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Validation of an analytical procedure for polychlorinated biphenyls, coplanar polychlorinated biphenyls and polycyclic aromatic hydrocarbons in environmental samples

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Abstract

This work describes an efficient analytical procedure for the analysis of PCBs (polychlorinated biphenyls), coplanar PCBs and PAHs (polycyclic aromatic hydrocarbons) from the same sample. The method includes a solvent extraction followed by a combined purification–separation step on an alumina–silica column. Coplanar PCBs are isolated from the first fraction (PCBs) by a further high-performance liquid chromatography (HPLC) fractionation on a PYE [2-(1-pyrenyl)ethyldimethylsilylated silica gel] column. PCBs are identified and quantified by gas chromatography (GC) with electron-capture detection whereas GC with flame ionization detection or mass spectrometry are used for PAH determinations. This method allows the measurement of these contaminants in biota and sediment at trace levels as low as 1 pg g^{-1} for coplanar PCBs with a precision better than 20%.

Keywords: Validation; Environmental analysis; Sediment; Fish; Polychlorinated biphenyls; Polynuclear aromatic hydrocarbons

1. Introduction

PCBs (polychlorinated biphenyls) and PAHs (polycyclic aromatic hydrocarbons) are widespread contaminants in the environment. Hazards associated with these compounds are due to their persistence, their hydrophobic character and their toxic properties. The high bioaccumulation potential of PCBs has been recognized for a long time. In this group of 209 congeners [1], several compounds have received increasing attention because of their toxic properties. These non-*ortho*-substituted congeners (CB77, 126 and 169), or so called coplanar CBs, and several mono-*ortho*-substituted compounds possess the same toxic mechanism and enzyme induction as 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). For these reasons, toxic equivalent factors (TEFs) have been given to the non-*ortho*- and mono-*ortho*-substituted PCB congeners [2,3].

PAHs are other major contaminants mainly produced by processes involving combustion and by petrochemical industries. Environmental risks associated with these compounds are related to the carcinogenic character of several individual hydrocarbons [4].

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For these reasons, there is a need for specific and sensitive measurements of PCBs and PAHs in various matrices, either in pollution monitoring programmes or in environmental studies dealing with their distribution, their fate and their effects. The analysis of organic compounds at trace levels in complex matrices usually involves several steps. Basically, an extraction step is followed by an extensive clean-up and then a fractionation of the clean extract may be required before the final instrumental separation of the analytes by chromatographic techniques [5–9]. Such tedious and time-consuming procedures might be advantageously replaced by a multi-residue approach [5,8,10,11]. In this case, an appropriate clean-up of the same extract and its preseparation provides well-defined fractions containing the different group of analytes. This approach gives the maximum information with only one sample extraction. The present work aims at establishing and validating an analytical protocol for the determination of PCBs, coplanar PCBs and PAHs from the same environmental sample.

2. Experimental

2.1. Samples

Two types of samples were used to test the various steps of the analytical protocol: a stock of tissue from blue mussels (*Mytilus edulis*) collected in the Seine estuary (France) and a cod liver oil purchased at a chemist's (Laboratoire Salver, Paris).

2.1.1. Reference materials

The final protocol was validated using two reference materials. The certified reference material CRM 349 is a cod liver oil available from the Community Bureau of Reference (CEE-BCR). It is certified for seven PCB congeners [12] and additional information on other CBs and chlorinated pesticides has been published [13].

The standard reference material SRM 1941a, provided by the United States National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA), is a well-characterized marine sediment which is certified for 23 individual PAHs and 21 PCB congeners [14,15].

2.1.2. Samples for the QUASIMEME intercomparison exercises

The test materials for the analysis of PCBs and non-*ortho*-PCBs in marine biota were supplied by The Netherlands Institute for Fisheries Research (RIVO-DLO), Ijmuiden, The Netherlands. They consist of liver extracts from cod (*Gadus morhua*) from the Southern North Sea (QOR056BT and QPL003BT), and of extracts of muscle tissue of mackerel (*Scomber scombrus*) from the Shetland Islands (QOR057BT and QPL004BT). The test materials for analysis of PAHs in marine sediment were supplied by the Institute for Environmental Studies, Vrije Universiteit, Amsterdam, The Netherlands and were collected off the Ayrshire coast of the Clyde Sea, Scotland, UK (QPH018MS) and at Haringvliet, The Netherlands (QPH019MS).

2.2. Solvents, reagents and adsorbents

2.2.1. Solvents

Solvents used for the analytical procedure including *n*-hexane, acetone, *n*-pentane, methylene chloride, 2-propanol and isooctane (2,2,4-trimethylpentane) were of Pestipur quality and purchased from SDS (Peypin, France). Ethanol used for saponification was purchased from Carlo Erba (Nanterre, France). Deionized water was obtained from a Milli-Q water purification system.

2.2.2. Reagents

The TBA sulfite reagent used for elimination of elemental sulfur [16] was prepared from a solution of 3.39 g tetrabutylammonium (TBA) hydrogensulfate (Fluka, France) in 100 ml of water which was extracted three times with 20-ml portions of hexane and then saturated with 25 g sodium sulfite (Merck, France).

2.2.3. Adsorbents

The alumina (Alumina B, Super I, particle size 50–200 μm) (Alltech, France) and the silica (silica gel, particle size 70–230 mesh, 60 Å) (Aldrich, France) were activated overnight at 400°C and then deactivated with 5% of Milli-Q water.

2.3. Preparation of standards

Twenty individual PCB congeners were used as

standards (CBs No. 28, 31, 52, 77, 101, 105, 110, 118, 126, 128, 132, 138, 149, 153, 156, 169, 170, 180, 187 and 194) [1,17]. They were purchased from Promochem, France. A primary solution was prepared from which working standard solutions were obtained in isooctane with concentrations in the range 1–500 pg μl^{-1} for the *ortho*-substituted CBs and in the range 1–50 pg μl^{-1} for the non- *ortho*-substituted CBs. PAH standard solutions at different concentration levels in the range 1–60 ng μl^{-1} were prepared from the standard reference material SRM 2260 solution, containing 23 certified aromatic hydrocarbons in toluene and provided by NIST. Among the 16 PAHs recommended as priority pollutants by the US EPA (Environmental Protection Agency), 14 parent compounds were studied: fluorene (F), phenanthrene (P), anthracene (A), fluoranthene (Fluor), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Chrys), benzo[*b*]fluoranthene (BbF), benzo[*k*]-fluoranthene (BkF), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IPyr), dibenzo[*a*,*h*]anthracene (DahA) and benzo[*ghi*]perylene (BPer).

The compounds used as internal standards were 1,2,3,4-tetrachloronaphthalene (TCN) for the analysis of PCBs and $[^2H_{10}]$anthracene and $[^2H_{12}]$benzo[*a*]pyrene for the analysis of PAHs and were purchased from Promochem, France. These compounds have been chosen as internal standards because they are not present in environmental samples and because of their good chromatographic resolution from analytes and interferences. They were added in the cleaned extracts just before the analysis by gas chromatography (GC).

3. Analytical procedure

The determination of *ortho*-, non-*ortho*-chloro-substituted PCBs and PAHs in environmental samples was performed according to a protocol including several steps, i.e., extraction, clean-up, fractionation and finally instrumental quantification by GC (Fig. 1).

3.1. Extraction and sulfur removal

The quantification of contaminants like PCBs and PAHs in environmental samples requires an extraction step in order to isolate these compounds from the bulk of the matrix.

The solid–liquid extraction is derived from the classical soxhlet extraction method and was per-

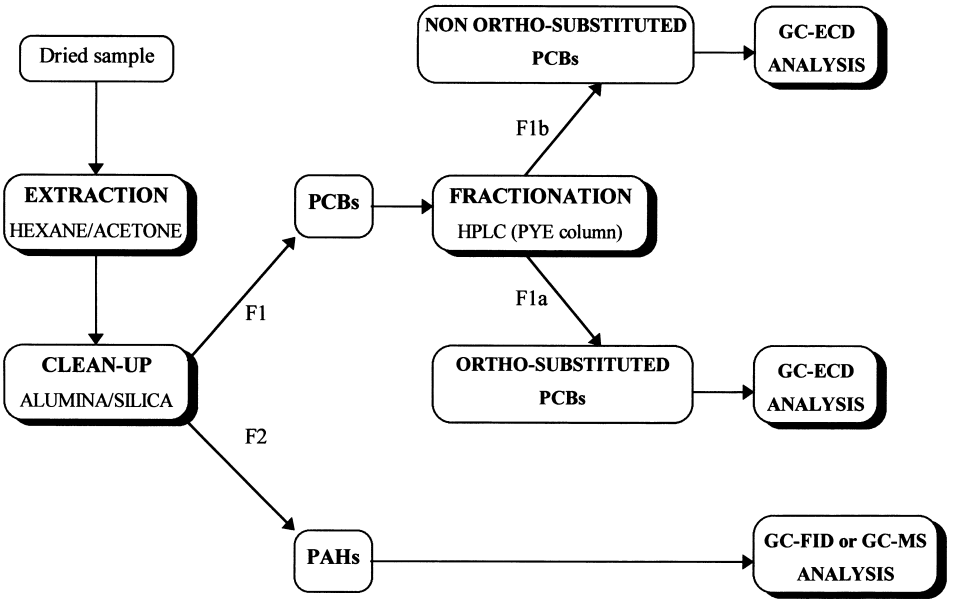

Fig. 1. Analytical protocol.

formed in a Soxtec System HT6 Tecator (France). Using this apparatus, exactly weighed dried samples were submitted to a two-stage extraction with 50 ml of *n*-hexane–acetone (80:20, v/v) for 3 h. Prior to the extraction step, environmental samples such as sediment or fish tissue can be treated in different ways depending on the purpose of the programme [8]. In this work, samples are first freeze–dried in order to achieve an efficient extraction. In the initial stage, the dry sample is placed in a cellulose thimble and then immersed into the boiling solvent mixture to achieve a rapid intimate contact during 2 h. Secondly, the thimble is elevated above the solvent for 1 h while the sample is continually rinsed by condensing solvent. Then the solvent is evaporated using a rota-evaporator (Rotavapor, Büchi) and the extract is concentrated under a nitrogen stream before the clean-up step.

In the case of sediment, it is necessary to remove the elemental sulfur which is co-extracted with the organic compounds because it can significantly interfere with many compounds during gas chromatographic analysis with electron-capture detection (ECD). According to Jensen et al. [16], the sulfur interfering in the gas chromatographic determination is removed in an efficient, rapid, non-toxic and non-destructive treatment of the extract with tetrabutylammonium sulfite. The solid sulfur is converted from the organic phase into water soluble thiosulfate according to the reaction: $(TBA^+)_2SO_3^{2-} + S(s) \rightarrow 2TBA^+ + S_2O_3^{2-}$. The reagent (1 ml) and 2 ml of 2-propanol are added to a 2-ml sample extract in hexane and shaken for at least 1 min and then placed at 4°C until a solid residue appears. If necessary, additional reagent is added. After this treatment, 5 ml of Milli-Q water are added, the mixture is shaken. Then the overlaying organic phase is removed and concentrated to approximately 500 μl before the clean-up step.

3.2. Clean-up

The aim of the clean-up step is to remove other unwanted co-extracted materials like biogenic macromolecules, lipids and pigments which may interfere in the final determination and quantification of the compounds of interest.

In the case of fatty matrix, the classical method used to remove co-extracted lipids is the dehydration and oxidation reactions with concentrated sulfuric acid [18]. This method is efficient for the most unreactive chemical groups like PCBs but cannot be used in the case of compounds of lower chemical stability like PAHs and some organochlorine pesticides which may be partially destroyed by the acid treatment. In order to prevent this problem, physico–chemical treatments are the best non-destructive alternatives to clean-up the extract before the simultaneous determination of PCBs and PAHs. Adsorption chromatography on open alumina–silica columns allows the removal of lipids and other organic material from environmental extracts and the separation of the groups of analytes into appropriate fractions for analysis.

Alumina and silica gel are activated overnight in an oven at 400°C and then deactivated with 5% Milli-Q water prior to their use for column chromatography. The chromatography columns used have an effective height of 30 cm, 10 mm internal diameter and are fitted with a sintered glass disc to retain the adsorbent above a PTFE stopcock. Adsorption columns prepared according to Law et al. [19] are slurry-packed, both alumina and silica being prepared in *n*-pentane. Each column to be packed is filled with 5 g of alumina over 5 g of silica gel, and is washed with 20 ml of *n*-pentane prior to the addition of the extract. The extract (500 μl) is then transferred to the top of the column. The first fraction (F1) is eluted with 40 ml of *n*-pentane and contains all PCBs and aliphatic compounds. The second fraction (F2) is eluted with 20 ml of methylene chloride–*n*-pentane (10:90, v/v) followed by 25 ml of methylene chloride–*n*-pentane (20:80, v/v) and contains aromatic compounds like PAHs. Both fractions are concentrated to a suitable volume prior to the next step. Persistent organochlorinated pesticides, like compounds from the hexachlorocyclohexane (HCH) and DDT groups, have not been considered in this study. If they are to be analyzed using this multi-residue protocol, DDE will be found in the first fraction together with PCBs whereas the other contaminants will be in the second.

3.3. HPLC preseparation of PCBs

The aim of the group separation step is to isolate non-*ortho*-chlorosubstituted congeners (CB77, CB126 and CB169) from the *ortho*-chlorosubstituted

congeners of PCBs. This separation is necessary because these toxic congeners are present at very low concentrations in environmental samples in comparison with the remaining *ortho*-substituted CBs, and because some of the key CBs may co-elute with other congeners on a gas chromatographic column (for example, CB77 coelutes with the CB110 on a DB-5 type column). The different methods available for the isolation of the CBs into separate fractions, prior to the gas chromatographic analysis, according to the spatial planarity of these compounds were reviewed by Hess et al. [5], Creaser et al. [6] and Wells [20]. In this work, PCB congeners have been separated according to their degree of planarity and chlorination level using a Cosmosil 5-PYE high-performance liquid chromatography (HPLC) column [2-(1-pyrenyl)ethyldimethylsilylated silica gel], particle size 5 μm, 150×4.6 mm (Nacalai Tesque, distributed by Macherey–Nagel). This column enables the separation of structurally similar molecules with different π-electron densities resulting from the spatial configuration of the aryl rings, and has sufficient resolution to isolate the non-, mono- and other *ortho*-chlorosubstituted CBs in different fractions [11,21–24]. The HPLC system consisted of a quaternaire gradient pump system (Hewlett-Packard, Model HP1100) and a manual injector (Hewlett-Packard, Model 7725, Rheodyne) equipped with a 200-μl loop. Pestipur-grade hexane (SDS, France) was used as mobile phase at a flow-rate of 0.5 ml min^{-1}. The separations were performed at room temperature. The first fraction (F1) collected from the alumina and silica clean-up was concentrated to 200 μl and manually injected into the HPLC system. Elution resulted in two fractions, noted F1a and F1b, which corresponded to 0–4.5 ml and to 4.5–15 ml of hexane, respectively. The F1a fraction contains all the *ortho*-chlorosubstituted CBs, whereas the F1b fraction contains the non-*ortho*-chlorosubstituted CBs. The mono-*ortho*-substituted congener CB156 was split between the first and second fractions. Each of these two fractions was carefully concentrated under a nitrogen stream. Before the final instrumental analysis, the internal standard TCN is added to the extract which is diluted or concentrated to an exact volume of isooctane depending on the expected concentration and the linear range of the detector.

3.4. Chromatographic conditions

The different fractions were analyzed by GC–ECD for the PCB containing fractions (F1a and F1b) and by GC–flame ionization detection (FID) or GC–mass spectrometry (MS) for PAHs (F2). The gas

Table 1
Gas chromatographic conditions for the determination and quantification of PCBs and PAHs in environmental samples

	PCBs		PAHs		
Instrument	HP 5890 Series II		HP 5890 Series II		
Column type	CP-SIL5C18 CB	CP-SIL19 CB	DB-5	DB-608	HP-5MS
Length	100 m	60 m	60 m	30 m	60 m
Internal diameter	0.25 mm	0.25 mm	0.25 mm	0.53 mm	0.25 mm
Film thickness	0.10 μm	0.15 μm	0.25 μm	0.5 μm	0.25 μm
Injection	On column, 1 μl autosampler	On column, 1 μl autosampler	On column, 1 μl autosampler	On column, 1 μl autosampler	Splitless, 4 μl autosampler
Carrier gas	Hydrogen	Hydrogen	Hydrogen	Hydrogen	Helium
Linear velocity	30 cm s^{-1}	35 cm s^{-1}	40 cm s^{-1}	136 cm s^{-1}	26.5 cm s^{-1}
Pressure	1.8 bar at 75°C	1.2 bar at 75°C	1.4 bar at 65°C	0.5 bar at 65°C	1.03 bar at 50°C
Make-up gas	Argon–methane	Argon–methane	Nitrogen	Nitrogen	
Detector	Electron-capture, ^{63}Ni	Electron capture, ^{63}Ni	Flame ionization	Flame ionization	Mass spectrometer
Detector temperature	330°C	330°C	300°C	300°C	305°C
Oven temperature programme	75°C for 1 min, 45°C min^{-1} to 180°C, 2.5°C min^{-1} to 280°C, 3°C min^{-1} to 300°C, 300°C for 2 min	75°C for 2 min, 30°C min^{-1} to 180°C, 2.5°C min^{-1} to 280°C, 280°C for 2 min, 10°C min^{-1} to 300°C	65°C for 2 min, 25°C min^{-1} to 140°C, 6°C min^{-1} to 310°C, 310°C for 10 min	65°C for 2 min, 25°C min^{-1} to 140°C, 4°C min^{-1} to 260°C, 260°C for 15 min	50°C for 1 min, 6°C min^{-1} to 300°C, 300°C for 20 min

chromatograph was a Hewlett-Packard 5890 series II equipped with an on-column injector, an autosampler injector 7673 HP and either an ECD system for CBs analysis or a FID system for PAH analysis. The GC–ECD or GC–FID analyses were performed on two columns with different selectivities for the separation of PCBs and of PAHs: CP-SIL19 CB and CP-SIL5C18 CB (Chrompack) for PCBs and DB-5 and DB-608 (J&W Scientific) for PAHs. For the electron impact GC–MS analysis, the GC system was coupled to a HP mass-selective detector and the column used was an HP-5MS (Hewlett-Packard). The samples were monitored in selected ion monitoring (SIM) mode recording the molecular ion for each PAH.

The gas chromatographic conditions for the determination and quantification of PCBs and PAHs in environmental samples are given in Table 1.

4. Results and discussion

4.1. Extraction

A dried blue mussel tissue homogenate was used to test the efficiency of the extraction procedures. This material is highly contaminated by PCBs. It has been spiked by PAHs at a high level (10 ng μl^{-1}) in order to facilitate the determination of the hydrocarbons by GC–FID.

Three extraction procedures have been tested:

Procedure 1: Soxtec extraction with 50 ml of hexane for 3 h.

Procedure 2: Soxtec extraction with hexane–acetone (40:10, v/v) for 3 h.

Procedure 3: Saponification with 50 ml of ethanolic KOH for 4 h.

For each procedure of extraction, six replicates were carried out.

Saponification combines extraction and destruction of lipids; this method is known as a very efficient extraction method in the case of PCBs [25,26]. Based on the measured concentrations of CBs in the extract, the three procedures revealed very similar efficiencies (Fig. 2A). On the contrary, differences between the three extraction procedures were observed for PAHs and lower values expressed as percent of the spiked concentrations were obtained by saponification (Fig. 2B). PAHs might be partially destroyed by the treatment with ethanolic KOH. Moreover this technique needs further extraction steps to isolate the cleaned extract from the saponified residue. These additional stages might also cause a loss of compounds. Therefore, the Soxtec methods appear more simple and efficient. Because a polar–apolar solvent mixture generally leads to better recoveries, the method using an acetone–hexane mixture was finally chosen. Various extraction times from 0.5 to 8 h were tested without significant improvement of the recovery of PCBs. The total duration of the extraction was fixed at 3 h (2 h extraction and 1 h rinsing). For example, recovery varied from 82 to 108% for the PAHs, the lowest values being found for the volatile hydrocarbons (fluorene, phenanthrene and anthracene) which are partially lost.

4.2. Clean-up

At this stage of the protocol, the environmental extracts contain a high proportion of co-extracted material such as lipids, sulfur, pigments and organic macromolecules which may interfere in the final determination by GC. As already mentioned in the Experimental section, the removal of sulfur was performed by reaction with sodium sulfite in tetrabutylammonium hydroxide [16].

In this work, adsorption chromatography on open column was preferred to destructive chemical methods for the removal of lipids and other interfering compounds. In order to test the efficiency of the clean-up stage, 250 mg of cod liver oil spiked with PCB and PAH mixtures was laid on the top of the column and then eluted according to the scheme described before. The measured concentrations and recoveries of the added compounds from each fraction (F1 and F2) are presented in Fig. 3.

The clean-up procedure performed over 12 replicates shows good recoveries ranging between 92.6 and 102.6% for all PCBs in fraction F1 and between 82.1 and 101.4% for the high-molecular-mass PAH compounds in fraction F2. Lower recoveries were obtained for the three-rings PAHs which are volatile and then partially lost during the concentration of solvent extract (fluorene: 52.8%; phenanthrene: 82.1% and anthracene: 86.7%). In addition, the low

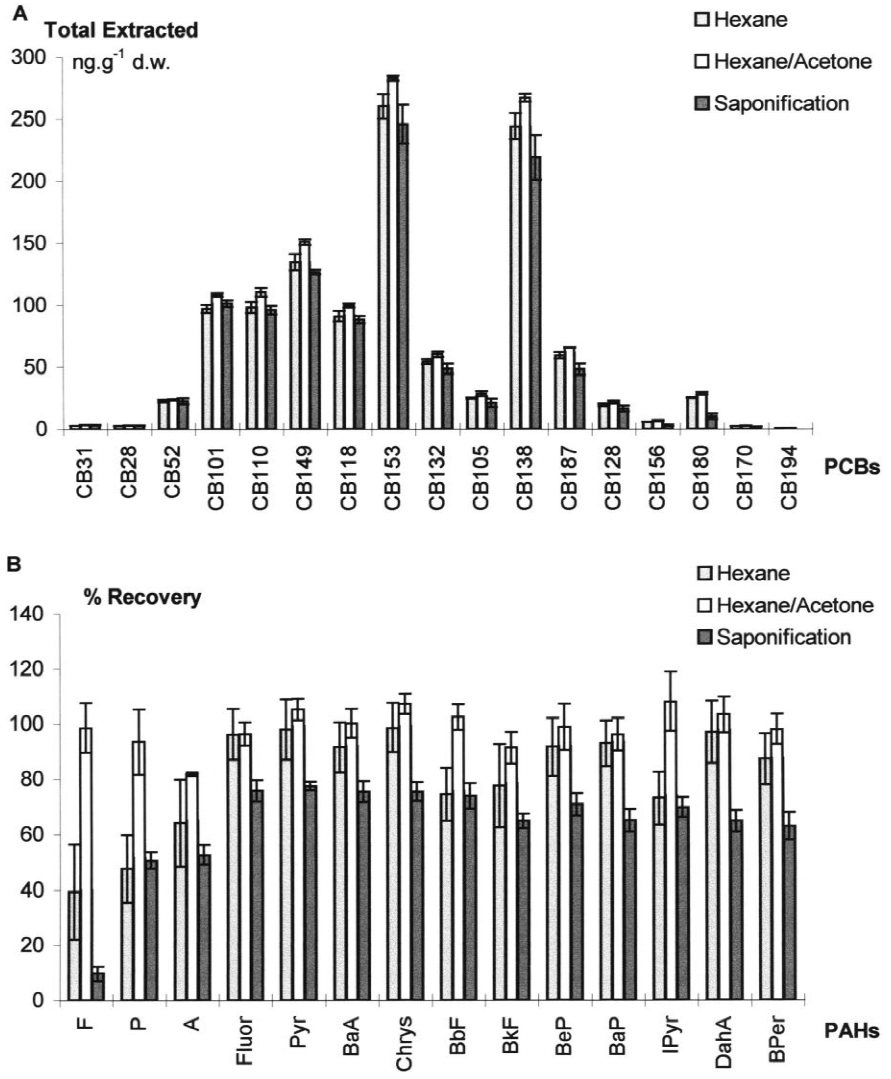

Fig. 2. Recoveries of PCBs (A) and PAHs (B) from a mussel tissue ($n=6$) using different extraction procedures. (Compounds classed according to their elution order on apolar stationary phase).

relative standard deviations (RSDs) from 2 to 9% for PCBs and from 1 to 12% for PAHs demonstrate the high reproducibility of this clean-up step.

The efficiency of the adsorption column for the clean-up also depends on the amount of lipids in the extract. Increasing amounts of lipids, from 100 up to 500 mg of spiked cod liver oil, were laid on the top of the column. Whatever the amount of fatty material within the 100–500 mg range, we do not observe any change in the elution order of the compounds, or interferences in the gas chromatograms. Consequently, the protocol may be used for relatively high lipid amounts in the samples. This point is very important for the analysis of trace level contaminated environmental samples, particularly when non-*ortho*-CBs are to be found, and when the analyst uses more sample in an attempt to obtain detectable amounts of the compounds of interest.

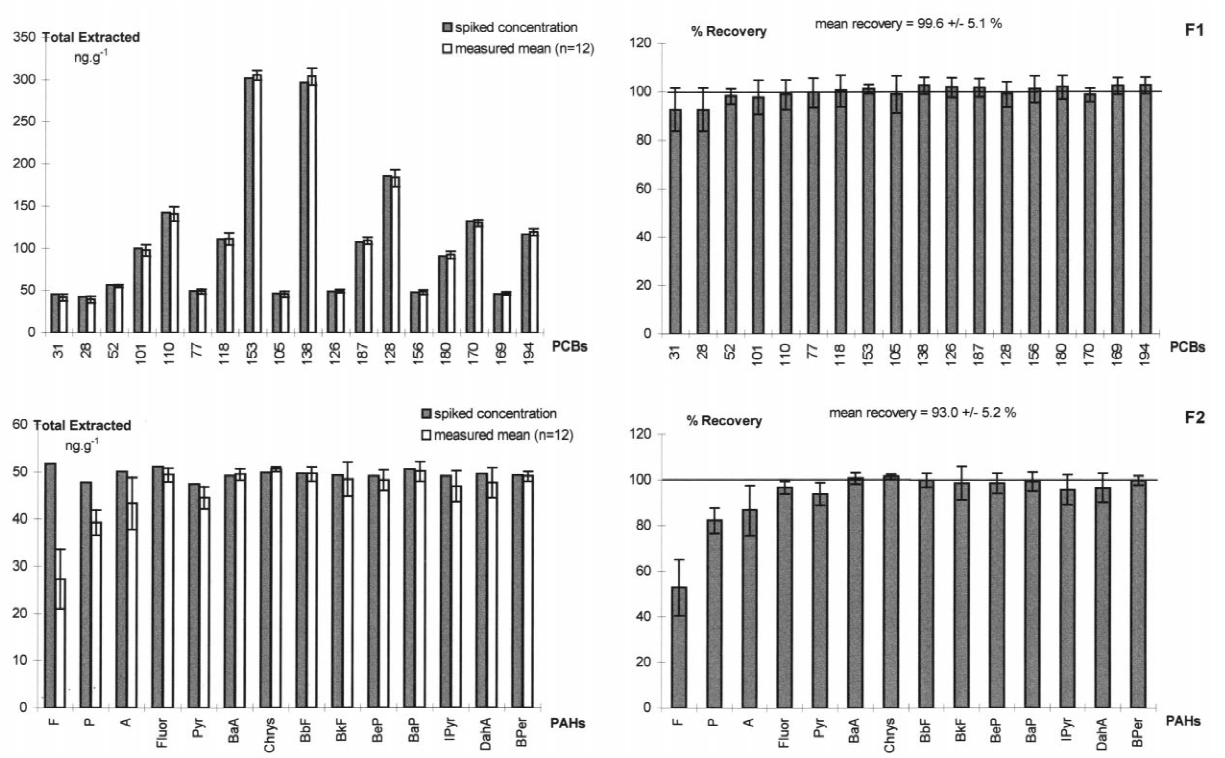

Fig. 3. Measured concentrations and recoveries of PCBs and PAHs after fractionation of spiked cod liver oil on alumina–silica ($n = 12$).

4.3. HPLC preseparation of PCBs

The PCB fraction (F1) was chromatographed on a Cosmosil 5-PYE HPLC column in order to separate the *ortho*- and non-*ortho*-chloro CBs on the basis of their degree of planarity and chlorination [11,21–24]. The appropriate fractionation is obtained (Fig. 4): the first fraction (F1a: 0–4.5 ml) contained PCB congeners with one or more chlorine atoms in the *ortho* positions of the biphenyl ring while non-*ortho*-chloro congeners were eluted in the second HPLC fraction (F1b: 4.5–15 ml) except for the mono-*ortho* congener CB156 which was split between the two fractions.

This fractionation step was carried out over 12 replicates of a PCB standard mixture. The results presented in Fig. 5 show good recoveries for all PCBs with values ranging between 84 and 98%. These data demonstrate a very low loss of compounds during the preseparation step.

In the same way, six replicates of cod liver oil spiked with PCBs, previously cleaned on the alumina–silica column, have been fractionated. The results presented in Table 2 show that the measured concentrations are in agreement with the spiked concentrations with a mean recovery higher than 90%, and consequently demonstrate the efficiency of this fractionation step in the case of environmental matrices.

4.4. GC determination

4.4.1. Calibration and linearity of the instrumental response

The calibration technique is the internal standard multipoint calibration using eight standard solutions. The compounds are quantified using the ratio of the analyte and internal standard response. The internal standard was added to the sample extract just prior to the instrumental analysis. Quantification of studied compounds must be conducted in the linear range of the calibration curve. In order to determine the linear

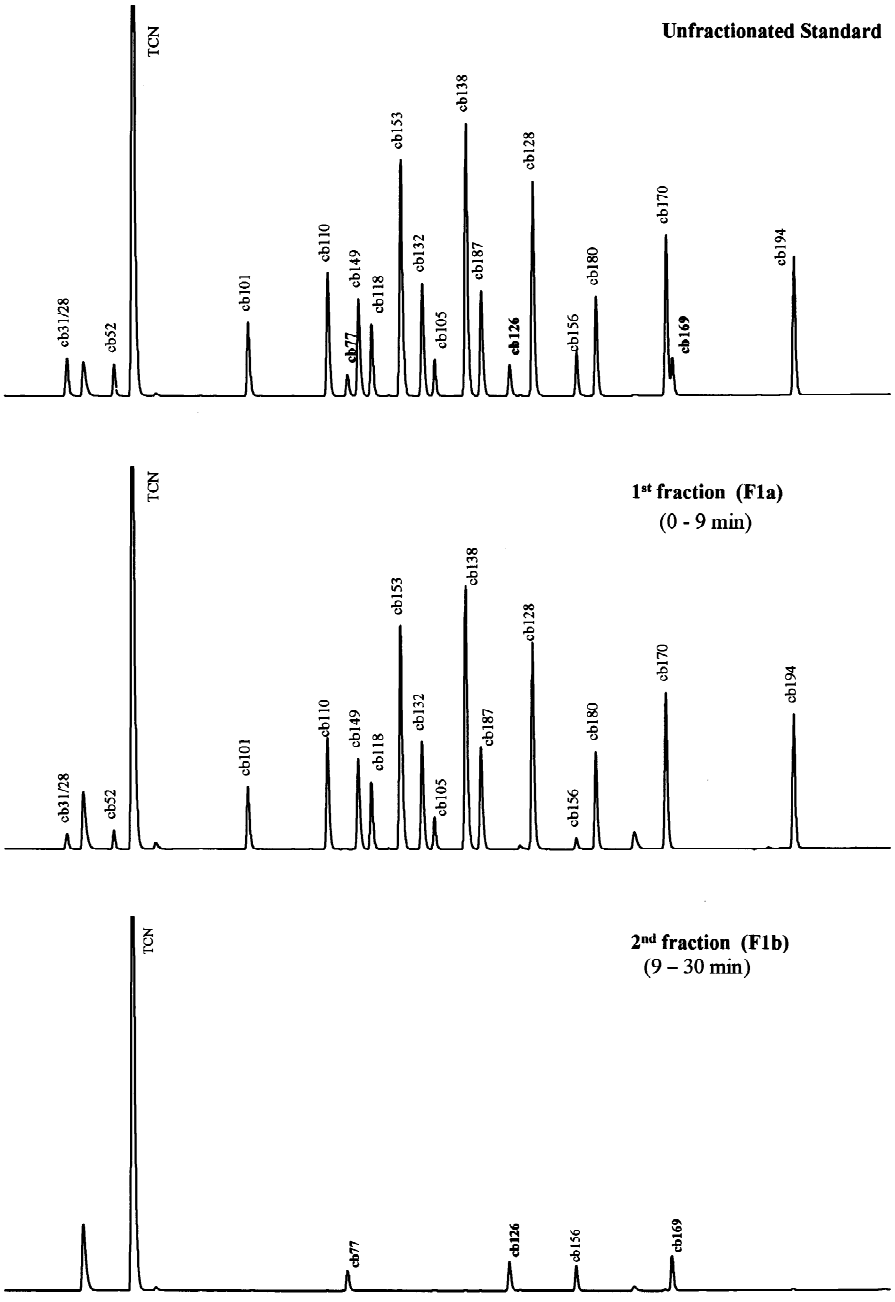

Fig. 4. Gas chromatograms on CP-SIL19 CB column of the unfractionated mixture and of the two fractions from the PYE column. Conditions: isocratic mode, mobile phase: hexane, flow-rate: 0.5 ml min^{-1}.

range of the detector, several standard solutions were prepared and injected at different concentration levels. For each PCB congener determined by GC–ECD, the range of concentrations was appropriated to the levels usually found in environmental samples. For example, in standard solutions, the concentra-

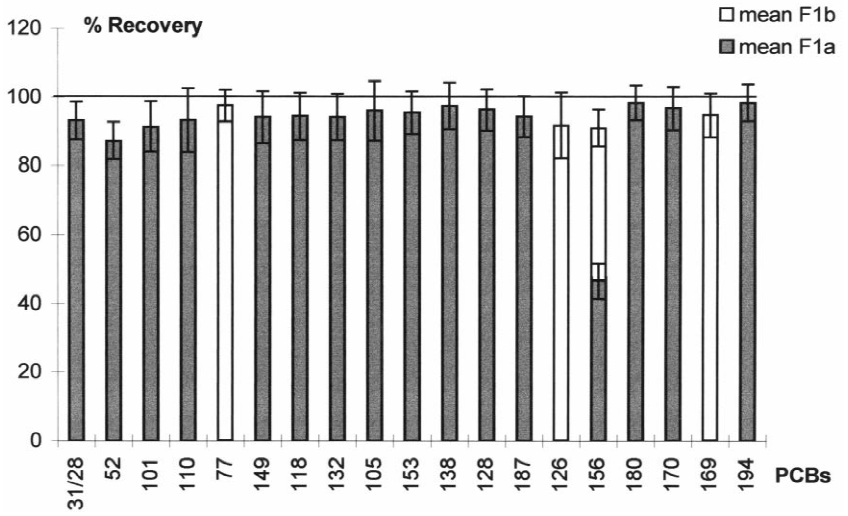

Fig. 5. Recoveries of *ortho*-CBs (F1a) and non-*ortho*-CBs (F1b) from the standard mixture after fractionation on the PYE column by HPLC ($n=12$).

tions of major congeners like CB153 and CB138 ranged between 6 and 1000 pg μl^{-1} whereas in the case of coplanar PCBs (CB77, 126 and 169) which are present at trace levels, the concentrations ranged between 1 and 100 pg μl^{-1}. For PAHs quantified by GC–FID or GC–MS, the concentrations tested were in the range 2–40 ng μl^{-1} and 0.1–1 ng μl^{-1}, respectively. The linear ranges used for PCBs and

Table 2
PCB recoveries ($n=6$) in a spiked cod liver oil after fractionation on a PYE column

CB	Spiked concentration (ng g^{-1})	Measured mean in F1a (ng g^{-1})	SD F1a (ng g^{-1})	Measured mean in F1b (ng g^{-1})	SD F1b (ng g^{-1})	Mean recovery (%)	RSD[a] (%)
31	45.1	40.7	4.6			90.1	11.2
28	42.3	33.0	2.9			77.9	8.7
52	56.7	45.8	4.1			80.7	8.9
101	99.9	103.7	3.9			103.9	3.8
110	142.3	135.1	7.2			95.0	5.4
77	49.4			42.8	2.7	86.7	6.4
149	122.8	130.8	5.8			106.5	4.4
118	111.1	108.7	2.5			97.8	2.3
105	46.1	41.6	2.4			90.3	5.8
153	301.9	298.4	6.0			98.8	2.0
138	296.6	307.5	6.5			103.7	2.1
128	185.3	195.9	10.3			105.7	5.3
187	107.8	98.5	6.1			91.3	6.2
126	48.6			47.3	1.6	97.1	3.3
156	47.9	19.7	5.1	21.6	4.7	86.3	3.6
180	90.7	95.0	5.3			104.8	5.6
170	131.8	115.3	4.5			87.5	3.9
169	45.8			41.3	1.4	90.3	3.5
194	116.8	102.2	5.5			87.5	5.3
Mean						93.8	5.1

[a] RSD: relative standard deviation = (SD/measured mean)·100.

Table 3
Linear ranges, limits of detection and limits of quantification for PCBs and PAHs

	GC–FID			GC–MS				GC–ECD		
	Linear range (ng μl^{-1})	LOD (ng μl^{-1})	LOQ (ng μl^{-1})	Linear range (ng μl^{-1})	LOD (ng μl^{-1})	LOQ (ng μl^{-1})		Linear range (pg μl^{-1})	LOD (pg μl^{-1})	LOQ (pg μl^{-1})
PAHs							*PCBs*			
F	2.0–40	1.00	3.33	0.1–1.0	0.06	0.22	31	0.9–270	0.30	1.00
P	2.0–40	1.39	4.64	0.1–1.0	0.07	0.23	28	0.9–270	0.30	1.00
A	2.0–40	1.06	3.53	0.1–1.0	0.05	0.17	52	1.1–100	0.25	0.83
Fluor	2.0–40	1.27	4.24	0.1–1.0	0.08	0.25	101	2.0–180	0.69	2.30
Pyr	2.0–40	1.14	3.79	0.1–1.0	0.10	0.33	110	10.0–310	1.31	4.35
BaA	2.0–40	1.12	3.73	0.1–1.0	0.11	0.36	77	1.0–90	0.64	2.13
Chrys	2.0–40	0.90	3.00	0.1–1.0	0.06	0.21	149	3.4–310	0.93	3.09
BbF	2.0–40	1.06	3.52	0.1–1.0	0.15	0.49	118	2.0–180	1.15	3.84
BkF	2.0–40	1.70	5.68	0.1–1.0	0.08	0.28	153	6.0–540	1.23	4.11
BeP	2.0–40	0.98	3.26	0.1–1.0	0.10	0.32	132	3.5–320	1.14	3.80
BaP	2.0–40	1.86	6.20	0.1–1.0	0.09	0.31	138	5.9–535	2.65	8.84
IPyr	2.0–40	2.47	8.25	0.1–1.0	0.16	0.54	105	2.0–80	0.35	1.18
DahA	2.0–40	1.03	3.44	0.1–1.0	0.19	0.62	187	3.1–280	1.08	3.59
BPer	2.0–40	2.40	8.00	0.1–1.0	0.15	0.50	126	1.0–90	0.55	1.83
							128	10–300	2.32	7.73
							156	2.0–85	0.69	2.30
							180	9.0–160	1.19	3.96
							170	3.2–285	2.58	8.60
							169	0.9–80	0.20	0.67
							194	3.4–300	2.42	8.08

PAHs are presented in Table 3. Within these linearity ranges, calibration curves plotted from eight points were obtained for all compounds by regression analysis of peak areas versus injected concentrations. In all cases, regression coefficients were higher than 0.99 for all the analytes.

4.4.2. Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to Taylor [27]. Triplicate analyses of five dilution standard solutions were performed. For each standard solution, a standard deviation was obtained and the mean of standard deviations (SDs) was determined for each analyte. The instrumental LOD, expressed in pg μl^{-1} and in ng μl^{-1} for PCBs and PAHs, respectively, was obtained from the mean SD multiplied by a factor of 3 and the LOQ by the same mean but using a factor of 10 [27]. The results obtained are summarized in Table 3. LOD values ranged from 0.25 to 2.65 pg μl^{-1} for PCBs analyzed by GC–ECD, from 0.9 to 2.47 ng μl^{-1} and from 0.05 to 0.19 ng μl^{-1} for PAHs analyzed by GC–FID and GC–MS, respectively. These last values are in agreement with the limits given for PAHs by Means [28].

4.4.3. Blank

The purpose of the analytical blank is to check the absence of contamination by interfering compounds, which will result in quantification errors [27,29]. Careful measurements of blank values are essential when determining organic compounds at the trace level and most particularly in the case of planar CBs. A procedural blank was analyzed periodically for each batch of 10 samples. It was prepared using the entire analytical procedure as well as the same reagents and solvents as for the samples. For example, in the case of PCBs, concentrations in the blank extract do not exceed 1 pg μl^{-1} for all congeners.

4.4.4. GC columns

Selection of appropriate chromatographic columns is of major importance for correctly and unambiguously identifying and quantifying trace organic contaminants. The resolution capacity of a column

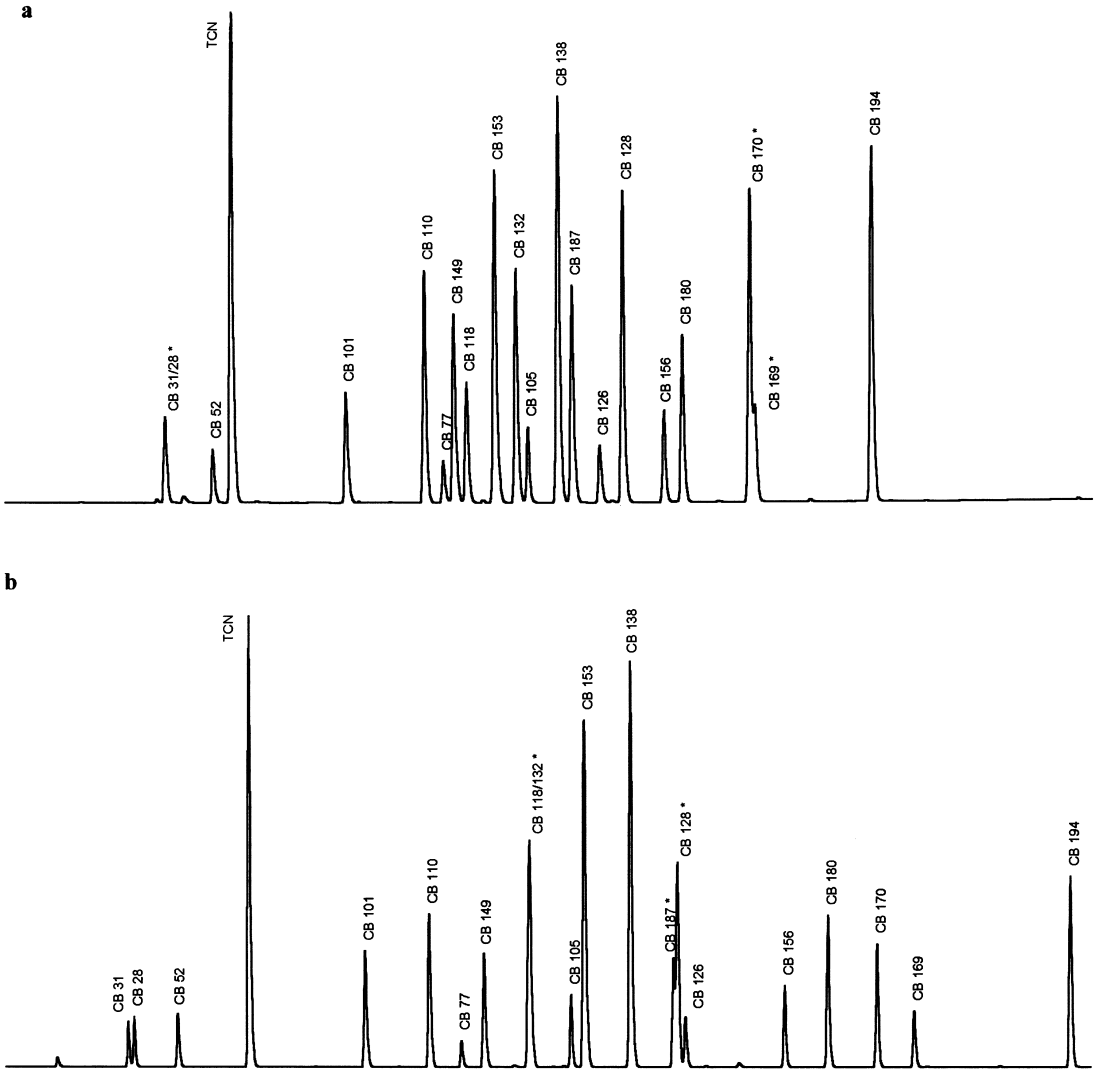

Fig. 6. Gas chromatograms of a PCB mixture on two columns with different polarities, CP-SIL19 CB column (a) and CP-SIL5C18 CB (b) Co-eluting compounds.

depends on its characteristics such as the nature of the stationary phase, the length and the internal diameter of the column. Various column types have been used for PCB determination [30–34] but no one allowed the separation of all congeners in a single chromatographic run.

In this study, the use of two columns with different polarities (CP-SIL19 CB and CP-SIL5C18 CB type columns) provided different elution patterns enabling separation and quantification of coeluting congeners. The chromatograms in Fig. 6 show that co-elution occurred for a number of CB pairs, i.e., CB 31/28 and CB170/169 on CP-SIL19 CB like column and CB 118/132 and CB 187/128 on CP-SIL5C18 CB like column, therefore demonstrating the need for using two chromatographic columns with different polarities. Similar problems occurred for the PAH determination by GC–FID, e.g.,

chrysene/triphenylene and benzo[*j*]fluoranthene/benzo[*k*]fluoranthene. For that reason, HPLC was considered more appropriate for PAH analysis.

5. Application to environmental samples

Once the various steps of the protocol were characterized and validated, it was necessary to check the performances of the whole analytical procedure. For that purpose, we first analyzed reference materials to gain information on the ability of that new analytical procedure to provide the expected concentrations. At the final stage, we participated in intercomparison exercises to have the opportunity to test the whole analytical procedure in real situations, i.e., on totally unknown samples.

5.1. Reference materials (CRMs and SRMs)

Two reference materials, CRM 349 ''Chlorobiphenyls in Cod Liver Oil'' and SRM 1941a ''Organics in Marine Sediment'' were analyzed by applying the full analytical procedure in order to determine the accuracy and precision of the method. The results obtained for PCBs and PAHs are given in Table 4.

5.1.1. Accuracy of PCB and PAH determinations

Results obtained in our work were compared to expected data given for these certified materials [12–15]. Recoveries, calculated as percent of the true value for each analyte, provide an estimation of the accuracy of the whole procedure.

In the CRM 349 (cod liver oil), recoveries for PCBs ranged between 81 and 242% for all analyzed congeners (Table 4), giving an average value (six replicates) of 114%. Whatever the column used and the chromatographic conditions, the recovery for CB105 was always very high (242%) thus suggesting a problem of coelution with another compound or a matrix effect. Excluding this congener, mean recoveries were very much closer to the expected values (81–123%, with a mean recovery of 104%).

The analysis of the other reference material (SRM1941a), a marine sediment containing both PCBs and PAHs, required an extraction step. In that material, PCB contamination is approximately 50-fold lower than in the cod liver oil. The results of five measurements are given in Table 4. Recoveries for PCBs vary from 72 to 166%, with an average value of 105%. Interferences probably occurred in the case of CB194. Conversely, in the sediment, the concentration of CB105 was very close to the expected value. These two examples, CB105 and CB194 in two different materials, show again, if necessary, that in trace analysis, each kind of sample must be questioned.

Among these two reference materials, only the sediment sample (SRM1941a) has been certified for PAHs [14,15]. Five replicates were analyzed and results are compared to the expected concentrations (Table 4). The recoveries for all PAHs ranged from 51 to 96% with a mean of 77%. The very low value obtained for fluorene (51%) is due to its volatility and consequently to its partial loss during evaporation. Two compounds (chrysene and benzo[*k*]-fluoranthene) were not well resolved and they co-eluted, respectively, with their isomers: triphenylene and benzo[*j*]fluoranthene. The results reported here take into account the sum of these unresolved PAHs. HPLC with programmed fluorescence detection might be a better alternative for the determination of these compounds [35,36].

5.1.2. Precision of PCB and PAH determinations

Generally speaking, and whatever the reference material considered (CRM349 or SRM1941a), the precision is estimated by the RSD. The RSD values ranged from 0.9 to 22% with a mean of 7.9% for all the PCBs in CRM349 and from 0.1 to 15% with a mean of 4.4% for all the PCBs in SRM1941a. In the case of PAHs in SRM1941a, the mean RSD was 12.6% and ranged from 2.9 to 28.9% for individual PAHs. These RSDs are in agreement with certified data.

The study of these two types of reference materials was of particular interest. The results obtained indicate first, that our protocol is suitable whatever the nature of the sample (cod liver oil or marine sediment), and secondly, that it allows the determination of most compounds at different concentration levels with a recovery close to 100%.

Table 4
Measured concentrations and recoveries of PCBs and PAHs in CRM349 and SRM1941a

	CRM349							SRM1941a						
	Certified values (ng g^{-1})	SD[a] (ng g^{-1})	RSD[b] (%)	Experimental mean ($n=6$) (ng g^{-1})	SD[c] (ng g^{-1})	Recovery[e] (%)	RSD[d] (%)	Certified values (ng g^{-1})	SD[a] (ng g^{-1})	RSD[b] (%)	Experimental mean ($n=5$) (ng g^{-1})	SD[c] (ng g^{-1})	Recovery[e] (%)	RSD[d] (%)
PCB														
CB31								6.2[f]	2.4	38.7	7.6	0.1	123.3	0.8
CB28	68	7	10.3	54.9	11.9	80.8	21.7	9.8[f]	3.7	37.8	7.4	0.1	75.9	0.9
CB52	149	20	13.4	156.0	14.5	104.7	9.3	6.9	0.6	8.1	7.3	1.1	105.4	14.8
CB101	370	17	4.6	373.7	3.2	101.0	0.9	11.0	1.6	14.5	10.7	0.3	97.4	3.3
CB118	454	31	6.8	465.1	68.7	102.5	14.8	10.0	1.1	11.0	10.4	0.05	104.3	0.5
CB153	938	49	5.2	956.4	24.0	102.0	2.5	17.6	1.9	10.8	12.9	1.2	73.0	9.2
CB105	50[f]	0	0.0	120.8	10.2	241.6	8.5	3.7	0.3	7.4	3.5	0.2	95.4	4.4
CB138	765[f]	74	9.7	883.0	27.7	115.4	3.1	13.4	1.0	7.2	13.5	0.01	100.7	0.1
CB156	40[f]	0	0.0	47.9	9.5	119.8	19.8	0.9	0.1	15.1	0.8	0.1	86.6	13.2
CB180	280	22	7.9	279.2	39.1	99.7	14.0	5.8	0.6	9.9	4.2	0.1	72.0	3.0
CB110	180[f]	0	0.0	222.0	7.4	123.3	3.3	9.5	0.9	9.0	11.9	0.4	125.1	3.0
CB149	284[f]	55	19.4	270.5	3.3	95.2	1.2	9.2	1.1	12.0	11.5	0.1	124.5	0.6
CB187	276[f]	14	5.1	298.1	3.3	108.0	1.1	7.0[f]	2.6	37.1	6.1	0.4	86.4	5.8
CB128	104[f]	9	8.7	109.8	–	105.6	–	1.9	0.3	17.1	2.3	–	123.0	–
CB170	149[f]	15	10.1	137.1	4.2	92.0	3.1	3.0	0.5	15.3	3.7	0.1	123.3	3.8
CB194								1.8	0.2	12.9	3.0	0.1	166.3	2.9
Mean						113.7	7.9						105.2	4.4
PAH														
F								97	8.6	8.9	49.0	1.4	50.5	2.9
P								489	23	4.7	367.4	72.7	75.1	19.8
A								184	14	7.6	142.7	41.2	77.5	28.9
Fluor								981	78	8.0	771.8	92.3	78.7	12.0
Pyr								811	24	3.0	641.1	48.0	79.0	7.5
BaA								427	25	5.9	330.6	38.9	77.4	11.8
Chrys+Triph								577	35	6.1	476.9	48.2	82.7	10.1
BbF								740	110	14.9	563.2	76.6	76.1	13.6
BkF+BjF								702	35	5.0	533.6	13.6	76.0	2.5
BeP								553	59	10.7	472.4	32.7	85.4	6.9
BaP								628	52	8.3	424.3	38.5	67.6	9.1
IPyr								501	72	14.4	405.8	65.5	81.0	16.1
DahA								74	9.7	13.1	70.9	17.3	95.7	24.4
BPer								525	67	12.8	423.1	45.0	80.6	10.6
Mean													77.4	12.6

[a] Standard deviation of certified values.
[b] Relative standard deviation of certified values, (SD/mean)·100.
[c] Standard deviation of experimental values.
[d] Relative standard deviation of experimental values.
[e] Recovery (experimental value/certified value)·100.
[f] Non-certified concentration.

In addition, the low values of relative standard deviations are indicative of a good precision for the methodology. At present, there are no certified reference materials for non-*ortho*-substituted PCBs.

5.2. Intercomparison exercises

A different approach to validate the developed methodology and to assess the reliability of PCB and

PAH measurements in environmental samples has been the participation in intercomparison exercises. During this study, we took part in the QUASIMEME programme (Quality Assurance of Information in Marine Environmental Monitoring in Europe). The purpose of this collaborative project is to improve the quality of informations and the quality of measurement of the data submitted to these programmes [37].

More precisely, in this Round 14 exercise, undertaken to improve the measurement of PCBs in marine biota and PAHs in marine sediment (Table 5), six samples from various matrices were to be analyzed.

The participating laboratories were asked to analyze the samples by their own methods, using their own calibration solutions, and single measurements were requested for each determinant in each sample. The full protocol was performed over four replicates for each test sample. The assessment report of the QUASIMEME Laboratory Performance Study for each exercise, sent to each participating laboratory and contains the individual participant's results and performance (assigned values, total errors allowable and information on robust statistics such as the *Z*-scores for all the determinants). The performance of individual laboratories was calculated as a *Z*-score using the assigned values and allowable error which were established independently [38]. The *Z*-score is defined for each determinant by:

$$Z = (x_i - x)/s_b$$

where x_i is the measured value, x is the assigned value, s_b is the allowable error. Comparison between the assigned values and the measured values is presented in Table 6. In this exercise, the assigned values for each test material were obtained from the robust means of the data from all the laboratories, excluding those submitting data which were extreme. In previous exercises, the assigned values were determined by the robust mean of a group of reference laboratories which have demonstrated the reliability of their analysis by performance in other previous interlaboratory tests for the same matrix-determinant combinations, or which participate successfully in certification exercises.

According to Wells [8], freeze–drying of samples prior to extraction is not appropriate if relatively volatile contaminants are to be determined. In this exercise, the different materials have been freeze–dried before the extraction step. The final results have been given on a wet mass basis after correction by the water content. Our results are very consistent with the expected values which means that freeze–drying technique does not cause any important loss of compounds.

5.2.1. PCBs in biota

Two fish samples were to be analyzed for PCBs. The cod liver sample (QOR056BT) contains more than 50% of extractable lipids and high levels of PCBs ranging from 13.6 to 383 ng g^{-1} wet mass (Table 6A). For most of the PCBs, the measured values ($n=4$) were consistent with the assigned

Table 5
Description of the six samples analyzed in the intercomparison exercises

Exercise No.	Sample code	Nature	Determination of	Indicated compounds	Number of participating laboratories
376	QOR056BT	Cod liver homogenate	Chlorinated organics in biota	9 CBs and 10 organochlorine pesticides[a]	32
376	QOR057BT	Mackerel muscle tissue	Chlorinated organics in biota	9 CBs and 10 organochlorine pesticides[a]	32
377	QPL003BT	Cod liver homogenate	Non-*ortho*-CBs, PCDDs and PCDFs in biota	3 non-*ortho*-CBs, 7 PCDDs and 9 PCDFs[a]	4
377	QPL004BT	Mackerel muscle tissue	Non-*ortho*-CBs, PCDDs and PCDFs in biota	3 non-*ortho*-CBs, 7 PCDDs and 9 PCDFs[a]	4
374	QPH018MS	Marine sediment	PAHs in sediment	17 PAHs	22
374	QPH019MS	Marine sediment	PAHs in sediment	17 PAHs	22

[a] In our work, only PCBs, non-*ortho*-PCBs and PAHs were analyzed. PCDD = Polychlorinated dibenzodioxin; PCDF = polychlorinated dibenzofuran.

Table 6
Concentrations and recoveries for PCBs (A) and non-*ortho*-PCBs (B) in cod liver and mackerel tissue and for PAHs (C) in two marine sediments

	Cod liver homogenate (QOR056BT)								Mackerel muscle tissue (QOR057BT)							
	Assigned values (ng g^{-1} wet mass)	Assigned error (%)	Assigned error (ng g^{-1} wet mass)	Measured values[a] (ng g^{-1} wet mass)	Measured SD[a] (ng g^{-1} wet mass)	Recovery (%)	RSD (%)	Z^b	Assigned values (ng g^{-1} wet mass)	Assigned error (%)	Assigned error (ng g^{-1} wet mass)	Measured values[a] (ng g^{-1} wet mass)	Measured SD[a] (ng g^{-1} wet mass)	Recovery (%)	RSD (%)	Z^b
A																
CB28	13.6	12.9	1.8	7.3	1.3	53.5	17.2	-3.6	0.7	19.8	0.1	0.2	0.1	30.4	50.1	−3.5
CB52	34.7	12.6	4.4	34.2	0.7	98.7	2.2	−0.1	1.1	17.2	0.2	0.9	0.2	84.0	23.6	−0.9
CB101	106.8	12.6	13.4	117.2	1.1	109.8	0.9	0.8	2.3	14.7	0.3	1.7	0.2	76.7	13.3	−1.6
CB118	139.0	12.5	17.4	135.1	0.1	97.2	0.1	−0.2	1.8	15.3	0.3	2.8	0.2	155.4	7.9	3.6
CB153	382.9	12.5	47.9	412.1	5.8	107.6	1.4	0.6	3.8	13.8	0.5	4.0	0.5	104.2	13.8	0.3
CB105	38.9	12.6	4.9	49.3	0.8	126.8	1.7	2.1	0.6	21.6	0.1	0.3	0.1	61.8	15.7	−1.8
CB138	284.0	12.5	35.6	364.9	8.3	128.5	2.3	2.3	3.1	14.1	0.4	3.2	0.5	103.9	14.2	0.3
CB156	16.6	12.8	2.1	17.7	0.6	106.6	3.2	0.5	0.2	38.7	0.1	0.1	0.0	63.2	19.1	−1.0
CB180	90.0	12.6	11.3	85.6	1.0	95.1	1.2	−0.4	1.1	17.0	0.2	0.9	0.2	79.5	22.9	−1.2
Lipids[c]	52.6	12.6	6.6	55.1	1.0	104.9	1.8	0.4	29.0	12.7	3.7	31.8	2.6	109.6	8.3	0.8
Mean[d]						102.6	3.3							84.3	20.1	
B	Cod liver homogenate (QPL003BT)								Mackerel muscle tissue (QPL004BT)							
	Assigned values (pg g^{-1} wet mass)	Assigned error (%)	Assigned error (pg g^{-1} wet mass)	Measured values[a] (pg g^{-1} wet mass)	Measured SD[a] (pg g^{-1} wet mass)	Recovery (%)	RSD (%)	Z^b	Assigned values (pg g^{-1} wet mass)	Assigned error (%)	Assigned error (pg g^{-1} wet mass)	Measured values[a] (pg g^{-1} wet mass)	Measured SD[a] (pg g^{-1} wet mass)	Recovery (%)	RSD (%)	Z^b
CB77	1248.0	12.5	156.0	1272.0	182.1	101.9	14.3	0.2	51.4	12.6	6.5	40.6	1.7	79.0	4.1	−1.7
CB126	965.8	12.5	120.8	939.3	83.1	97.3	8.8	−0.2	11.6	12.9	1.5	10.9	0.9	93.4	8.7	−0.5
CB169	139.9	12.5	17.5	210.9	20.5	150.8	9.7	4.0	1.9	15.2	0.3	2.8	0.7	151.3	25.5	3.4
Lipids[c]	59.0	12.6	7.4	61.0	6.1	103.4	10.0	0.3	33.2	12.7	4.2	32.4	1.1	97.9	3.3	−0.2
Mean[d]						116.7	11.0							107.9	12.7	

C	Marine sediment (QPH018MS)								Marine sediment (QPH019MS)							
	Assigned values (ng g^{-1} dry mass)	Assigned error (%)	Assigned error (ng g^{-1} dry mass)	Measured values[a] (ng g^{-1} dry mass)	Measured SD[a] (ng g^{-1} dry mass)	Recovery (%)	RSD (%)	Z[b]	Assigned values (ng g^{-1} dry mass)	Assigned error (%)	Assigned error (ng g^{-1} dry mass)	Measured values[a] (ng g^{-1} dry mass)	Measured SD[a] (ng g^{-1} dry mass)	Recovery (%)	RSD (%)	Z[b]
F	65.2	12.5	8.2	77.0	14.0	118.2	18.2	1.5	153.5	12.5	19.2	150.0	29.2	97.7	19.4	−0.2
P	483.5	12.5	60.4	410.0	30.4	84.8	7.4	−1.2	947.5	12.5	118.4	840.0	34.6	88.7	4.1	−0.9
A	128.9	12.5	16.1	105.0	17.5	81.5	16.6	−1.5	278.6	12.5	34.8	230.0	17.3	82.5	7.5	−1.4
Fluor	851.4	12.5	106.4	780.0	17.3	91.6	2.2	−0.7	1534.6	12.5	191.8	1500.0	72.3	97.7	4.8	−0.2
Pyr	878.6	12.5	109.8	900.0	90.7	102.4	10.1	0.2	1111.5	12.5	138.9	1200.0	52.0	108.0	4.3	0.6
BaA	448.6	12.5	56.1	460.0	30.6	102.5	6.6	0.2	741.5	12.5	92.7	700.0	28.9	94.4	4.1	−0.4
Chrys	448.8	12.5	56.1	470.0	47.3	104.7	10.1	0.4	883.4	12.5	110.4	990.0	63.3	112.1	6.4	1.0
BbF	612.8	12.5	76.6	620.0	7.1	101.2	1.1	0.1	1034.7	12.5	129.3	1020.0	64.1	98.6	6.3	−0.1
BkF	367.8	12.5	46.0	550.0	63.6	149.6	11.6	4.0	570.8	12.5	71.4	900.0	94.5	157.7	10.5	4.6
BeP	596.1	12.5	74.5	680.0	58.5	114.1	8.6	1.1	797.5	12.5	99.7	850.0	22.7	106.6	2.7	0.5
BaP	656.5	12.5	82.1	660.0	66.8	100.5	10.1	0.0	670.4	12.5	83.8	680.0	9.3	101.4	1.4	0.1
IPyr	617.1	12.5	77.1	620.0	47.7	100.5	7.7	0.0	648.5	12.5	81.1	750.0	83.6	115.6	11.2	1.3
DahA	98.7	12.5	12.3	120.0	63.5	121.6	52.9	1.7	131.4	12.5	16.4	90.0	25.2	68.5	28.0	−2.5
BPer	595.2	12.5	74.4	610.0	47.3	102.5	7.7	0.2	600.8	12.5	75.1	600.0	60.4	99.9	10.1	−0.0
Mean[d]						105.4	12.2							102.1	8.6	

[a] Mean of measured values performed over four replicates.

[b] *Z*-scores have been calculated to give each participant a normalized performance score for bias and can be interpreted as follows [38]: $Z<2$ satisfactory value, $2<Z<3$ questionable value, $Z>3$ unsatisfactory value.

[c] Extractable lipids removed from the sample during the extraction with hexane–acetone.

[d] Mean only performed on PCBs.

values and the recoveries ranged from 95 to 110% excepted for a few congeners, CB28 (53.5%), CB105 (126.8%) and CB138 (128.5%). These compounds are more difficult to analyze due to coelution problems, despite the use of two chromatographic columns having different polarities. CB138 coeluted with other PCBs (CB163, 160 and 158) on most of the stationary phases [39]. According to Frame et al. [33], the use of the DB-XLB type column which presents a good ability for separating many PCB congeners allows to resolve this problem of coelution. With the exception of these compounds, the *Z*-scores which assess the performance of the laboratory, were less than 2 and demonstrated the good agreement between the assigned and the measured values. In the mackerel tissue (QOR057BT), which contains approximately 30% of extractable lipids, the PCB concentrations were much lower in comparison with those in cod liver and ranged from 0.2 to 3.8 ng g^{-1} wet mass (Table 6A). The results show a relatively good agreement between the measured and the assigned values for most of the PCBs. The recoveries calculated from the measured concentrations and the expected concentrations, were very satisfactory except for CB28 and CB118. They ranged between 62 and 104% when these last compounds were excluded. In this matrix, the major difficulty was to analyze the mono-*ortho*-substituted PCBs present at very low concentrations (1.8, 0.6 and 0.2 ng g^{-1} wet mass, respectively for CB118, 105 and 156). The concentrations were strongly underestimated for both CB105 and CB156 (recoveries close to 60%) and overestimated for CB118 (recovery close to 155%). The high recovery for CB118 can be explained by the fact that it coelutes with CB132 on the CP-SIL5C18 CB type column. However, it is important to emphasize that the assigned errors for PCBs in the mackerel tissue were higher compared to those in cod liver. Generally, the uncertainty of the measurements increases with low concentration levels: noise increases on the baseline of chromatograms, interferences have a more pronounced effect and variability of the results increases. Despite these low recoveries, the *Z*-scores which take into account the assigned errors were less than 2 for all PCBs excepted for CB28 and CB118 and consequently demonstrate the good performances of the protocol.

5.2.2. Non-ortho-PCBs in biota

In this exercise, participants were asked to determine the non-*ortho*-substituted CBs (CB77, 126 and 169) both in cod liver (QPL003BT) and in mackerel tissue (QPL004BT). Because of the very low concentrations of these compounds in environmental samples, the quantitative analysis of the non-*ortho*-PCBs presents several difficulties and requires particular care during the full procedure. Only four laboratories carried out this exercise, thus illustrating the present difficulty to correctly quantify these compounds in complex matrices. The assigned concentrations ranged from 140 to 1250 pg g^{-1} wet mass and from 2 to 51 pg g^{-1} wet mass, for the cod liver and the mackerel tissue, respectively (Table 6B). Fig. 7 shows gas chromatograms of the two fractions (F1a and F1b) obtained after extraction, clean-up and HPLC fractionation of cod liver (QPL003BT). Generally speaking, a good agreement can be observed between our results and the assigned concentrations excepted for the congener CB169 which was overestimated. The high values obtained for this compound could be attributed to its very low concentration and coelution with other compounds or interfering materials not removed during the clean-up step. In our case, results were obtained by GC–ECD after HPLC fractionation. Elution problems might be solved using GC–MS with negative chemical ionization which combines both specificity and high sensitivity [5,40].

5.2.3. PAHs in marine sediment

Similarly, two marine sediments (QPH018MS and QPH019MS) were analyzed using the protocol in order to determine the concentrations of PAHs (Table 6C). Fig. 8 presents the gas chromatogram of the final extract of marine sediment (QPH019MS). The measured concentrations were consistent with the assigned values for all the PAHs in the two sediments. In the QPH018MS sediment, all the PAHs are recovered at good rates ranging from 81.5 to 121.6% with a mean of 105.4±12.2%, except for BkF (149.6%). Similarly, the recoveries of PAHs in the QPH019MS sediment are satisfactory ranging from 82.5 to 115.6% with a mean of 102.1±8.6% excepted for BkF (157.7%) and DahA (68.5%). The high recovery for BkF could be explained by the fact that it coelutes with benzo[*j*]fluoranthene despite the

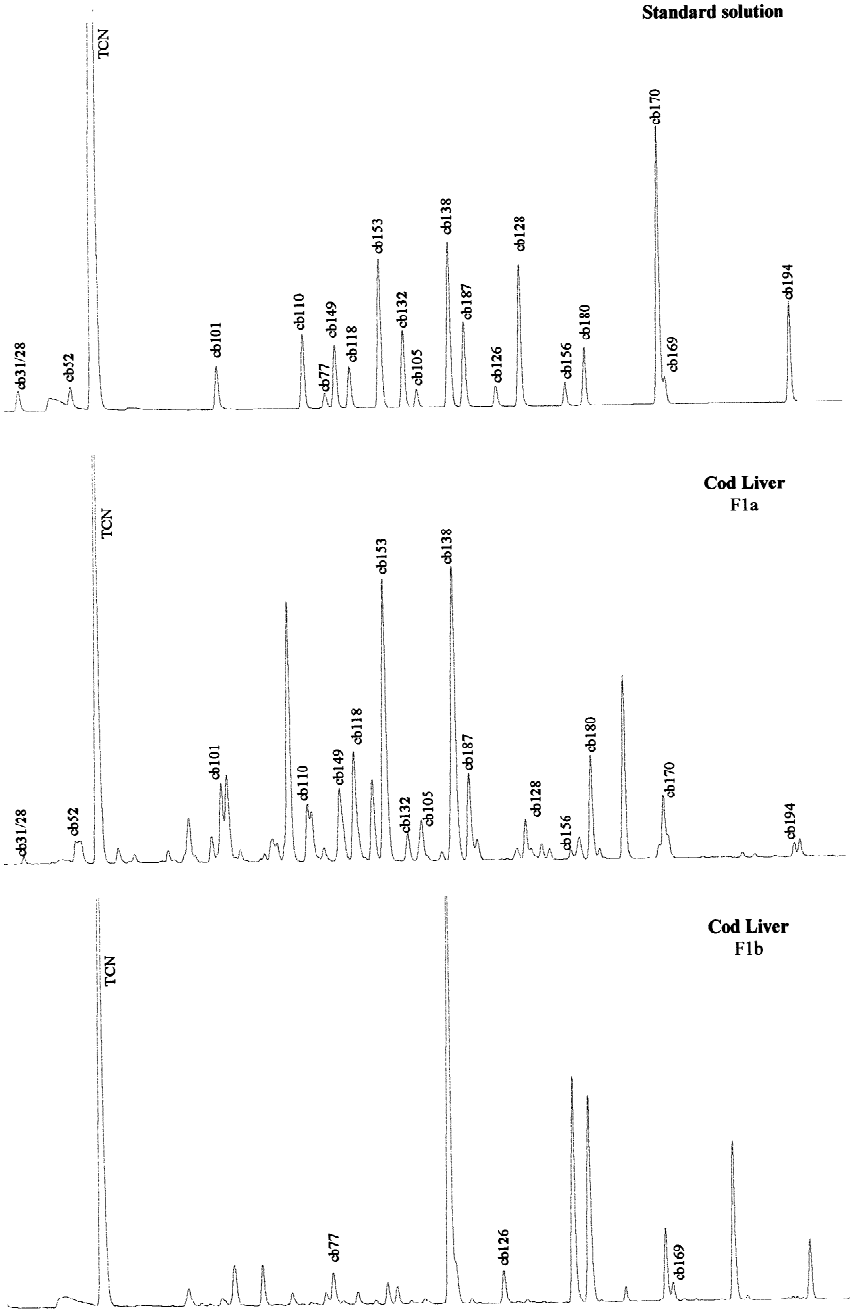

Fig. 7. Gas chromatograms on CP-SIL19 CB of a standard solution of PCBs and the two fractions (F1a and F1b) obtained after extraction, clean-up and HPLC fractionation of cod liver (QPL003BT).

use of two chromatographic columns. However, the large differences of recoveries as well as the high standard deviation found for DahA are difficult to explain. Finally, the relative standard deviation of our measurements close to the assigned errors demonstrate the accuracy of the protocol for the de-

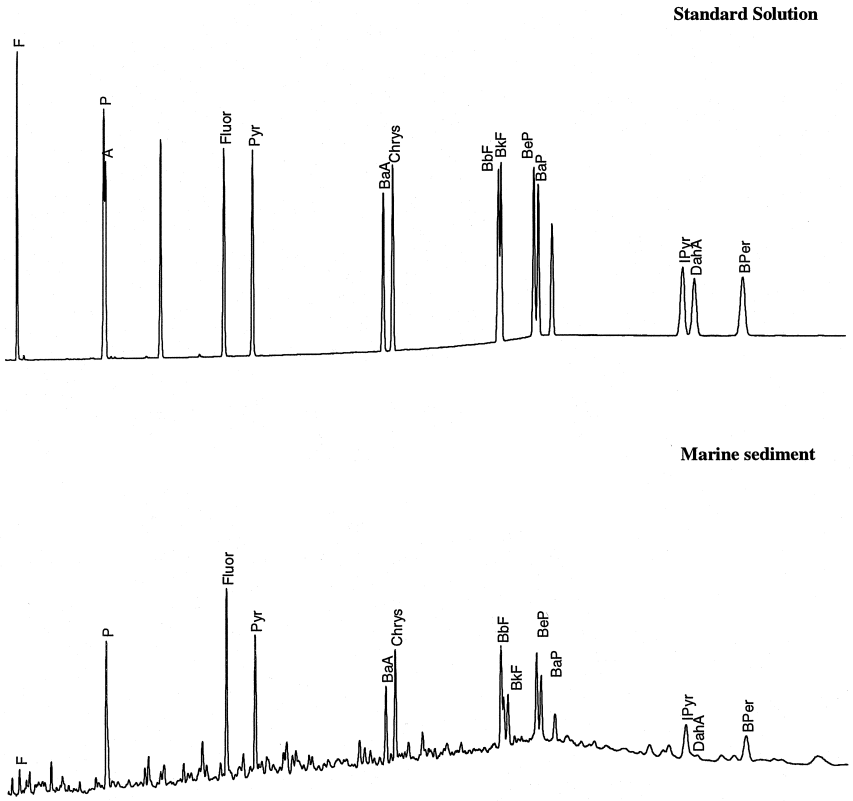

Fig. 8. Gas chromatograms on DB-608 of a standard solution of PAHs and the final extract of marine sediment (QPH019MS).

termination of PAHs in sediments. The satisfactory *Z*-scores, with few exceptions, demonstrate the capacity of our protocol to measure PAHs in sediments.

During the intercomparison exercises, PAHs were only measured in sediments. The analytical protocol remains the same whatever the matrix: sediments or biological tissues. Its applicability to biota mainly depends on the actual PAH concentrations in organisms. Due to their presumed rapid metabolisation, those compounds are present only at very low concentrations in biological tissues. In fact, within our ongoing work on the distribution of PCBs and PAHs in the organisms from the Seine estuary, PAHs were found and these results will be published later.

6. Conclusion

A multi-residue analytical method has been set and validated for the analysis of PCBs, coplanar PCBs and PAHs in environmental samples. This protocol includes the solvent extraction of the samples in a soxtec apparatus, the clean-up of the extract and its separation by adsorption chromatography on an open column into two fractions, the first containing the PCBs and the second PAHs. Next, coplanar PCBs are separated from the other PCBs by HPLC on a PYE column. Finally, the contaminants are identified and quantified by GC–ECD, GC–FID or GC–MS. This protocol enables the determination of these compounds at concentrations as low as 0.5 ng g^{-1} for PCBs, 1 pg g^{-1} for coplanar PCBs in biota and 50 ng g^{-1} for PAHs in sediments with small sample sizes, usually a few grams dry mass. The precision of the method is better than 20% depending on the contamination levels. The participation in international intercomparison exercises have proven the accuracy of this protocol. The multi-residue protocol is less time-consuming than many other protocols. Moreover its use enabling the determination of these three groups of contaminants in

the same sample provides a better information of the chemical contamination and may facilitate the interpretation of data. Further studies will be devoted on the distribution and behavior of PCBs and PAHs in the marine environment using this methodology.

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