusions

A SFE method for the extraction and clean-up of 15 OCPs and 11 PCBs in mussel samples has been developed. The method loaded Florisil sorbent on top of the sample in the extraction cell, followed by

Table 2
Certified and measured values of CRM MA-A-1/OC

Analyte	Certified value (ng/g)	Range (ng/g)	Measured value mean±standard deviation (ng/g) ($n=3$)
α-BHC	10	1.6–18.4	ND (2±0)[a]
γ-BHC	8.2	1.9–14.7	3±1
Aldrin	14	0–33	ND (0)[a]
4,4′-DDE	6.1	1.5–17.1	9±1
Dieldrin	6.6	NA[b]	14±2
4,4′-DDD	5.5	6–11	9±3
4,4′-DDT	8.3	3.4–13.2	14±2

[a] Not detected, the measured value shown in parenthesis is below the MDL.
[b] Not available, impossible to apply test, less than 5 accepted values.

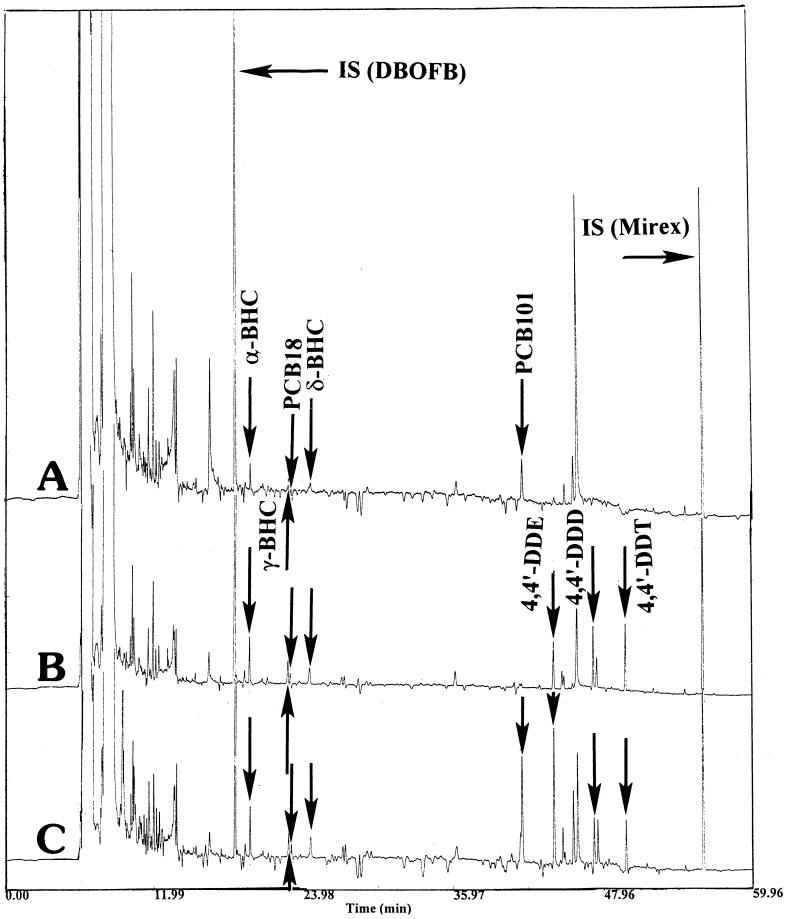

Fig. 3. GC–ECD chromatograms of SFE extracts of mussel samples: (A) T2; (B) K2 and (C) M2.

GC–ECD detection and GC–MS confirmation. The MDLs vary from 1 to 10 ng/g for OCPs and from 2 to 15 ng/g for PCBs. The mean reproducibilities expressed as average standard deviation are 11% and 10%, respectively, which are very desirable for long-term environmental pollution monitoring study. The mean recoveries of 80% for OCPs and 53% for PCBs are not as good as those obtained using comprehensive analytical methods [26,29,30]. However, the proposed SFE method is simple, rapid and requires only small amount of samples and solvents. The total amount of time needed from SFE ex-

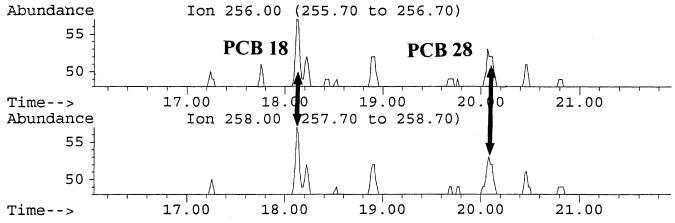

Fig. 4. Representative GC–MS-SIM chromatogram of PCBs from a Machu mussel M2.

traction to GC–ECD analysis is less than 2 h. Preliminary results indicate that the proposed SFE method can be successfully applied to fortified mussels (*Crassostrea gigas*) and real mussels (*Crassostrea gigas* and *Mytilus smarrangdinus*) contaminated with native OCPs and PCBs at ng/g levels. The method may serve a screening protocol for the determination of OCPs and PCBs in mussels on a routine basis.

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