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Determination of mono- to octachlorobiphenyls in fish oil using Florisil adsorption followed by headspace solid-phase microextraction and gas chromatography with time-of-flight mass spectrometric detection

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Abstract

A simple and reliable method for the determination of polychlorinated biphenyls (PCBs) from mono- to octachlorobiphenyls in fish oil for dietary supplement is described. The method combines Florisil clean up and headspace solid-phase microextraction on 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB). Analyte detection was carried out using GC–time-of-flight mass spectrometry (GC–TOF-MS). Fifty three PCB congeners including the seven indicator PCBs (IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180) were analyzed. Under optimal conditions, the method detection limit (MDL) of each congener in the range from 0.8 to 31 ng/g was found. A certified reference material (BCR-349) was analyzed and it showed good agreement with the certified data.

Keywords: Fish-oils; PCBs; Headspace solid-phase microextraction; Florisil

1. Introduction

The manufacture of dietary supplement from fish oil is becoming popular. In the UK alone, pharmaceutical and food grade nutritional supplements shared a market of 50 million pounds sterling in 1992 [1]. Whole-fish oils but more popularly cod-liver oils are processed for this purpose. However, studies revealed that these substances are contaminated with PCBs in the range from 1.2 to 2.6 μg/g wet mass suggesting that cod-liver oil poses a health risk for human consumption because the figure exceeded the tolerance level of 2 μg/g set by the FDA (US Food and Drug Administration) [2]. Thus, a need to regulate these materials should be addressed.

Determination of PCBs in fatty matrices is of utmost relevance. PCBs tend to accumulate in fatty tissues due to their lipophilic character, thus extraction of them in the presence of extractable major sample components such as fats proved to be a challenge. Moreover, large amount of fat injected in a gas chromatograph can cause problems in the injector and the top of the column [3,4], it can also contaminate the ion source of the mass spectrometer causing poor analytical performance [5].

Saponification, gel permeation chromatography (GPC), sulfuric acid treatment, adsorption chromatography (alumina, silica gel, Florisil) were reported to have been used alone or in combination with other methods to remove interfering compounds [6,7]. However, use of concentrated sulfuric acid produces carbon that eventually adsorbs and degrade low chlorinated PCBs. On the other hand, column chromatography using alumina, silica gel and Florisil or combination of these adsorbents though widely used for clean up, cannot guarantee a lipid-free extract [8]. GPC is more efficient for this purpose but suffers drawbacks because it requires special equipment for a forced liquid flow [7].

Pressurized liquid extraction (PLE) was also reported to have been used for the extraction of fatty matrices [9]. It offers a fast and reliable one-step sample preparation but it requires a high cost of equipment and elevated temperatures that may degrade thermo labile analytes, possible blockage through

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concentration of matrix materials may also be a problem [10].

Solid-phase microextraction (SPME) is a relatively new extraction technique. Its work is based on the partitioning of the analytes between the aqueous phase and the organic coating (direct immersion mode) or between the gas phase above the sample and the SPME fiber (headspace mode) [11]. This method appears to be simple, fast and inexpensive. Because of these advantages, SPME methods have been developed to study persistent organic pollutants (POPs) in various matrices (water, soil, biota, food) of which PCB is a major component [10,12–14].

While PCB in aqueous matrices are easily detected by SPME, SPME in more complex matrices displays difficulty. As a matter of fact, there are a limited number of published papers concerning SPME in oily matrices [10,15,16].

For complex matrices, it is still possible to utilize SPME by using a suitable pre-extraction technique followed by an enrichment procedure using SPME prior to GC–MS analysis.

The aim of this study is to investigate the feasibility of using SPME as an enrichment technique in combination with clean up by adsorption chromatography for the analysis of PCBs in fish oil in dietary supplement. While electron-capture detection (ECD) is the most utilized detection method for PCBs, high-speed GC–time-of-flight (TOF) MS was used for this analysis to exploit the advantage of analyzing closely eluting PCBs, as it permits fast recording of mass spectra per second [10]. Fifty-three PCB congeners from mono- to octachlorobiphenyls including the seven indicator PCB congeners mandated by the European legislation to be monitored were studied. Parameters of adsorption chromatography (type of adsorbent and adsorbent weight) and SPME (fiber type, extraction time and temperature, desorption time, addition of salt) were separately optimized since they are independent of each other. A certified reference material was analyzed for PCBs (cod-liver oil, BCR-349) to validate the proposed method.

2. Experimental

2.1. Reagents and samples

A mixture of PCB congeners ranging from mono- to decachlorobiphenyls in nonane, BP-MS (Wellington Labs., Ontario, Canada) was used as PCB standard. The concentration was 2 μg/mL for each congener. Our preliminary investigations showed that nona- and decachlobiphenyls were not consistently recovered. However, since these PCB congeners are not widely used in commercial PCB mixtures and hence not found in most environmental samples, the experiment was carried out without quantifying them[17]. C-13 labeled PCBs, ranging from mono- to decachlorobiphenyl (5 μg/mL in nonane), MBP-CG (Wellington Labs.) were used as surrogate standards. Aroclor 1260, 2000 μg/mL in methanol was from Supelco (Bellefonte, PA, USA).

Florisil (60–100 mesh) was purchased from J.T. Baker (Phillipsburg, NJ, USA). It was activated at 130 °C overnight prior to use. Silica gel (75–150 μm) was from Wako (Japan) while aluminum oxide (50–200 μm) was purchased from Acros Organics (NJ, USA). These adsorbents were used for the preliminary test for the adsorption chromatography test.

Anhydrous Na_2SO_4 and NaCl from Kanto (Tokyo, Japan) were furnaced at 700 °C for 8 h before using. Hexane (ultraresi analyzed) was purchased from J.T. Baker. Glasswool was from Showa (Japan). Ultrapure water was obtained from Milli-Q water purification system (Millipore, Molsheim, France). N_2 gas used for all drying purposes was 99.9999% pure.

The certified reference material, BCR-349 was acquired from the Institute for Reference Materials and Measurement (Geel, Belgium).

The fish oil from dietary supplement was from Pharma-Rex (CA, USA). Preliminary analysis of these samples by a GC–MS method based on saponification using potassium hydroxide in methanol [6] showed no detectable amounts of PCBs under investigation making them appropriate for spiking experiments.

2.2. Chromatographic equipment and experimental conditions

Gas chromatography was carried out with a 6890 N Agilent gas chromatograph (Agilent Technologies, DE, USA). A 10 m × 0.18 mm I.D., 0.18 μm thickness DB-5 column (J&W Scientific, USA) was used with He (99.9999% purity) at 1.2 mL/min as carrier gas. The injector was equipped with an 11 mm, thermogreen LB-2 pre-drilled septa from Supelco. An injection sleeve with a 0.75 mm I.D. from Supelco was also used to improve peak shape. The injector was operated in the splitless mode at 260 °C. The oven temperature program was initially set to 50 °C and held for 3 min, then raised to 250 °C at a rate of 20 °C/min and finally raised to 300 °C at 10 °C/min.

The GC was attached to a Pegasus III TOF-MS system (LECO, MI, USA). Mass spectra were obtained in the electron ionization mode (70 eV) in the range from 10 to 530 *m/z*. The transfer line was heated to 320 °C while the ion source was set to 250 °C. Mass spectra were monitored using the extracted ion monitoring mode. The acquisition rate of MS spectra was 10 spectra/s and their average peak width was 0.7 s.

2.3. Clean up with adsorption chromatography

A 6 g amount of Florisil, silica gel and alumina was loaded in a 10 mL glass syringe, 15 mm I.D., plugged with glass wool. A stopcock valve was used to regulate the flow of eluant. Each was pre-conditioned with 15 mL hexane. When the solvent was about 1 mm above the adsorbent, 0.2 g of fish oil spiked with MBP-CG (final concentration is 60 ng/g oil) was added with 1.5 mL hexane and eventually loaded to the column. 5 min was allotted for equilibrium to be established.

Fractionation by 5 mL was made. The first 15 mL was full of impurities, however beyond the succeeding 15 mL, most of the PCBs were found. Hence, the first 15 mL was discarded and the subsequent 40 mL was used for the PCB determination. 3, 4 and 6 g of adsorbent were used for a 0.2 g fish oil sample for testing the adsorbent capacity.

2.4. Solid-phase microextraction

SPME fibers used for the optimization study were 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB) and 100 μm polydimethylsiloxane (PDMS) held in an SPME fiber holder, which were purchased from Supelco. Each fiber was conditioned based on the supplier's recommendations.

SPME enrichment was carried out as follows: 40 mL of the eluate was concentrated via rotary evaporation. The concentrate was transferred to a 40 mL vial with a PTFE lined septum. The concentrate was dried under a gentle stream of nitrogen. Twenty milliliters of ultrapure water was added and the vial was heated at 90 °C for 40 min. The container used was a 40 mL screw cap vial. The cap is lined with Teflon. Water used was 20 mL and headspace volume was 20 mL. Increasing the water to more than 20 mL causes too much pressure to the screw cap. Water condensation at the top of the cap, and in worst cases, leaks around the hole in the cap were observed, causing poor data precision and short SPME lifespan due to contamination and SPME fiber damage. 10, 8 and 2 mL vials were also tested, maintaining a 1:1 water volume: headspace volume. But these volumes proved to be too small for the needle to be exposed completely, especially with the 2 mL vial (thus, this was not tested further). However, with the 8 mL vial, it was observed that the SPME needle was wet by the water that condenses around SPME fiber because of the small headspace that is allowed for the fiber exposure. Again, this incident is not very good for the method precision. Hence, 20 mL water and 20 mL headspace was used due to technical practicality. Headspace extraction was done and the fiber was immediately injected to the GC–TOF-MS system. Procedure blanks were done periodically to check possible fiber contamination.

3. Results and discussion

3.1. Extraction optimization

Preliminary SPME experiments showed that PCBs are not transferred from the fish oil to the fiber satisfactorily. This may be explained by the high affinity of PCBs to the oil owing to its lipophilic characteristic. As a result of this, an intermediate step was made to address this problem. Fig. 1 shows the flowchart of the proposed procedure.

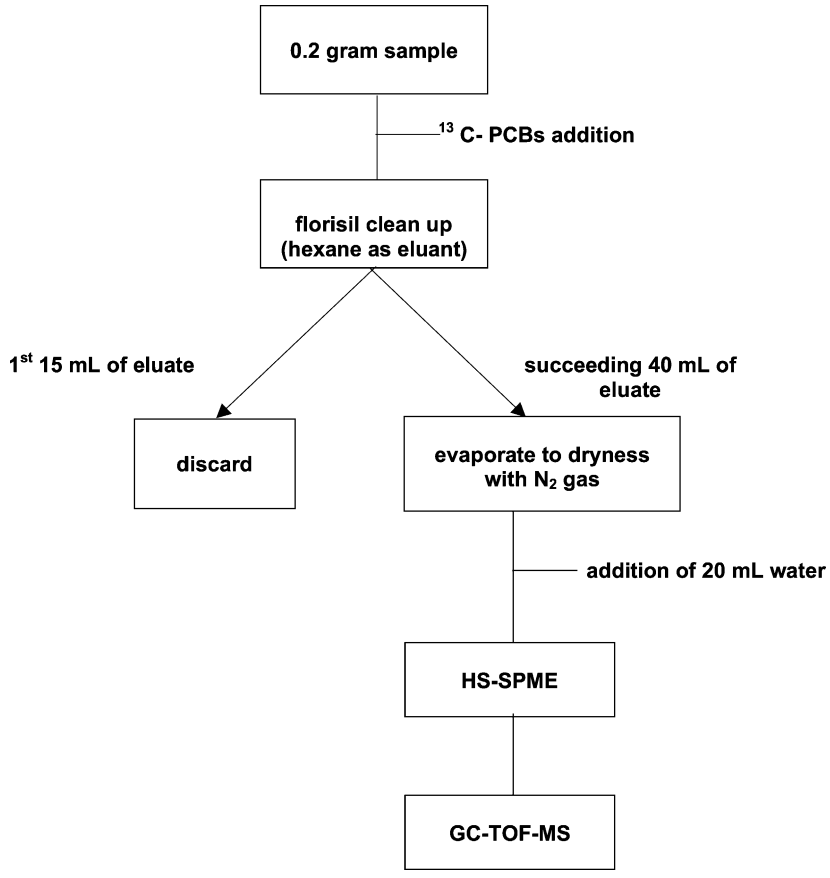

Fig. 1. Procedure for the analysis of PCBs in fish oil.

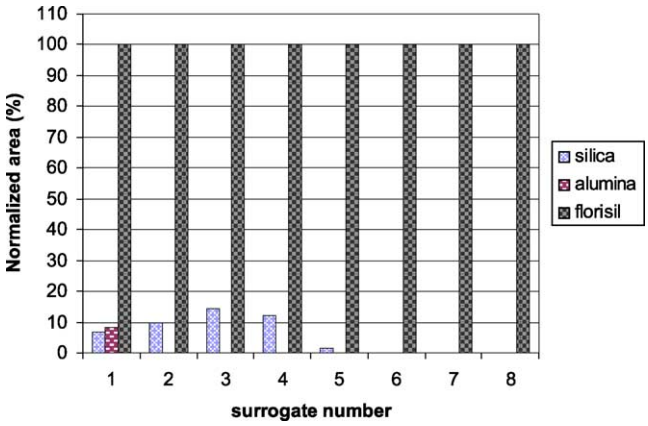

Fig. 2. Comparison of adsorbent performance in recovering [^{13}C]PCBs. 60 ng MBP-CG/g oil was used in the comparison study. *Surrogate number (*x*-axis) indicates the [^{13}C]PCBs (i.e. surrogate 1 = ^{13}C-monochlorobiphenyl; surrogate 2 = [^{13}C]dichlorobiphenyl and so on) used in the study.

Adsorption chromatography was chosen to be the intermediate step because of its simplicity and cost. From the experiment, it was found out that Florisil is the best adsorbent that can satisfactorily recover surrogates 1–8 (Fig. 2) as well as the mono- up to the octachlorobiphenyls (data not shown), hence, it was chosen to be used as the adsorbent throughout the experiment. Moreover, 6 g of adsorbent was found acceptable for the purpose. Weights lower than this gave oily residues that interfered with analyte detection.

An optimization procedure concerning the factors that affect SPME performance was carried out using spiked pure HPLC water. Two fibers were chosen for this purpose based on their data applications found in the literature [15,18,19]. From the investigation, PDMS–DVB was chosen over PDMS since the former gave higher responses with respect to the seven indicator PCBs at the extraction temperature investigated (not shown). This result was consistent with those found by Criado and Rodriguez [18].

Although agitation was recommended to establish rapid transfer of analytes, preliminary study showed that stirring did not significantly improve the transfer of analytes (data not shown). Moreover, use of magnetic stirrer was reported to cause cross contamination [19]. Hence, sample stirring was not carried out. Moreover, direct immersion of SPME fiber was not further evaluated since residues from the sample may cause severe damage to the fiber.

Optimum desorption condition was investigated by testing different injection depths and desorption times. The injection depth that gives the best peak response (area counts and peak shape) and the time for all analytes to desorb from the fiber with minimal carry-over were the criteria for the most suitable desorption conditions. An injection depth corresponding to number 3 in the SPME holder needle guide gauge and a desorption time of 3 min at 260 °C injector temperature gave the best result.

SPME extraction time was also studied using an aqueous solution of Aroclor 1260 (1 ng/mL). 60 min was not enough to achieve equilibrium. But for practical purposes, 40 min was chosen to have a compromise between sensitivity and analysis time. 50, 70, 90 and 100 °C were investigated as the extraction temperatures. Even though 100 °C gave the biggest analyte response, it was observed that droplets of water condenses at the SPME septum piercing needle and fiber giving inconsistent analysis results. For this reason, 90 °C was chosen on the basis of sensitivity and result precision.

The effect of salt addition was also evaluated. 0, 1, 2 and 6 g of NaCl was added to 20 mL of 1 ng/mL Aroclor 1260. One gram salt/20 mL PCB solution gave the best result. However, when this condition was applied to the fish oil eluate, low recoveries were observed. Non-addition of salt gave a better result in the case of the fish oil eluate.

GC–MS chromatograms showing the PCB congeners analyzed at optimimal conditions are shown in Fig. 3.

3.2. Method evaluation

After establishing the experimental procedures, evaluation of the SPME–GC–TOF-MS was carried out. Linearity, linear range, method detection limit and RSD were determined. Evaluation of the method was tested using the blank fish oil as the matrix. Two levels of calibrations were made (low and high) to cover all the analytes of concern. Isotope-labeled PCBs were used as internal standard in the level of 100 ng/g oil sample. The calibration curves (ratio of analyte peak area to internal standard peak area versus concentration) were linear over the tested concentration range with correlation coefficients (R) of 0.99 or better (Table 1). The method detection limit and RSD were evaluated using seven replicate analysis of spiked fish oil in the level of 20 and 100 ng/g for the low and high level, respectively. As can be seen from Table 1, although some RSDs were quite high (above 15%) these results are within the ranges observed using SPME [19]. The sum of method detection limits (MDLs) of the seven indicator PCBs were low enough to satisfy the tolerance level set by the European Union (200 ng/g), while the sum of MDLs for the 53 PCB congeners were enough to satisfy the tolerance level set by the FDA (2000 ng/g). In both cases, the proposed method will be highly relevant and useful.

Another advantage of this method is the separation of the mono-ortho PCBs and coplanar PCBs. Mono-ortho PCBs and coplanar PCB usually appeared as two peaks, that is, a pool of mono-ortho appearing as one peak and coplanar PCBs appearing as another single peak. From the results in Fig. 3, it was shown that PCB Nos. 126 (coplanar PCB) was successfully separated from PCB Nos. 114, 118, 123, 156, 157, 167 and 189 (mono-ortho PCBs). To separate mono-ortho PCB Nos. 118 and 156 from interfering compounds PCB Nos.149 and 171, respectively, an extensive sample preparation using Lipidex followed by fractionation is essential [7]. However, with the combination of simple SPME technique and GC–TOF-MS, PCB Nos. 118 and 156 were separated

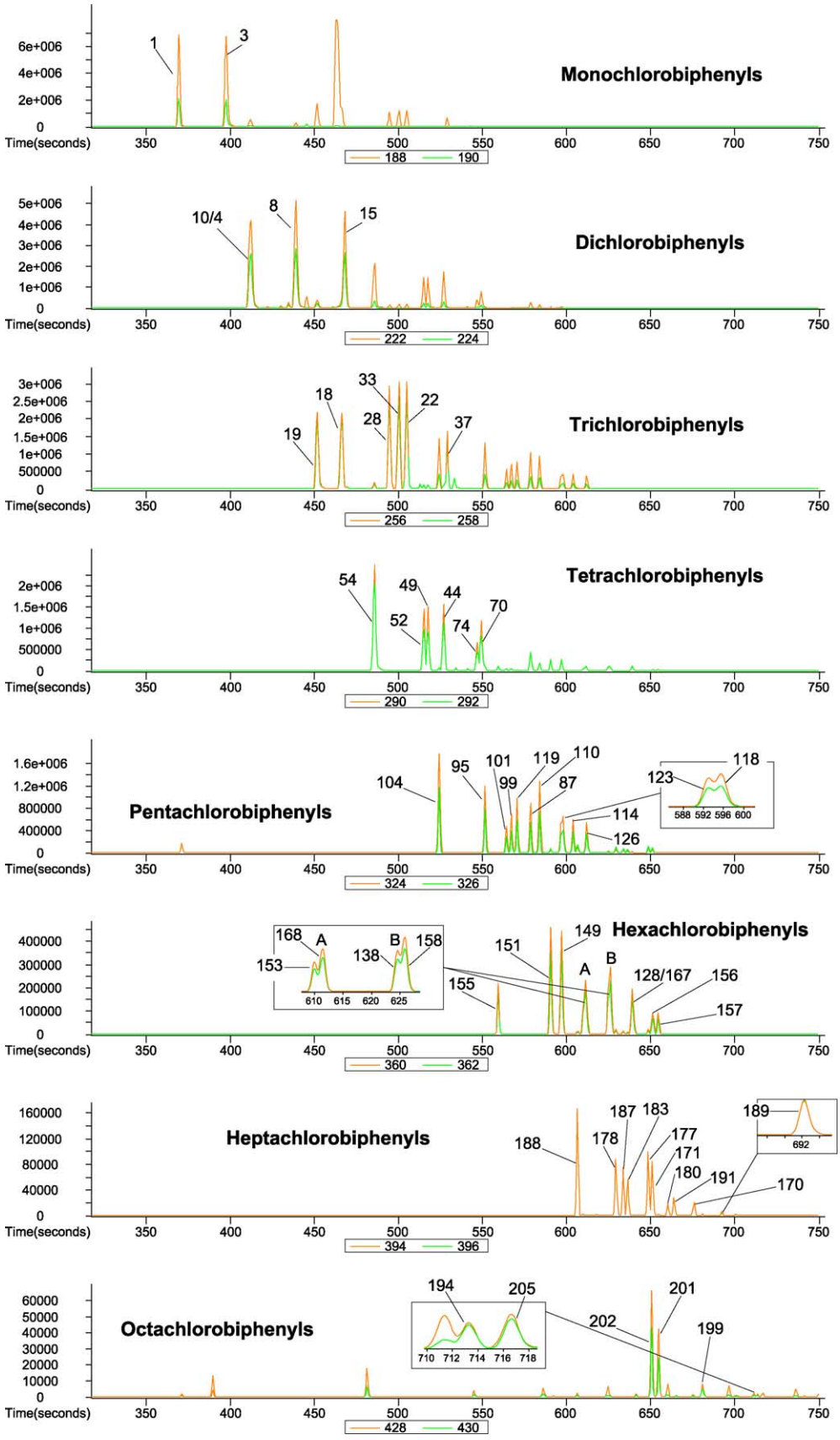

Fig. 3. PCB chromatograms of 500 ng/g oil using the optimized conditions. (——) quantification ion, (——) identification ion.

Table 1
Method validation data for the 53 PCB congeners

PCB congener number	Quantitation ion (*m*/*z*)	Correlation coefficient (*R*)	RSD (%)	MDL (ng/g) (*n* = 7)
1	Monochlorobiphenyl (*m*/*z* = 188)	0.9962	17	9
3		0.9982	2	0.8
10/4	Dichlorobiphenyl (*m*/*z* = 222)	0.9995	16	8
8		0.9996	25	11
15		0.9993	6	2
19	Trichlorobiphenyl (*m*/*z* = 256)	0.9998	22	8
18		0.9995	7	3
28*		0.9998	6	2
33		0.9999	6	3
22		0.9992	11	5
37		***0.9909***	***17***	***31***
54	Tetrachlorobiphenyl (*m*/*z* = 290)	0.9999	16	6
52*		0.9999	3	1
49		0.9999	5	2
44		0.9999	8	3
74		0.9981	15	5
70		0.9998	16	5
104	Pentachlorobiphenyl (*m*/*z* = 324)	***0.9984***	***35***	***23***
95		0.9901	25	14
101*		***0.9992***	***10***	***20***
99		0.9991	18	8
119		0.9994	17	8
87		0.9993	16	8
110		0.9998	16	8
123		0.9994	8	4
118*		***0.9999***	***5***	***10***
114		0.9990	6	3
126		0.9974	6	10
155	Hexachlorobiphenyl (*m*/*z* = 360)	0.9989	21	12
151		0.9994	13	6
149		0.9995	10	4
153*		0.9995	5	2
168		0.9993	9	3
138*		0.9980	10	19
158		***0.9993***	***5***	***8***
126/167		***0.9995***	***12***	***21***
156		0.9983	15	11
157		0.9968	14	11
188	Heptachlorobiphenyl (*m*/*z* = 394)	0.9999	18	11
178		0.9999	9	4
187		0.9999	9	4
183		0.9999	11	5
177		0.9999	11	4
171		0.9992	13	5
180*		***0.9982***	***2***	***4***
191		***0.9960***	***9***	***15***
170		***0.9952***	***7***	***11***
189		***0.9900***	***11***	***19***
202	Octachlorobiphenyl (*m*/*z* = 428)	0.9993	27	12
201		0.9928	22	12
199		***0.9912***	***1***	***26***
194		***0.9938***	***11***	***24***
205		***0.9999***	***13***	***31***

Data written with (*) indicate that they are part of the seven PCB indicators. Data written in bold, italics indicate that high level (*n* = 5) calibration curve was used (20–500 ng/g) otherwise, low level calibration (*n* = 4) was used (2–20 ng/g) for the determination of MDLs and RSDs. MDL = 1.943S.D.

Table 2
Recoveries of PCBs extracted from 0.2 g BCR-349 using the proposed method

Chlorobiphenyl IUPAC no.	Certified value (μg/kg)	Experimental results (μg/kg) ($n = 3$)	Recovery (%)	RSD (%) ($n = 3$)
28	68 ± 8	88	129	2.3
52	149 ± 21	153	102	1.7
101	372 ± 18	418	112	5.2
118	460 ± 40	408	89	0.29
153	940 ± 40	1054	112	1.7
180	282 ± 23	305	108	4.4

from PCB Nos. 149 and 171, respectively. Interferences especially from early eluting compounds can affect early eluting PCBs (such as PCB Nos. 28, 101 and 104). The extent of the interference from these peaks causes the large difference in detection limits between the two CB congeners (e.g. CB 101 and CB 114) with similar retention times and the same degree of chlorination. Interference with signals coming from an unidentified compound in milk sample was also reported to interfere with PCB 101 [14]. These problems can be solved by doing additional clean up procedures. This action would need considerable time, which will forfeit the objective of method rapidity, and so we decided to accept the data in favor of the rapidity of the method.

In order to complete the method validation, the proposed method should be used to test a certified reference material. BCR-349 was used for this purpose. One hundred nanograms of MBP-CG was spiked to 1 g of the reference material as internal standard. It was then analyzed following the proposed methodology. Results from the analysis conforms excellently with the certified data indicating that the developed method can be used reliably for the analysis of PCBs from fish oils (Table 2). But the concentration or CB28 was significantly higher than the certified value, this difference in concentrations is due to the interferences by coextratives eluting with early eluting PCBs (such as PCB Nos. 28, 101 and 104).

4. Conclusions

The successful development of the procedure based on SPME technique from the Florisil extract for the detection of PCBs in fish oils has been established. This procedure provided easy sample manipulation, less glassware consumption without sacrificing data accuracy and precision. However, internal calibration is recommended to overcome matrix to matrix influence in the SPME performance.

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