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Matrix solid-phase dispersion extraction and gas chromatographic screening of polychlorinated biphenyls in fish

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Abstract

A matrix solid-phase extraction (MSPD) and gas chromatographic–electron-capture detection method for determining polychlorinated biphenyls (PCBs) in fish (grass carp) is described. The method uses an octadecylsilyl-derivatized silica and acidic silica gel-based MSPD co-column for direct in-line clean-up. The performance of the method compares favourably with that of the traditional approach but the sample size, analysis time and overall cost are lower. The method may serve as a screening protocol for the isolation and determination of PCBs in fish.

1. Introduction

Environmental contamination from polychlorinated biphenyls (PCBs) is widely recognized. The presence of trace amounts of PCBs in fish is of concern because of their risks to human health [1]. In response to this concern, the tolerance levels of PCBs in fish are limited to 5.0 μg/g by the US Food and Drug Administration [2] and 1.0 μg/g by the Taiwan Department of Health [3]. In addition, fish are commonly used as bioindicators of environmental pollution. Thus, PCBs are constantly monitored in fish samples.

A variety of analytical methods involving pretreatment by solvent extraction, column chromatography, distillation and saponification with base followed by instrumental analyses using gas chromatography (GC) with electron-capture detection (ECD) or mass spectrometry (MS) have been developed [4]. The pretreatment step becomes the limiting factor when analysing large numbers of samples with recent advances in instrumental techniques [5–7]. The benefit of decreasing the number of samples required in more comprehensive analytical procedures by first applying a simple, rapid and inexpensive screening method becomes apparent. Recently, a method of matrix solid-phase extraction (MSPD) of trace organic compounds from homogeneously dispersed solid matrices by adsorbing them on suitable solid adsorbents followed by desorption with a small amount of organic solvent was developed for this purpose. MSPD has been used successfully to determine trace amounts of drugs and chemical residues in various biological matrices [8–12]. The aim of this work was to develop a similar method as a screening protocol

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for the isolation and determination of PCBs in fish.

2. Experimental

2.1. Materials

All solvents used were of Fisher Optima grade. The PCB mixtures KC-300 and KC-500 were obtained from Gasukuro Kogyo. Isotope-labelled PCBs were obtained from AccuStandard. Standard calibration mixtures were prepared by diluting a stock standard solution in hexane by volume. Octadecylsilyl (C_{18})-derivatized silica (ODS) (40 μm) was obtained from Supelco and 35–70-mesh silica gel from Merck. Fish (grass carp) used for fortified and blank studies were obtained from the local market and found to be free from PCBs. Real fish samples used for comparison studies were collected from water ponds near a warehouse where discharged PCB-containing transformers and capacitors were stored. ODS was prewashed by continuously refluxing it in hexane for 24 h in a Soxhlet apparatus followed by oven drying at 150°C for 1 h. Acidic silica gel (44%, w/w) was prepared by homogeneously mixing the silica gel with an appropriate amount of concentrated H_2SO_4.

2.2. Preparation of sample extracts

A 2-g portion of C_{18} was placed in a glass mortar and *ca.* 0.5 g of edible fillet (*i.e.*, muscle tissue) of fish sample was added. The C_{18}–tissue mixture was then gently blended for *ca.* 2 min with a glass pestle with clockwise circular motion to yield a semi-dry, homogeneous-appearing material. The homogeneous mixture was placed in a pre-weighed 5-ml glass syringe barrel column containing a frit and 1 g of acidic silica gel packing at the bottom. A frit was placed at the top of the column to serve as a retainer. The column was lightly tamped to remove air pockets, then tightly compressed to a final volume of *ca.* 5 ml using a syringe plunger. The exact mass of the fish muscle tissue was obtained by subtracting the mass of the silica gel column from the mass of the sample-loaded column. The column was then placed in a rack on top of a Supelco solid-phase extraction vacuum manifold ready for elution.

A 10-ml erlenmeyer flask was positioned below each column to collect the eluate. The column was eluted with 10 ml of hexane by gravitational flow. When the flow had ceased, excess of hexane was removed by applying suction pressure to the vacuum manifold using an Elyea A-3S aspirator. The hexane eluate was then transferred into a concentration tube and purged with nitrogen to a final volume of 1.0 ml.

2.3. Apparatus

The equipment for the extraction procedure consisted of four main parts: a sample reservoir, the extraction columns, the extraction vacuum manifold and an aspirator connected in series.

All GC analyses were carried out using a HP-5890 Series II gas chromatograph equipped with a J & W DB-1 capillary column (30 m × 0.53 mm I.D., 1.0 μm film thickness) and a ^{63}Ni electron-capture detector. Samples were introduced into the GC column via the splitless mode of a split–splitless injector system. The GC injector and detector were operated at 280 and 300°C, respectively. Argon–methane (95:5) was used as the carrier gas and make-up gas at flow-rates of 8 and 15 ml/min, respectively. The column temperature was initially held at 170°C for 3 min, then programmed at 5°C/min to 300°C.

GC–MS analyses were carried out using an HP-5971 instrument equipped with an HP-5890 Series II gas chromatograph and an HP-7673 autosampler. The column used was a J & W DB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). The injector was operated in the splitless mode and at 280°C. Effluents from the GC column were transferred via a transfer line held at 300°C and fed into a 70 eV electron impact (EI) ionization source. The column temperature was initially held at 70°C for 5 min, then programmed at 20°C/min to 180°C and held for 2 min, then at 10°C/min to 320°C and held for 6 min.

3. Results and discussion

The MSPD method used in this study differs from traditional methods in that the sample is dispersed over a large surface area on C_{18} by mechanical and hydrophobic forces to form a thin-layer sample. Non-polar materials such as lipids and other membrane components associate with the ODS polymer and facilitate disruption and unfolding of structural components of the fish muscle tissue. The hydrophilic components and more polar protein ends are associated with the silica support. Accordingly, 10 ml of extraction solvent can result in an efficient extraction. The recoveries and method detection limits using fortified fish are summarized in Table 1.

The fortification levels used in this study were chosen because they covered the ranges of regulatory tolerance levels. For KC-300-fortified samples, the recovery varied from 81.1 to 103.3% with an average of 94.8%. The reproducibility expressed as relative standard deviation varied from 2.1 to 8.6% with an average of 5.9%. The method detection limit (MDL) was 0.17 μg/g, which is below the regulatory tolerance levels of 5.0 and 1.0 μg/g. Comparable results were obtained when analysing KC-500-fortified samples. The recovery varied from 82.1 to 85.7% with an average of 84.1%. The reproducibility varied from 3.2 to 6.1% with an average of 4.9%. The MDL was 0.13 μg/g. The correlation coefficients for the calibration graphs of both KC-300 and KC-500 were better than 0.999. Using fortified fish samples, the recovery and reproducibility obtained from the MSPD method were comparable to those obtained with the standard method (saponification with base followed by GC–ECD, denoted SB–GC–ECD) [13], as shown in Table 2. A higher precision was always obtained with the MSPD approach. The preliminary results indicate that the MSPD method yields satisfactory extraction and determination of PCBs in fish samples.

In Tables 1 and 2, the average recoveries of KC-300 fortified samples are higher than those of KC-500-fortified samples. On the other hand, the MDL of KC-500-fortified samples is lower than that of KC-300-fortified samples. The lower MDL of KC-500-fortified samples is ascribed to the higher sensitivity of ECD towards PCBs containing a larger number of chlorine atoms such as KC-500. The difference in recovery was first studied from the viewpoint of co-extractants. The presence of co-extractants was serious during the early experiments when acidic silica

Table 1
Detection of PCBs by MSPD–GC–ECD in KC-300- and KC-500-fortified fish

KC-300		KC-500	
Concentration (μg/g)	Recovery (%) (mean ± S.D.[a])	Concentration (μg/g)	Recovery (%) (mean ± S.D.[a])
0.37	81.1 ± 7.8[b]		
0.90	103.3 ± 6.6[c]	0.89	82.1 ± 5.5[c]
1.69	98.5 ± 8.6[c]	1.70	84.6 ± 6.1[c]
5.60	93.7 ± 4.2[c]	5.62	85.7 ± 3.2[c]
9.17	97.7 ± 2.1[b]		
Average	94.8 ± 5.9	Average	84.1 ± 4.9
MDL (μg/g)	0.17	MDL (μg/g)	0.13
R^d	0.9992	R^d	0.9999

[a] Standard deviation.
[b] Four replicate analyses.
[c] Seven replicate analyses.
[d] Correlation coefficient.

Table 2
Detection of PCBs by SB–GC–ECD and MSPD–GC–ECD in KC-300- and KC-500-fortified fish

Method	Recovery (%) (mean ± S.D.[a])	
	KC-300 (0.60 μg/g)	KC-500 (0.60 μg/g)
SB–GC–ECD	85.2 ± 8.4	80.6 ± 11.7
MSPD–GC–ECD	83.2 ± 5.3	79.8 ± 6.3

[a] Standard deviation; four replicate analyses.

gel was not added to the column. The hexane eluate displayed a distinct yellow colour. No visible colour in the eluate was observed with acidic silica gel in the column. It appeared that the interfering compounds might be trapped by the silica gel as a yellow-orange color developed in the underlying silica gel layer during hexane elution. It was noted that small amounts of co-extractants still existed when hexane elution was carried out using suction pressure. The dominant co-extractant was tentatively identified as palmitic acid using GC–MS. The palmitic acid was not detected in the eluate when hexane elution was carried out using gravitational flow (Fig. 1). In short, both the in-line clean-up capability of the acidic silica gel and the proper use of gravitational flow for hexane elution were critical. Nevertheless, the presence of co-extractants as a possible cause of the difference in recovery was excluded.

The difference in recovery was next studied using GC–isotope dilution MS [14] to investigate whether it was because of the differences in recovery among PCB homologues. The composition and recovery of the KC-300 and KC-500 used in this study were determined and are given in Table 3. PCB homologues with two, three and four chlorines and with four, five and six chlorines were the major components of KC-300 and KC-500, respectively. The difference in composition between KC-300 and KC-500 was apparent. The recoveries were 79.0, 89.1 and 93.2% for two, three and four chlorine-containing PCBs from KC-300-fortified fish samples. For KC-500-fortified fish samples, the recoveries were 98.6, 92.8 and 84.9% for four, five and six chlorine-containing PCBs. Differences in recovery among PCB homologues were apparent. Using the raw data in Table 3, the ratio of the sum of the products of the composition and recovery from three major components was *ca.* 1.22 (KC-300 *vs.* KC-500). This value was close to 1.12, which

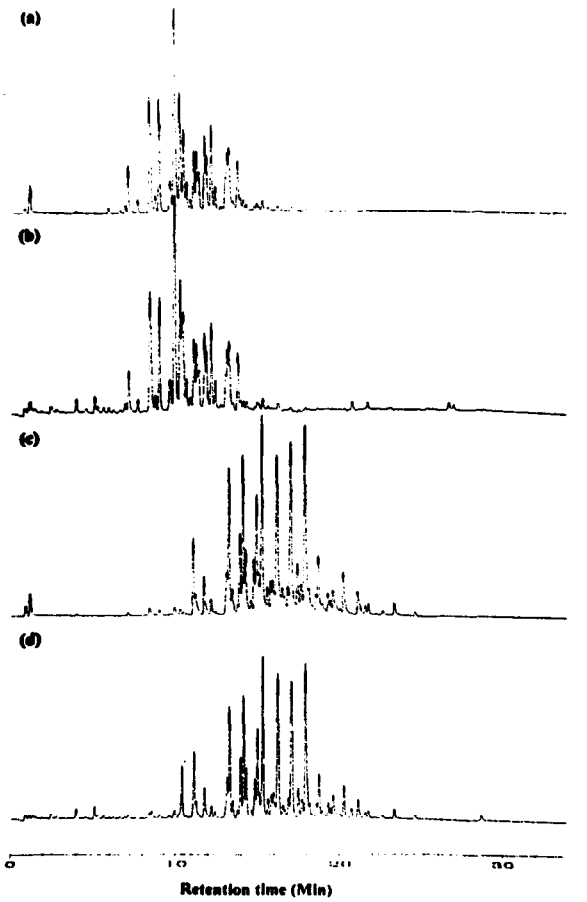

Fig. 1. GC–ECD of (a) KC-300 standard, (b) hexane eluate from KC-300-fortified fish, (c) KC-500 standard and (d) hexane eluate from KC-500-fortified fish.

Table 3
Composition and recovery of KC-300 and KC-500 by chlorine number

Homologue	Composition (%)		Recovery (%) (mean ± S.D.[a])	
	KC-300	KC-500	KC-300	KC-500
1Cl	0.09	–	71.7 ± 4.7	–
2Cl	8.67	0.42	79.0 ± 7.9	82.3 ± 5.6
3Cl	48.22	1.88	89.1 ± 2.0	92.2 ± 5.2
4Cl	38.55	16.74	93.2 ± 4.3	98.6 ± 1.7
5Cl	3.79	50.19	64.5 ± 5.4	92.8 ± 3.5
6Cl	0.68	26.78	10.3 ± 11.9	84.9 ± 5.8
7Cl	–	3.01	–	79.0 ± 6.7
8Cl	–	0.98	–	16.8 ± 1.9
9Cl	–	–	–	–

[a] Standard deviation; four replicate analyses.

was the ratio of the average recovery of KC-300 *vs.* KC-500 using the raw data in Table 2. Therefore, the different recoveries in Table 1 might be ascribed to the combined effects of different compositions between KC-300 and KC-500 and different recoveries among PCB homologues.

The MSPD method was applied to determine PCBs in real fish (grass carp). The results are given in Fig. 2. The total PCB content ranged from 0.12 to 0.29 μg/g, which are below the tolerance levels. Fish samples 1 and 2, which

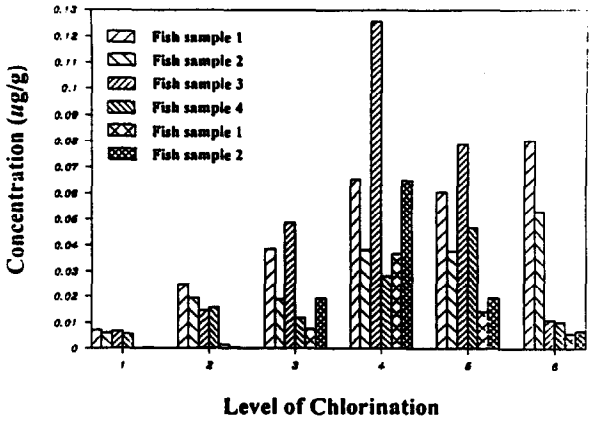

Fig. 2. PCB concentrations in real fish samples determined using the MSPD–GC–ECD method (samples 1–4) and the SB–GC–ECD method (samples 1 and 2) and expressed by level of chlorination.

were of larger size and could yield the 25 g of muscle tissue as required by the SB–GC–ECD method, were also analysed using that method. The total PCB contents were 0.07 and 0.11 μg/g, which were lower than the 0.28 and 0.17 μg/g determined using the MSPD–GC–ECD method. The difference is ascribed to the distribution of the various PCBs in fish body. The muscle tissue analysed by the MSPD–GC–ECD method was collected from the fish body close to the abdominal fin. This portion of fish body generally contained higher concentrations of lipid than the entire fish body. Higher concentrations of PCBs were usually observed in this portion of fish body because of the hydrophobic nature of lipids. Nevertheless, the advantage that the small sample size required by the MSPD method could be exploited to establish the distribution of PCBs in fish was demonstrated. The apparent accumulation of PCBs with three, four and five chlorines in all fish samples indicated that the PCB source might consist of a mixture of KC-300 and KC-500.

4. Conclusions

The determination of PCBs in fish can be achieved with an ODS and acidic silica gel-based MSPD co-column for direct in-line clean-up. The hexane eluate can be subsequently introduced into a GC–ECD system for rapid determination of PCBs. The MDLs ranged from 0.13 to 0.17 μg/g, which are well below the regulatory tolerance levels. The results obtained with the MSPD–GC–ECD method compare favourably with those from the classical SB–GC–ECD method. Further, the proposed method requires a smaller sample size (0.5 g *vs.* 25 g), has a shorter analysis time (40 min *vs.* 300 min) and is more economic (10 ml *vs.* 400 ml of solvent). Preliminary results indicate that the method may be successfully applied to fortified and real fish (grass carp) samples. The method presented here may serve as a screening protocol for the isolation and determination of PCBs in fish to protect human food supplies and to monitor environmental pollution. Studies to extend the

application fields and to lower the MDLs are in progress.

5. Acknowledgements

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