SFE as clean-up technique for ppt-levels of PCBs in fatty samples

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An off-line SFE method was developed for the supercritical fluid extraction (SFE) of polychlorinated biphenyls (PCBs) from fatty samples. The SFE method can be used for the analyses of large amounts of fats, rendering it suitable for samples with ppt-levels of PCB and other contaminants. Selective extraction of PCBs was achieved using supercritical CO_2 with a density of 0.65 g/mL (134 bar and 50 °C) and a flow of 4 mL/min. The dynamic extraction time is dependent on the amount of fat and the group of selected PCBs. In the case of SFE, PCBs in eel were found at levels 10 - 40 % higher than those found with conventional extraction techniques; for fish oils, levels of PCBs of 0.05 to 0.1 ng/g of fat could be determinated. The method was applied on the SFE extraction of ppt-levels of PCBs from total diet samples.

1. Introduction

The interest in PCBs and other contaminants in fat containing samples originates from the bio-accumulation of these components in the food chain and at last, in man. In the last decades, special attention was paid to the sensitivity of detection techniques and the chromatographic separation of PCBs. Because PCBs are found in the lipid fraction of the various samples, removal of the lipids is an important and laborious step in sample pretreatment.

Recently, the possibilities for selective extractions with SFE have been investigated. Several studies have reported methods for PCBs in fatty samples as fish and human tissue using on-line SFE-GC techniques[1,2,3] or off-line methods[4-8]. SFE conditions cannot be too strong to prevent co-extraction of lipids and typical densities are around 0.6 g/ml, except for Van Bavel et al.[5] and Lindström et al.[6], who use 0.9 g/ml. Samples were either freeze dried or mixed with sodium sulfate and subsequently mixed with - in most studies - (basic) alumina in different ratios as fat retainer. Because of the high mixing ratios from 1:6 to 1:20, and the relatively small sizes of extraction cells, the amount of fat which can be handled is only a few hundreds of mg and limits of determination will be enlarged. Hale[4] reported LODs of 0.1-0.5 ng/g of fish; on the other hand Lindström et al.[6] could detect ppt levels of PCDD/Fs in human tissue.

In this study, special attention was paid to the mixing ratio of the fatty sample and the fat retainer and the amount of fat to be handled in SFE was maximized; the influence of SFE parameters, especially the extraction time was investigated. The method was applied on the extraction of PCBs at low ppt levels from fish, fish oil and total diet.

2. Materials and methods

Samples
Blank herring samples were freeze dried and homogenized before extraction. Eel samples from the river Rhur in the Netherlands were homogenized and directly extracted. Refined fish oils were provided by the food industry.
The conventional clean-up procedure for eel samples consisted of two column chromatography steps on alumina 10 % de-activated and silica, after addition of the internal standards. All solvents used were HPLC-grade.

Supercritical fluid extraction
Supercritical fluid extractions were performed on a Hewlett Packard SFE 7680T (Wilmington, DE, USA) instrument using CO_2 (Hoek Loos 5.3, Amsterdam, The Netherlands) as supercritical fluid. Samples, mixed in different weight ratios with silica/$AgNO_3$ 10%, were placed in 7 ml extraction cells. Spikes with PCBs 28, 52, 101, 118, 153, 138 and 180 (at an absolute level of 30 and 75 ng) were added on top of the extraction cell to check retention behaviour of fat/adsorbent combinations. SFE parameters were optimized giving the following final SFE conditions: extractions were performed using a pressure of 134 bar at 50 °C resulting in a supercritical density of 0.65 g/ml with a flow of 4 ml/min, with a variable dynamic extraction time; all analytes were trapped on a solid trap packed with florisil (Merck, Darmstadt, Germany). The nozzle and trap temperature were 45 and 20 °C, respectively. Analytes were recovered by elution with 1.5 to 1.8 ml of hexane. Samples were concentrated or directly injected on GC-ECD.

Analysis
An HP 5890 gas chromatograph, equipped with an HP 7673A autosampler, an electron-capture detector (ECD), and an Ultra 2 (50m * 0.2 mm; 0.33 µm; HP) column was used for chromatographic separation, interfaced to an HP 3365 Chemstation (Hewlett Packard, Palo Alto, California). Helium was used as carrier gas (2 mL/min) and argon/methane as purge gas (60 mL/min). After injection of 3 µL, the temperature program consisted of an initial temperature of 80 °C in several steps to the final temperature of 290 °C. The injector temperature was 260 °C and detector temperature was 325 °C. Quantification was performed by comparison with an external standard mixture. Limits of determination for each component were 0.05 - 0.1 ng/g of fat depending on matrix and using the conditions specified above for sample preparation and analysis.

3. Results

Method development
Extraction parameters were chosen to achieve selective extraction of PCBs from the fat matrix by varying different SFE parameters, *i.e.* extraction time, pressure, temperature and flow resulting in different densities and swept volumes and the adsorbent for fat retaining (results will be published elsewhere). Especially the amount and type of fat determine the optimal fat:silica ratio. In Figure 1, chromatograms are shown of 1, 2 and 3 g of herring, which were spiked with PCBs, mixed with 1 g of silica/$AgNO_3$ and subsequently extracted with SFE. By using too high fat:silica ratios, GC-ECD chromatograms were heavily disturbed at the baseline with interferences and negative peaks, resulting in reduced recoveries of the individual PCBs. Especially the higher chlorinated PCBs were not fully extracted at higher amounts of fat. With the correct fat:silica ratio and SFE conditions, no additional clean-up is necessary for GC-ECD analysis.

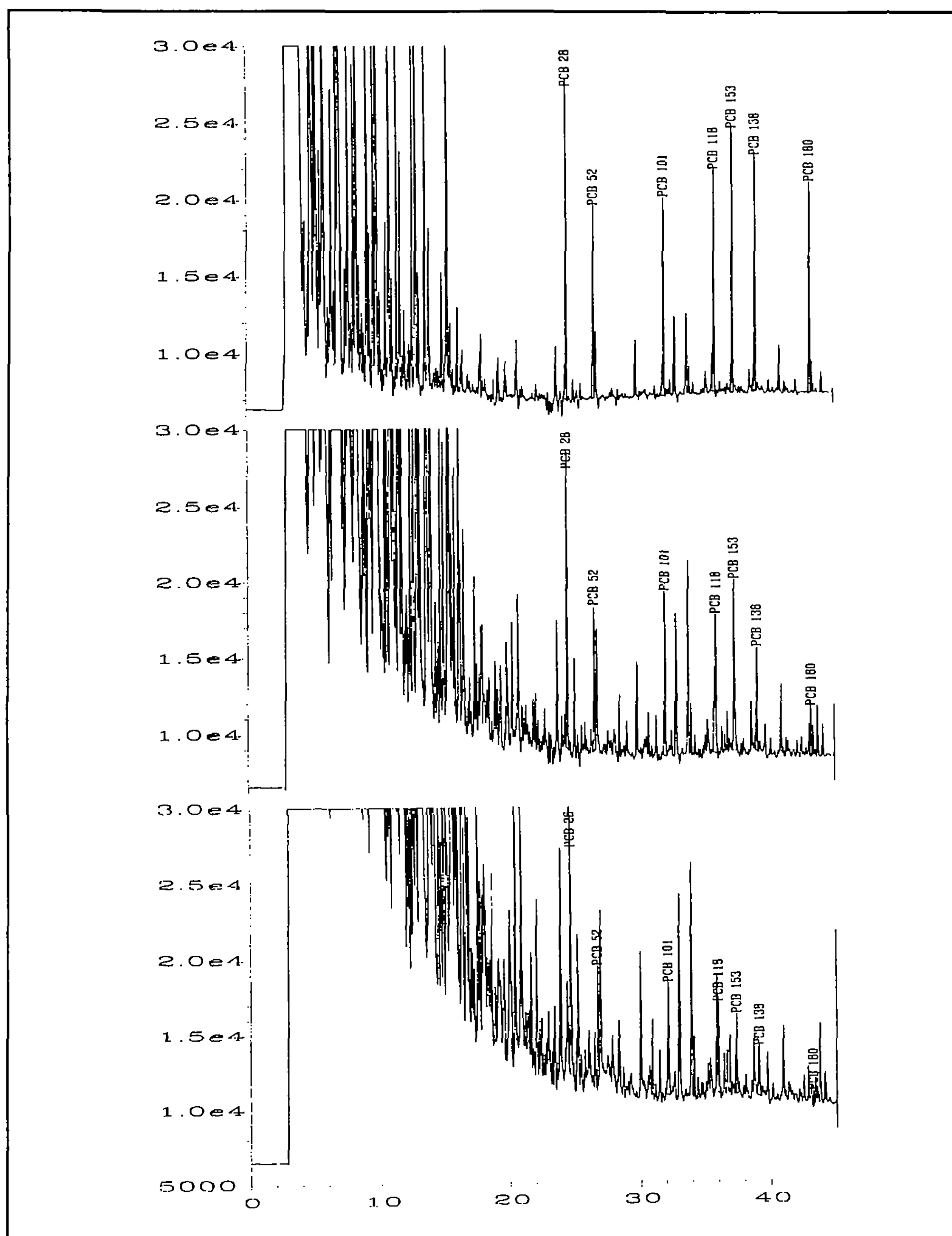

Figure 1. GC-ECD chromatograms of spiked herring mixed in different fat:silica/AgNO3 (w:w) ratios. Top) 1:1; middle) 2:1 and bottom) 3:1. SFE and GC-ECD conditions specified in materials and methods.

The SFE method was applicable up to 2 g of pure fish oil. In Figure 2, recoveries of PCBs are presented for 2 g of spiked fish oil, mixed with 3 g of silica/AgNO3 and extracted with increasing dynamic extraction times from 20 to 120 min. Higher chlorinated PCBs need longer extraction times to obtain sufficient recoveries. Retention of components appeared to depend on the amount of fat, and to a lesser extend on the amount of silica, *i.e.* the column length in the extraction cell.

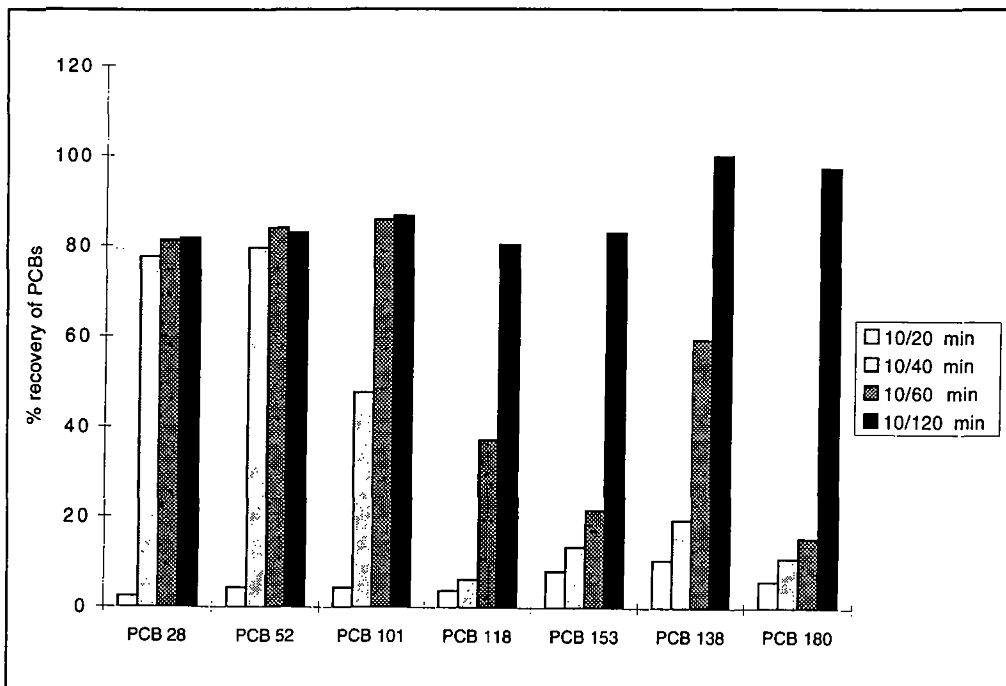

Figure 2. Recoveries of PCBs in 2 g of fish oil with increasing dynamic extraction times.

The presented method can be applied on fatty samples, as well as on pure fat or extracted fat from other matrices. The advantage of the latter is that also samples with ppt levels of organic contaminants - which otherwise cannot be extracted with SFE as a result of the small extraction cell - can be extracted and on-line cleaned from the lipid fraction using this method.

Applications

This method was applied on eel samples from the river Ruhr in The Netherlands, suspected to be contaminated with PCBs from several mining areas. Eel samples were mixed with hydromatrix to bind the water and with silica/AgNO3 (1:1:1) and subsequently dynamically extracted for 20 min. In Table 1, levels of PCBs in eel are compared for SFE and a conventional extraction technique. SFE yielded 10 - 40% higher levels of PCBs. RSDs were relatively high, probably as a result of inhomogeneity of the eel sample by insufficient mixing.

In native fish oils, levels of PCBs of 0.05 to 0.1 ng/g of fat could be determinated (results not shown here).

Table 1. Comparison of SFE with conventional extraction for PCBs in eel (in ng/g of fish (wet weight)).

PCBs	SFE extraction (in ng/g of fish (wet weight)					SFE (n=5)		convent. extr. (n=2)
	1	2	3	4	5	ng/g fish	RSD	ng/g fish
PCB 28	15	13	12	11	9	12	18	9
PCB 52	383	240	243	239	241	269	24	243
PCB 101	228	157	147	150	149	166	21	142
PCB 118	395	268	270	288	282	300	18	235
PCB 153	442	282	283	285	298	318	22	235
PCB 138	351	278	266	277	263	287	13	219
PCB 180	176	116	115	117	114	128	21	89

SFE conditions: 1 g of eel (13.9% of fat) mixed with hydromatrix and silica/$AgNO_3$ 10% (1:1:1); 20 min. dyn. extraction. Conventional extraction: see materials and methods.

In Figure 3, the GC-HRMS chromatogram of native PCBs in a total diet sample is shown; levels are between 1 and 10 ng/g of fat for the indicator PCBs to 0.1 and 1 ng/g of fat for the mono-ortho PCBs. A total diet sample, originating from a 24 h consumption study, contains in general 20 % of fat. For conventional techniques, 100 g of total diet (around 4 g of fat) has to be extracted and the lipid fraction is removed by a laborious alkaline saponification clean-up or other techniques. In this case, a few hundreds of mg of extracted fat from the total diet was extracted with SFE, concentrated to 50 µl and directly analyzed with GC-HRMS. Only the ratio fat:silica had to be adjusted to 1:5, to retain the different lipids in the extraction cell.

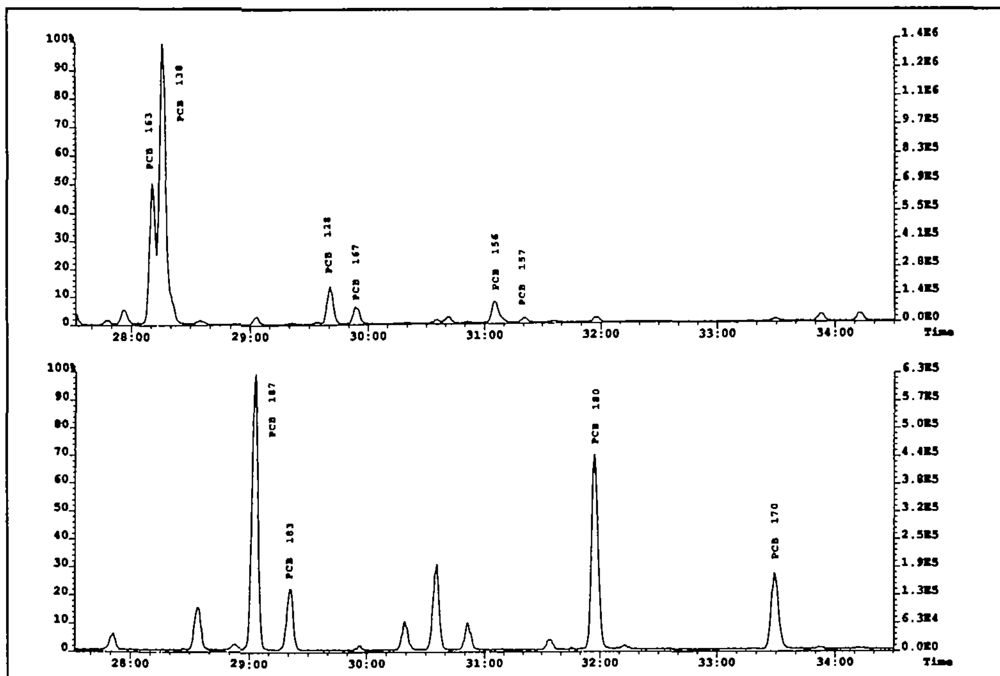

Figure 3. GC-HRMS chromatogram of PCBs in total diet. Upper trace) m/z 359.8 for hexa PCBs and lower trace) m/ z 393.8 for hepta PCBs.

4. References

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