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Journal of Chromatography A, 675 (1994) 189–204

JOURNAL OF CHROMATOGRAPHY A

Supercritical fluid extraction of polychlorinated biphenyls from lyophilized fish tissue

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(First received January 28th, 1994; revised manuscript received March 22nd, 1994)

Abstract

A method for the rapid interference free analysis of polychlorinated biphenyl congeners (chlorinated biphenyls, CBs) in lyophilized fish tissue is presented. The method was developed on a lyophilized tuna muscle tissue that contained 2.8% lipid (dry mass based), and native CB concentrations in the range of 3–84 ng/g. Sample preparation was made by supercritical fluid extraction using pure CO_2 as extraction fluid. Analysis by high-resolution gas chromatography–electron-capture detection analysis was carried out with on-column injection on two parallel coupled columns, a 60 m DB-17 column and a series combination of a 25 m SIL-8 column and a 25 m HT-5 column. Supercritical fluid extraction was compared with Soxhlet extraction and found to give quantitative recoveries, detection limits of 0.5–2 ng/g and standard deviations of less than 5% on average. The developed method was confirmed on nine different lyophilized fish samples which contained 6.1–26.5% lipid (dry mass based), and native CB concentrations in the range 0.8–134 ng/g.

1. Introduction

The analysis of polychlorinated biphenyls (PCBs) plays an important role in the monitoring of environmental contamination [1]. Because fish are the main source of PCBs in the diet, they constitute a key matrix in the monitoring of these compounds [2]. PCBs accumulate in the lipid (fat) fraction of the tissue and previous extraction procedures anticipate the lipids and the PCBs to be extracted together [3]. This, however, also creates the major problem associated with the analysis of lipid-containing samples *i.e.* the tedious separation of lipids from analytes of interest in order not to ruin the final determination. Lipid separation is normally performed by gel permeation or column chromatography using Florisil or alumina [3–5]. The lipids can be divided into different groups, ranging from non-polar to more polar. Some lipids are bound to the tissue while others form the group of "free lipids" [3].

The most common method for the extraction of PCBs from lipid-containing matrices is Soxhlet extraction applied with a mixture of polar and non-polar solvents. Another generally applied

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SSDI 0021-9673(94)00290-P

the PCBs with a light aliphatic hydrocarbon. This method is believed to give the most thorough extraction of PCBs from fatty tissues, especially when the ratio of bound to "free" lipids is high [3]. Unfortunately, saponification is rather laborious. Also, some chlorinated pesticides that are often determined simultaneously, are destroyed during saponification [3]. For this reason Soxhlet has been a more popular choice for extraction of PCBs from animal tissues. As long as Soxhlet extraction is carried out with a mixture of polar and non-polar solvents for an adequate amount of time, this method is also thought to give quantitative recovery of PCBs [3].

Supercritical fluid extraction (SFE) has become increasingly popular in the recent years, and the number of reports on applications for different analytes in diverse matrices is rapidly growing [6–10]. There has, however, been a lack of thoroughly tested SFE methods for the routine monitoring of PCBs in environmental matrices which could replace conventional procedures. This is evident, as most of the published data on SFE have been acquired on spiked samples. When applied to real contaminated samples, SFE was found to be much more difficult than initially thought [11,12]. Therefore further effort is still required in the method development of SFE. It has previously been demonstrated that SFE with solid-phase trapping has the potential of simultaneous extraction, clean-up and concentration of PCBs from different matrices [13–16]. Although somewhat more complicated than conventional trapping in SFE (cryo and liquid trapping), solid-phase trapping is highly efficient and seems well suited for automation and consequently for routine analysis.

SFE of fatty matrices has been carried out on a number of occasions [7,13,17–20]. But in several of these reports the purpose of the extraction has been to get a quantitative yield of lipids rather than the associated analytes. This would also in most cases give high recoveries of PCBs and other lipid-soluble compounds but has the drawback of necessitating a following clean-up step before the final analysis by gas chomatography (GC) with electron-capture detection (ECD) or mass spectrometry (MS). The application of SFE to the analysis of PCBs in fish has only been reported a few times [21–23], and until now never for quantitative analysis of tissues contaminated with native PCB congeners (CBs) at low ng/g levels.

The principal objective of the work presented here was to investigate the use of SFE with solid-phase trapping in the analysis of fish tissues contaminated with native CBs at levels down to a few ng/g. Because many biological samples today are lyophilized in order to facilitate storage, lyophilized fish tissues were to be used instead of raw tissues. A secondary aim was to develop a method suitable for routine usage necessitating a minimum of labour and time consumption.

2. Materials and methods

2.1. Chemicals

The CBs used in this study were obtained as neat crystals from the Community Bureau of Reference (BCR), Brussels, Belgium (IUPAC numbers 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170 and 180). The DDE and DDT standards were obtained from Supelco in a solution of known purity and concentration. All dilutions were made gravimetrically in isooctane.

The solvents used (acetone, *n*-hexane, *n*-heptane, isooctane and dichloromethane) were all pesticide grade (Merck, Darmstadt, Germany). The CO_2 and the methanol (MeOH)-modified CO_2 were all obtained as SFE/SFC grade from SIAD, Milan, Italy.

2.2. Fish samples

Nine fish samples (see Table 2) were collected at different sites of Lake Lugano. Depending on the size of the fish one or more were used in the following process. The edible parts of the fish were filleted, grinded with a meat grinder and lyophilized. The lyophilisation was done at 5°C for 48 h. After lyophilisation the dry muscle

tissue was ground in a mechanical grinding device (contact surfaces in ZrO_2) until a fine homogeneous powder was obtained. The powder was then sieved to remove remaining fibres, filled into sealed glass containers and stored at 5°C in darkness. In cases where more than one fish were used for one sample, dry mass and lipid content (based on dry mass) were calculated as an average (Table 2) [24].

The same procedure was used for the tuna fish (*Katsuwonus pelamis)* that was collected in the Mediterranean Sea. This fish had a dry mass of 18.5% and a lipid content of 2.8% (based on dry mass). This lyophilized tuna muscle tissue has been produced at the Environment Institute JRC Ispra, and a certification procedure for various elements and organic compounds is in course[a].

All lake fish (except perch III) were subjected to acid silica clean-up after SFE. This was essential because otherwise gradual column deterioration following on-column injection would occur. This step can be omitted if splitless injection is used instead of on-column. Extracts were loaded on a 10 cm × 3 mm column with activated silica impregnated with 40% (w/w) sulphuric acid (conc.) and eluted with 50 ml *n*-hexane. The eluent was evaporated and the residues were re-dissolved in 1.8 ml isooctane.

2.3. Supercritical fluid extraction

All the work presented was performed with a Hewlett-Packard 7680A supercritical fluid extractor. Fish extractions were prepared as follows: 2-g portions of lyophilized fish powder were mixed with 7 g of anhydrous Na_2SO_4 and packed into 7-ml extraction cells. In our experience on SFE with other types of matrices [14,15], pure CO_2 with a density of 0.75 g/ml (218 bar and 60°C) and a supercritical fluid flow of 1 ml/min normally gives high recoveries of PCBs with very little interfering compounds in the final *n*-heptane eluent. Therefore pure CO_2, without any kind of modifier, was chosen for the SFE to minimize the solubilisation of lipid from the lyophilized muscle tissues.

The method was developed on a tuna fish sample and the supercritical fluid extractions were compared using the following three sets of conditions:

COND-1: 30 min dynamic extraction with pure CO_2 at a density of 0.75 g/ml (218 bar) at 60°C with a flow of 1 ml/min.

COND-2: 10 min static extraction with pure CO_2 at a density of 0.75 g/ml (218 bar) at 60°C followed by 30 min dynamic extraction at the same density and temperature and with a flow of 1 ml/min.

COND-3: 10 min static extraction with pure CO_2 at a density of 0.75 g/ml (378 bar) at 97°C followed by 30 min dynamic extraction at the same density and temperature and with a flow of 1 ml/min.

The completeness of the extractions were examined using sequential extractions. Two different sequences were used:

SEQ-1: step A: identical to the one described under COND-2 above, followed by step B: 30 min dynamic extraction with CO_2 + 5% MeOH (same density, temperature and flow) followed by step C: 30 min dynamic extraction with pure CO_2 (density, temperature and flow as in COND-3).

SEQ-2: step A: identical to the one described under COND-3 above, followed by step B: 30 min dynamic extraction with CO_2 + 5% MeOH (same density, temperature and flow).

Finally the lakefish was analysed using the conditions COND-2 except for perch III, where the conditions COND-1 were used.

For all extractions the nozzle temperature was kept constant at 45°C and the trap was kept at a temperature of 20°C when pure CO_2 was used but 65°C when methanol was used as modifier [14].

The trap was filled with approximately 1 ml Florisil (0.16–0.25 mm particle size) as trapping material and was eluted with 2 × 1.5 ml *n*-heptane, then 1 × 1.5 ml dichloromethane followed by 2 × 1.5 ml *n*-heptane after the end of each individual extraction. A 50-μl volume of internal

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standard (PCB 35 and PCB 169, at 2.16 ng/μl and 0.43 ng/μl, respectively) was added to the individual fractions and the final volume was adjusted to 1.8 ml with *n*-heptane resulting in internal standard concentrations of *ca.* 60 pg/ml for PCB 35 and *ca.* 12 pg/ml for PCB 169.

2.4. Soxhlet extraction

Aliquots of 2 g of lyophilized fish powder were mixed with 7 g of anhydrous Na_2SO_4 and extracted with 250 ml of *n*-hexane–acetone (2:3) for 18 h. The solvents were evaporated on a rotary evaporator at 30°C, the residues were weighed (to determine the lipid content) and dissolved in 10 ml of *n*-hexane. Extracts were loaded on a 15 cm × 6 mm column with activated silica impregnated with 40% (w/w) sulphuric acid and eluted with 50 ml *n*-hexane. The eluent was evaporated and the residues were re-dissolved in 1.5 ml isooctane. Internal standards were added (PCB 35 and 169, as for the supercritical fluid extractions) and the final volume was adjusted to 1.8 ml with isooctane.

The extractions at the laboratory in IJmuiden were performed with 500 ml *n*-pentane–dichloromethane (1:1) for 12 h. Clean-up was carried out over 15 g alumina (6% water) and fractionation over 1.8 g silica (1.5% water) [25].

2.5. Dual-column gas chromatography

The extracts were analysed using a pressure-controlled Hewlett-Packard (HP) 5890 II gas chromatograph equipped with heatable on-column injector (run in oven track mode) and two ^{63}Ni electron-capture detectors held at 300°C [purged with 60 ml/min of argon–methane (90:10)] and a HP 7673A auto sampler.

Aliquots (1 μl) of the extracts were on-column injected on two parallel coupled columns, a 60 m × 0.25 mm, 0.25 μm 50% diphenyl-dimethyl-siloxane DB-17 column (J & W Scientific) and a series combination of a 25 m × 0.25 mm, 0.25 μm 5% diphenyl-dimethyl-siloxane SIL-8 column (Chrompack) and a 25 m × 0.22 mm, 0.10 μm 1,7-dicarba-closo-dodecarborane-dimethyl-polysiloxane HT-5 column (Scientific Glass Engineering). The columns were installed in the GC oven together with a deactivated 2 m × 0.53 mm fused-silica retention gap using a quick-seal glass "T".

The GC oven program was the following: initial temperature 90°C, retained for 2 min, then increased at a rate of 20°C/min to 170°C, retained for 7.5 min, then increasing at a rate of 3°C/min to 275°C retained for 10 min. Hydrogen linear velocity was approximately 43 cm/s, held constant by the pressure-controlled inlet throughout the whole temperature programme (starting pressure 1.7 atm at 90°C; 1 atm = 101 325 Pa). This choice of columns and GC conditions has previously been shown to give optimum separation of CBs and organochlorine pesticides [26].

Quantitative measurements of CBs and pesticides were performed using peak heights after a 7-point multilevel calibration curve (5-point for the pesticides) using the power fit calibration routine provided with the HP Chem 3365 software. CBs were calibrated in the concentration interval of 1.7 to 573 pg/μl where the intervals for the pesticides were 6.3 to 200 pg/μl (see Table 3 for the exact individual calibration range for the fish powder). Standards were injected after every fifth sample to determine deterioration of separation or drift. New calibrations were performed if the results for the standards drifted by more than 10%.

Chromatograms shown in Figs. 1–3 were performed on a 50 m × 0.22 mm, 0.25 μm 5% diphenyl-1,7-dicarba-closo-dodecarborane-dimethyl-polysiloxane HT-8 column (Scientific Glass Engineering) run under identical conditions as described above.

The GC analyses at the laboratory in IJmuiden were performed with a Perkin-Elmer 8320 gas chromatograph with splitless injection (injector temperature 270°C) and a ^{63}Ni electron-capture detector held at 360°C using 60 ml/min nitrogen as purge gas. The GC was equipped with a 50 m × 0.15 mm (0.30 μm film thickness) CP-Sil 19

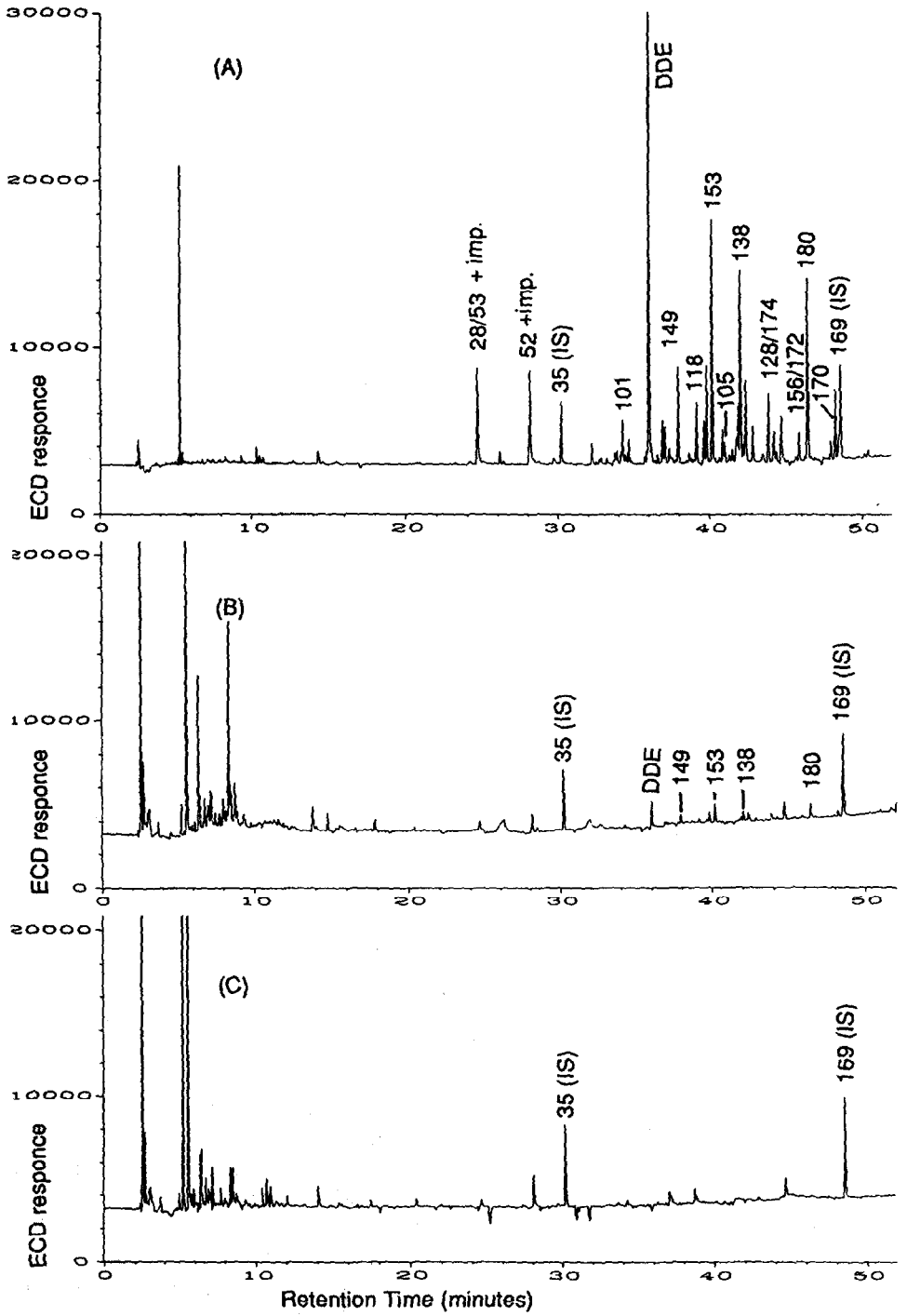

Fig. 1. GC–ECD chromatograms (HT-8) of sequential extractions (SEQ-1) of the lyophilized tuna muscle tissue: (A) 2 g of lyophilized tuna extracted by SFE with pure CO_2 (10 min static and 30 min dynamic, 0.75 g/ml, 218 atm, 60°C, 1 ml/min), (B) 30 min dynamic extraction with CO_2 + 5% MeOH of the tuna tissue already extracted in A (same SFE parameters as A), (C) 30 min dynamic extraction with pure CO_2 (0.75 g/ml, 378 atm, 97°C, 1 ml/min) of the tuna tissue already extracted in B.

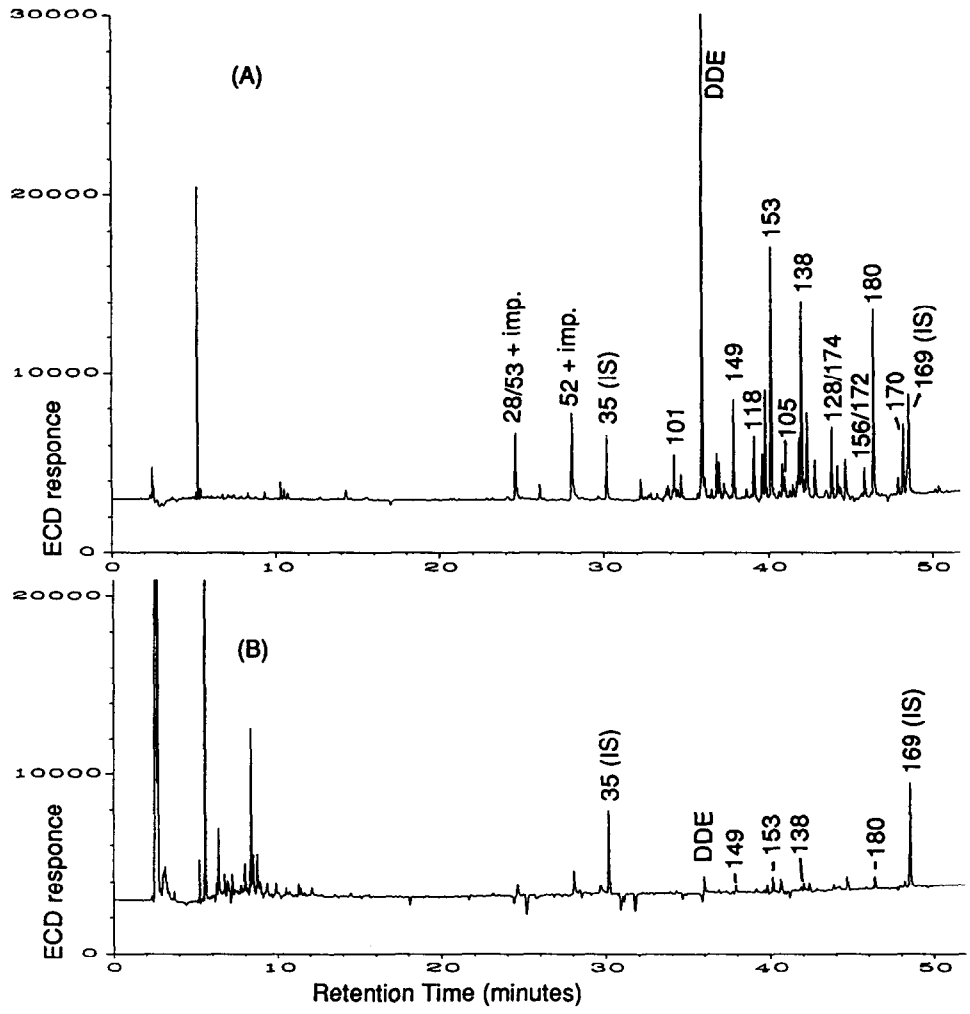

Fig. 2. GC–ECD chromatograms (HT-8) of sequential extractions (SEQ-2) of the lyophilized tuna muscle tissue: (A) 2 g of lyophilized tuna extracted by SFE with pure CO_2 (10 min static and 30 min dynamic, 0.75 g/ml, 378 atm, 97°C, 1 ml/min), (B) 30 min dynamic extraction with CO_2 + 5% MeOH of the tuna tissue already extracted in A (same SFE parameters as A).

column run with hydrogen as carrier gas at a linear gas velocity of 35 cm/s [27].

3. Results and discussion

3.1. Method development: SFE at different conditions compared with Soxhlet extraction

The developing experiments were carried out on a lyophilized tuna muscle tissue which was available in large quantities and was known to be contaminated with PCBs at easily detectable levels[a].

The supercritical fluid extractions were carried out under three rather similar conditions and the results compared with Soxhlet. The results are listed in Table 1. It can easily be seen that the fully dynamical extraction conditions (COND-1) give 10–25% lower recoveries than Soxhlet.

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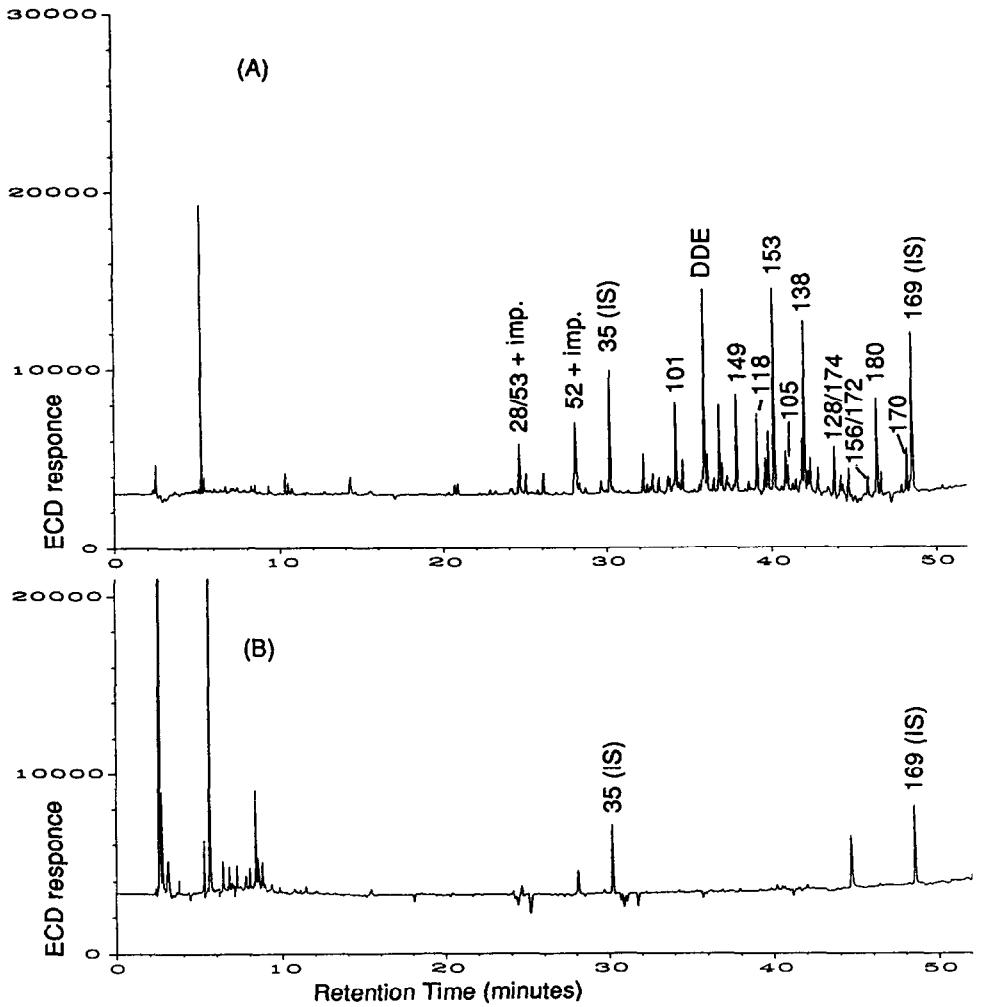

Fig. 3. GC–ECD chromatograms (HT-8) of sequential extractions (SEQ-2) of the lyophilized tench muscle tissue: (A) 2 g of lyophilized tench extracted by SFE with pure CO_2 (10 min static and 30 min dynamic, 0.75 g/ml, 378 atm, 97°C, 1 ml/min), (B) 30 min dynamic extraction with CO_2 + 5% MeOH of the tench tissue already extracted in A (same SFE parameters as A).

However by adding a 10-min static extraction step (COND-2), values virtually identical to the Soxhlet values were obtained. In the third set of experiments the temperature was raised to 97°C while keeping the density constant (COND-3). The reason for this was that it has been reported that extraction at higher temperature rather than higher density results in a more exhaustive extraction [11,28]. Also in our case we have higher recoveries of CBs, but the differences are generally too low to be significant. One drawback to the extraction at high temperature, however, was that the eluents contained interfering compounds in concentrations too high to inject directly for GC–ECD without a previous clean-up. Using these conditions results in the loss of easy automatization contrary to the extraction at lower temperature (60°C) where additional clean-up is not necessary.

From Table 1, it can also be seen, that the standard deviations for extractions performed at COND-1 and COND-3 are significantly higher than for COND-2 and Soxhlet which are of the same magnitude. For the data under COND-1

Table 1
Comparison of Soxhlet extraction with SFE using different extraction conditions for a tuna muscle tissue

PCB	SFE, COND-1		SFE, COND-2		SFE, COND-3		Soxhlet	
	Mean (ng/g dry mass)	S.D. (ng/g)	Mean (ng/g dry mass)	S.D. (ng/g)	Mean (ng/g dry mass)	S.D. (ng/g)	Mean (ng/g dry mass)	S.D. (ng/g)
28	<1	–	<1	–	<1	–	<1	–
52	2.1	0.5	3.1	0.1	3.4	0.2	3.1	0.1
101	12.7	1.3	16.9	0.3	18.6	1.1	16.9	0.2
105	6.8	0.3	6.9	0.1	7.7	0.2	7.2	0.3
118	20.3	1.6	23.5	0.4	23.4	0.8	23.4	0.3
128	6.3	0.3	7.1	0.2	7.8	0.2	7.1	0.1
138	59.1	3.3	62.0	1.4	58.8	2.1	62.4	1.4
149	25.5	1.7	34.4	1.1	36.5	1.3	33.1	0.9
153	67.0	4.2	84.0	2.2	83.1	3.1	79.6	2.6
156	4.6	0.3	4.5	0.1	4.9	0.3	4.4	0.1
170	9.7	0.5	12.0	0.4	13.7	0.2	11.5	0.2
180	41.7	1.7	50.2	1.0	50.9	1.7	49.0	0.7

COND-1 = 30 min dynamic extraction with carbon dioxide at 60°C, without clean-up (four replicates); COND-2 = 10 min static and 30 min dynamic extraction with carbon dioxide at 60°C, without clean-up (four replicates); COND-3 = 10 min static and 30 min dynamic extraction with carbon dioxide at 97°C, with clean-up (five replicates); Soxhlet = 18 h with 250 ml hexane–acetone (2:3), with clean-up (two replicates). S.D. = Standard deviation.

this is probably explained by an incomplete extraction, while for the data under COND-3 the more complex sample handling together with the larger amount of co-extracted compounds (lipids etc.) possibly leads to higher standard deviations.

3.2. Method development: effect of sequential extractions

In the past couple of years a number of results have been published proving that Soxhlet extraction does not necessarily give exhaustive extraction even at optimized conditions [11,29]. Recently a three-step method for the validation of a quantitative SFE method has been proposed [28]:

(1) Determination of the recovery with known concentrations of spiked compounds.

(2) Comparison of the recoveries of native analytes with those achieved using conventionally accepted extraction methods (including the use of standard reference materials).

(3) Performing multiple sequential extractions of the same sample with increasingly stronger extraction conditions.

The present SFE method (COND-2) was validated according to this three-step approach:

(1) For the system used in these experiments, studies on spiked samples demonstrating the trapping ability as well as on a certified reference material (CRM 392, sewage sludge) have been published elsewhere [14,15].

(2) The SFE method was compared with Soxhlet extraction (Table 1).

(3) Two different sequential extractions were conducted on the tuna fish.

The chromatograms resulting from the sequential extractions together with the different parameters used can be seen in Figs. 1 and 2. It is apparent that the first extraction, both at 60°C (SEQ-1) and 97°C (SEQ-2), is not completely exhaustive. In both cases the second extraction with CO_2 + 5% MeOH (known to increase extraction efficiency for most target analytes [8–12]) releases 5–8% additional CBs together with impurities and lipids. These findings demonstrate that relying on a single technique for validation of a method can result in misleading conclusions. The last extraction with pure CO_2 (Fig. 1C) at 97°C only extracts additional impurities and

lipids with no trace of PCBs. The conclusion is that it is only possible to talk about quantitative or near-quantitative recovery if a sequential extraction with increasing strength of extraction has been performed (this does not only apply to SFE but also to other methods).

When sequential extractions were performed on a fish with higher lipid content, however, no additional extractable PCB was observed. Fig. 3 shows the sequential extraction for the tench that has a lipid content of 9.4% (based on dry mass) —more than three times greater than the tuna fish (2.8%). As the bound lipid content in fish is normally around 2.5% [3], the ratio of bound to free lipid for the tuna is very high which makes this fish quite difficult to analyse in comparison with other more-lipid-containing fish. Given that the difference in extraction efficiency for COND-2 and COND-3 is almost negligible, and since the eluates from COND-2 are considerably cleaner, it was decided to use the parameters in COND-2 for method confirmation on nine different lake fish.

3.3. Method confirmation using unknown lyophilized fish samples

The developed SFE method was applied to nine different lyophilized fish sampled in Lake Lugano in the course of a larger survey [24]. The fish investigated are listed in Table 2 together with their respective dry mass, lipid content (based on the dry mass) and the total number of fish used for lyophilization. The fish were selected to represent the largest possible differences in dry mass and in lipid content. With this selection it was hoped also to find large differences in the contents of PCBs, DDT, DDE and DDD between the different fish species and in this way to provide the strictest possible test for the proposed method. As a comparison Soxhlet extraction for 18 h with 250 ml hexane–acetone (2:3) was selected, which was also used for the tuna fish.

During the analysis of the selected fish it was realised that there was a difference using the SFE method on lean fish and on fat fish. For the lean fish such as the tuna no problems concerning lipids in the eluent were experienced. But as the lipid content of the fish increased also the lipid content in the eluent increased. Even if the eluents only contained relatively small amounts of lipids, their prolonged injection (on-column) in GC–ECD were leading to a gradual deteriorating of the resolution of the columns and necessitating a replacement of the retention gap. Because no GC–ECD with split–splitless injection was available (that would minimize the problem substantially), it was decided to do a fast clean-up over acid silica (40% sulphuric acid) for all the lake fish concerned, except for the leanest fish (perch III) that was also ex-

Table 2
Details on the nine fish used for method confirmation

Fish	Dry mass (%)	Fat content (%)[a]	No. of fish used[b]
Bleak (*Alburnus alburnus*)	23.8	26.5	30
Largemouth bass (*Micropterus salmoides*)	22.3	17.0	4
Carp (*Cyprinius carpio*)	21.8	16.1	1
Burbot (*Lota lota*)	18.6	11.8	8
Pike perch (*Stizostedion lucioperca*)	25.0	9.7	1
Tench (*Tinca tinca*)	30.2	9.4	1
Perch I (*Perca fluviatilis*)	21.9	8.9	11
Perch II (*Perca fluviatilis*)	20.1	8.2	8
Perch III (*Perca fluviatilis*)	22.0	6.1	11

[a] Calculation of fat content is based on the dry mass.
[b] The number of fish used for lyophilization in the pooled samples.

tracted without the static step of 10 min. We did, however, analyse some of the extracts before clean-up by GC–MS with split–splitless injection without detecting any effect on the resolution of the column. To take full advantage of the method it is therefore recommendable to use splitless injection for routine analysis of lyophilized fish samples with lipid contents higher than 8–10%.

Table 3 shows the calibrated range for the lyophilized fish tissue according to the specifications in the Materials and methods section together with the detection limits for the method and the column used for quantification. The detection limits were established as the lowest amount of CBs as well as DDT, DDE and DDD detected in the lyophilized muscle tissue samples giving a signal-to-noise ratio greater than 10. No attempt was made to concentrate the extracts further, which means that a detection limit of 2 ng/g dry mass corresponds to an injected amount of approximately 1 pg/compound that is split (1:1) on the two columns. In environmental samples it is not likely that concentrations below our detection limits may cause any concern for the CBs considered in this study.

Generally, there was a good agreement between quantifications on the two different columns used. The choice of what congener to be quantified on which column was based on the knowledge of possible co-elutions [30] (Table 3). In a few cases where obvious interferences were encountered, the lowest results of the two columns were accepted as the results closest to the true value.

In Table 4 the quantitative results of the SFE are listed together with the values from Soxhlet extraction for comparison. The highest amounts of pollutants were generally found in largemouth bass with up to 134 ng/g of CB 153 and 129 ng/g of DDE, whereas the lowest amounts were found in burbut with down to 0.77 ng/g of CB 156 and less than 2 ng/g of DDT. The large dynamic range of concentrations for which the method works satisfactory is worth noting. The relative standard deviations are on average around 5%, lowest with 1–2% for the highest concentrations and highest with approximately 10% for the concentrations close to the detection limits. The relative standard deviations for the SFE experiments are generally of the same

Table 3
Analytical details on the method used for method confirmation

PCB	Calibrated range (ng/g dry mass)[a]	Detection limit (ng/g dry mass)[b]	Column used for quantitation[c]
28	2.8–300	1.5	SIL-8–HT-5
52	4.8–516	1.5	SIL-8–HT-5
101	3.4–366	1	SIL-8–HT-5
105	1.7–183	1	DB-17
118	2.0–216	1.5	SIL-8–HT-5
128	1.7–183	0.5	SIL-8–HT-5
138	2.2–236	1	DB-17
149	2.7–293	1	SIL-8–HT-5
153	1.9–210	1	SIL-8–HT-5
156	1.4–150	0.5	SIL-8–HT-5
170	1.7–181	1	SIL-8–HT-5
180	1.6–168	1	SIL-8–HT-5
DDE	5.9–183	2	SIL-8–HT-5
DDD	5.9–183	2	DB-17
DDT	5.9–183	2	SIL-8–HT-5

[a] The calibrated range for the fish powder according to the specifications in the Materials and methods section.
[b] Detection limits are the lowest amounts of CBs, DDT, DDE and DDD giving a $S/N > 10$.
[c] In general values quantified on the listed columns were used; when the other column was giving smaller values for one type of fish values from this column were used.

Table 4
Comparison of Soxhlet and SFE on nine fish samples

Fish	PCB (ng/g dry mass)	Soxhlet (mean ± S.D.), (ng/g dry mass)	SFE, COND-2 (mean ± S.D.), (ng/g dry mass)	Relative recovery (Soxhlet = 100) (%)
Bleak	28	4.0 ± 0.2	4.6 ± 0.1	114
	52	12.6 ± 0.9	15.3 ± 0.8	122
	101	30.7 ± 1.8	37.0 ± 1.2	120
	105	7.1 ± 0.3	8.1 ± 0.4	114
	118	24.3 ± 0.9	27.4 ± 1.0	113
	128	5.3 ± 0.3	6.0 ± 0.3	113
	138	34.7 ± 0.3	38.4 ± 0.8	111
	149	28.0 ± 1.9	33.8 ± 1.5	121
	153	44.9 ± 3.1	51.7 ± 1.8	115
	156	2.7 ± 0.1	3.2 ± 0.2	118
	170	4.9 ± 0.4	5.4 ± 0.2	111
	180	21.4 ± 0.6	23.2 ± 0.7	108
	DDE	82.5 ± 4.6	89.5 ± 2.8	108
	DDD	12.0 ± 0.8	13.1 ± 0.8	108
	DDT	3.6 ± 0.2	4.0 ± 0.2	110
Largemouth bass	28	5.7 ± 0.3	5.6 ± 0.2	98
	52	19.1 ± 0.3	19.1 ± 0.3	100
	101	62.1 ± 1.3	64.6 ± 1.6	104
	105	15.0 ± 0.7	16.2 ± 0.7	108
	118	52.5 ± 2.1	55.8 ± 1.7	106
	128	12.1 ± 0.6	13.1 ± 0.1	108
	138	98.6 ± 4.5	107 ± 4.6	108
	149	59.7 ± 1.4	62.6 ± 1.5	105
	153	126 ± 5.1	134 ± 3.8	106
	156	7.7 ± 0.2	8.0 ± 0.1	104
	170	14.9 ± 0.8	15.6 ± 0.3	105
	180	68.5 ± 3.6	74.7 ± 1.2	109
	DDE	129 ± 7.2	141 ± 6.4	110
	DDD	10.0 ± 0.2	18.1 ± 0.9	181
	DDT	12.2 ± 0.7	14.2 ± 0.5	116
Carp	28	3.4 ± 0.4	3.7 ± 0.1	107
	52	11.4 ± 1.3	12.0 ± 0.2	105
	101	24.0 ± 2.6	25.1 ± 0.7	104
	105	4.5 ± 0.5	5.0 ± 0.1	111
	118	20.2 ± 2.3	20.7 ± 0.4	102
	128	4.3 ± 0.5	4.6 ± 0.1	105
	138	34.8 ± 3.8	39.9 ± 0.6	115
	149	21.9 ± 2.2	22.2 ± 0.8	101
	153	43.8 ± 4.5	45.1 ± 0.6	103
	156	2.8 ± 0.3	3.3 ± 0.2	115
	170	5.9 ± 0.5	6.1 ± 0.1	104
	180	25.9 ± 2.7	27.9 ± 0.3	108
	DDE	43.8 ± 4.0	43.6 ± 0.6	100
	DDD	7.4 ± 1.0	8.1 ± 0.2	110
	DDT	3.7 ± 0.5	3.8 ± 0.1	103

(*Continued on page 200*)

Table 4 (*continued*)

Fish	PCB (ng/g dry mass)	Soxhlet (mean ± S.D.), (ng/g dry mass)	SFE, COND-2 (mean ± S.D.), (ng/g dry mass)	Relative recovery (Soxhlet = 100) (%)
Burbot	28	< 1	< 1	–
	52	2.4 ± 0.1	2.5 ± 0.2	104
	101	4.7 ± 0.2	4.7 ± 0.2	100
	105	1.4 ± 0.1	1.4 ± 0.1	100
	118	4.4 ± 0.4	4.2 ± 0.2	96
	128	1.2 ± 0.0	1.2 ± 0.1	98
	138	6.4 ± 0.1	7.0 ± 0.2	108
	149	2.7 ± 0.2	2.9 ± 0.1	108
	153	9.0 ± 0.3	9.0 ± 0.2	100
	156	0.8 ± 0.1	0.8 ± 0.1	103
	170	1.4 ± 0.0	1.4 ± 0.1	106
	180	4.7 ± 0.1	4.8 ± 0.1	102
	DDE	14.2 ± 0.2	13.3 ± 0.3	94
	DDD	2.7 ± 0.1	2.5 ± 0.1	93
	DDT	< 2	< 2	–
Pike perch	28	2.5 ± 0.1	2.6 ± 0.1	100
	52	7.1 ± 0.4	7.2 ± 0.4	102
	101	18.8 ± 0.8	19.2 ± 0.5	102
	105	4.5 ± 0.1	4.4 ± 0.1	99
	118	14.3 ± 0.3	15.2 ± 0.6	106
	128	3.4 ± 0.1	3.5 ± 0.1	103
	138	21.3 ± 0.1	22.6 ± 0.5	106
	149	16.5 ± 1.0	17.5 ± 0.5	106
	153	29.7 ± 1.2	31.1 ± 0.7	105
	156	1.7 ± 0.1	1.7 ± 0.1	102
	170	3.4 ± 0.2	3.7 ± 0.1	108
	180	13.5 ± 0.3	13.9 ± 0.3	103
	DDE	47.7 ± 1.6	49.9 ± 0.7	105
	DDD	6.6 ± 0.2	6.8 ± 0.1	103
	DDT	3.0 ± 0.1	3.4 ± 0.2	111
Tench	28	3.1 ± 0.2	3.1 ± 0.1	99
	52	9.5 ± 0.1	10.8 ± 0.2	114
	101	31.9 ± 1.5	36.4 ± 0.6	114
	105	7.2 ± 0.4	8.1 ± 0.2	113
	118	27.8 ± 1.1	30.4 ± 0.4	109
	128	6.4 ± 0.2	7.1 ± 0.1	111
	138	52.2 ± 2.1	59.8 ± 1.4	114
	149	28.8 ± 1.1	33.6 ± 0.5	117
	153	62.6 ± 2.6	68.9 ± 0.8	110
	156	4.0 ± 0.2	4.6 ± 0.1	116
	170	7.6 ± 0.3	8.6 ± 0.1	114
	180	32.3 ± 0.9	36.6 ± 0.8	113
	DDE	65.0 ± 2.2	70.4 ± 1.4	108
	DDD	6.7 ± 0.9	11.1 ± 0.2	166
	DDT	12.6 ± 0.3	15.2 ± 0.2	120

Table 4 (*continued*)

Fish	PCB	Soxhlet (mean ± S.D.), (ng/g dry mass)	SFE, COND-2 (mean ± S.D.), (ng/g dry mass)	Relative recovery (Soxhlet = 100) (%)
Perch I	28	3.0 ± 0.1	3.2 ± 0.1	106
	52	8.1 ± 0.1	9.6 ± 0.4	118
	101	29.7 ± 0.7	34.0 ± 1.0	115
	105	6.6 ± 0.1	7.3 ± 0.2	110
	118	26.1 ± 0.8	28.2 ± 0.1	108
	128	6.4 ± 0.1	6.9 ± 0.2	108
	138	54.8 ± 1.3	59.5 ± 1.7	109
	149	28.7 ± 0.4	33.6 ± 1.1	117
	153	62.6 ± 1.2	70.7 ± 2.0	113
	156	4.1 ± 0.1	4.7 ± 0.0	113
	170	8.0 ± 0.2	8.8 ± 0.4	110
	180	33.8 ± 0.9	36.8 ± 0.9	109
	DDE	62.5 ± 2.4	64.9 ± 1.6	104
	DDD	8.9 ± 0.1	10.4 ± 0.2	117
	DDT	11.2 ± 0.1	13.1 ± 0.2	117
Perch II	28	2.0 ± 0.1	2.0 ± 0.2	99
	52	7.3 ± 1.0	8.0 ± 0.4	110
	101	23.2 ± 2.6	26.0 ± 1.3	112
	105	5.1 ± 0.1	5.8 ± 0.4	114
	118	18.9 ± 1.4	21.2 ± 1.0	112
	128	4.8 ± 0.4	5.3 ± 0.2	110
	138	33.6 ± 0.6	40.9 ± 1.8	122
	149	23.5 ± 3.0	26.8 ± 1.2	114
	153	43.8 ± 4.7	48.9 ± 2.4	112
	156	2.6 ± 0.1	3.1 ± 0.1	117
	170	5.5 ± 0.6	6.3 ± 0.3	113
	180	22.6 ± 1.7	25.7 ± 1.0	114
	DDE	47.6 ± 3.6	51.9 ± 1.9	109
	DDD	9.6 ± 0.1	10.7 ± 0.7	111
	DDT	15.4 ± 1.2	17.8 ± 0.8	116
Perch III	28	1.7 ± 0.1	1.6 ± 0.1	96
	52	5.2 ± 0.2	4.8 ± 1.5	93
	101	16.2 ± 0.1	15.3 ± 3.2	94
	105	4.1 ± 0.1	4.1 ± 0.3	100
	118	14.2 ± 0.2	15.4 ± 1.5	108
	128	3.4 ± 0.1	3.6 ± 0.2	104
	138	27.2 ± 0.4	26.8 ± 0.9	99
	149	17.5 ± 0.4	17.6 ± 1.7	100
	153	31.2 ± 0.9	28.5 ± 3.4	91
	156	2.1 ± 0.1	2.1 ± 0.3	97
	170	4.2 ± 0.3	3.8 ± 0.3	89
	180	16.4 ± 0.6	15.9 ± 0.6	97
	DDE	30.4 ± 0.1	27.2 ± 2.7	89
	DDD	8.0 ± 0.2	8.5 ± 0.6	106
	DDT	6.0 ± 0.6	6.0 ± 0.2	101

COND-1 = 30 min dynamic extraction with carbon dioxide at 60°C, without clean-up (four replicates); COND-2 = 10 min static and 30 min dynamic extraction with carbon dioxide at 60°C, with clean-up (four replicates); Soxhlet = 18 h with 250 ml hexane–acetone (2:3), with clean-up (two replicates).

magnitude as for the Soxhlet extractions with a tendency to be a little smaller. The recovery ranges from 89 to 181% (in comparison with Soxhlet). The obvious outliers with a DDD recovery of 181 and 166% for largemouth bass and burbut most probably derives from the acid silica clean-up of the Soxhlet extracts. Usually the CBs elute first followed by DDE, DDT and finally DDD when silica clean-up is performed with a non-polar hydrocarbon as eluent [2]. It is likely that an elution volume of only 50 ml in some cases is to small for complete recovery of DDD and DDT from the larger amounts of acid silica necessary for the clean-up of the Soxhlet extracts.

The average recoveries of the SFE experiment in comparison with Soxhlet point to a significant higher recovery for SFE when performed with the COND-2 parameters. This means that the Soxhlet extractions under the given conditions are not quantitative for all the fish investigated. Furthermore, there does not seem to be any obvious reason for some of the fish giving higher recovery with SFE, because no correlation with either the relative dry mass or the lipid content is visible. Natural variations from one fish to another could be the reason. Comparing the average recoveries for perch III (lipid content 6.1%; extracted with COND-1) with the values for the other fish (extracted with COND-2), it is obvious that there is a difference. This difference, however, is not as large as should be expected from the data comparison on the tuna fish extractions. This could mean that the conditions required to extract PCBs with SFE from fatty fish are somewhat milder than conditions for lean fish.

3.4. Independent determination of PCB levels in tuna and tench

Quantitative determinations from analysis of the same sample with different methods are usually closer when performed by the same laboratory than those obtained from different laboratories. Evidently, this is because methods, calibration standards, GC system, injection technique and column choice vary between different laboratories. In order to have a final test of the present method, an extra independent analysis of the tuna and the tench was conducted in the laboratory of the Institute in IJmuiden. Independent means that the laboratory in IJmuiden was using their normal equipment and CB standards for the analysis without knowing the concentrations of individual CBs.

The result can be seen in Table 5. Overall, there is a good agreement between the results of the two laboratories. For some CBs, the values from Soxhlet 2 (IJmuiden) are a little higher than both Soxhlet 1 (Ispra) and SFE (COND-2). However, the difference is not significant, as the Soxhlet 2 analyses were performed on a single GC column (CP-Sil 19) [27,31], while the other analyses were performed on a dual-column system, from which the lowest value was selected to eliminate the possibility of interference. International intercomparisons on PCB analysis show a mean error of 10–15% [32,33]. Seen in this light, the comparison especially between SFE and Soxhlet 2 seems very reasonable.

4. Conclusions

Off-line SFE and GC–ECD (with the right choice of extraction parameters, GC injector and GC columns) has the potential of performing interference free congener specific analysis of native PCBs and related compounds on a routine basis in lyophilized fish tissues without the use of any manual work-up between extraction and GC analysis. With this combination of techniques the time ($\leq$2 h) and labour requirements can be reduced by nearly an order of magnitude in comparison with conventional methods for low ng/g levels of native pollutants. Also the analysis can be performed without losing accuracy and precision, because SFE in this respect is at least as good as Soxhlet extraction. This study demonstrates the importance of sequential extractions in the validation of quantitative SFE methods in addition to comparison with conventional procedures. Finally this study points to the need for sufficient similarity in sample composition when performing method development of a

Table 5
Comparison of SFE with Soxhlet extraction using different and independent methods

PCB	Tuna					Tench				
	SFE, COND-2		Soxhlet 1		Soxhlet 2	SFE, COND-2		Soxhlet 1		Soxhlet 2
	Mean (ng/g dry mass)	S.D. (ng/g)	Mean (ng/g dry mass)	S.D. (ng/g)	Single experiment (ng/g dry mass)	Mean (ng/g dry mass)	S.D. (ng/g)	Mean (ng/g dry mass)	S.D. (ng/g)	Single experiment (ng/g dry mass)
28	<1	–	<1	–	<1	3.1	0.1	3.1	0.2	4.1
52	3.1	0.1	3.1	0.1	3.0	10.8	0.2	9.5	0.1	11
101	16.9	0.3	16.9	0.2	22	36.4	0.6	31.9	1.5	38
105	6.9	0.1	7.2	0.3	8.8	8.1	0.2	7.2	0.4	11
118	23.5	0.4	23.4	0.3	26	30.4	0.4	27.8	1.1	32
128	7.1	0.2	7.1	0.1	8.9	7.1	0.1	6.4	0.2	8.6
138	62	1.4	62.4	1.4	65[a]	59.8	1.4	52.2	2.1	58[a]
149	34.4	1.1	33.1	0.9	37	33.6	0.5	28.8	1.1	34
153	84	2.2	79.6	2.5	100	68.9	0.8	62.6	2.6	77
156	4.5	0.1	4.4	0.1	5.0	4.6	0.1	4.0	0.2	4.6
170	12	0.4	11.5	0.2	22[b]	8.6	0.1	7.6	0.3	16[b]
180	50.2	1.0	49.0	0.7	51	36.6	0.8	32.3	0.9	32

COND-2 = 10 min static and 30 min dynamic extraction with carbon dioxide at 60°C (four replicates); Soxhlet 1 = 18 h with 250 ml hexane–acetone (2:3) (two replicates), Ispra; Soxhlet 2 = 12 h with 500 ml pentane–dichloromethane (1:1), single experiment, single column (CP-Sil 19), IJmuiden.
[a] PCB 163 constitutes more than 20% of the value of PCB 138.
[b] Most likely there is a non-PCB interference in the determination of PCB 170.

specific matrix, as just the difference in lipid content in a sample can be enough to change the optimal extraction conditions.

Acknowledgements

The authors are grateful to Hewlett-Packard Italiana S.p.A., Cernusco S/N, Milan, Italy and especially to Giuseppe Candolfi and Costanza Rovida, for making the HP 7680A SFE extractor available.

Finally, we would like to thank Herbert Muntau and Michele Bianchi (Environment Institute, JRC, Ispra, Italy) for valuable help and discussions throughout this work.

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