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Determination of coplanar polychlorobiphenyl congeners in biota samples

M. D. Pastor, J. Sanchez, D. Barceló and J. Albaigés

Environmental Chemistry Department, CID-CSIC, c/Jordi Girona 18, 08034 Barcelona (Spain)

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ABSTRACT

A method for the separation of coplanar congeners of polychlorobiphenyls (PCBs) (IUPAC Nos. 81, 77, 126 and 169) from other organic micropollutants such as hydrocarbons, organochlorinated pesticides and other PCBs is described. It involves a two-step clean-up procedure with sulphuric acid treatment and LC fractionation (Hypercarb column) and permits the isolation of the coplanar congeners of PCBs in biota samples. The separation of the coplanar PCBs in the Hypercarb column requires only 100 ml of *n*-hexane. Recoveries ranged from 84 to 97% for these and sixteen other PCB congeners. Gas chromatography (GC) with electron-capture detection of all the compounds was performed using capillary GC columns of different polarity, CP-Sil-8 CB or DB-5, DB-17 or DB-225, followed by confirmation by GC–mass spectrometry in the electron impact and selected ion monitoring mode. The whole analytical method permits the determination of individual PCBs in biota samples at a level of 1 ng/g wet tissue.

INTRODUCTION

Residue levels of polychlorobiphenyls (PCBs) have been found in aquatic biota, both continental and marine, as a result of their widespread distribution, environmental persistence and high lipophylicity [1–3]. Among the 209 possible PCB congeners, only *ca.* 150 have been reported in to the total environment. Seven of them (IUPAC Nos. 28, 52, 101, 118, 138, 153 and 180) were selected as the most relevant because of their distribution throughout the chromatogram and coverage of the chlorination range, use in technical mixtures, relative ease of determination and proved toxicity. Consequently, they are commonly mentioned in environmental regulations [4].

In the last few years, increasing attention has been paid to congeners having two *para*-chlorines and at least one *meta*-chlorine that show the same type of toxicity as 2,3,7,8-tetrachloridibenzo-*p*-dioxin (2,3,7,8-TCDD). These congeners are also called "coplanar" PCBs, as they lack *ortho*- and contain *meta*- and *para*-chlorine atoms, thus making a coplanar conformation more probable [3]. They show particularly high "dioxin-like" toxicity, *e.g.*, PCBs with IUPAC Nos. 81, 77, 126 and 169. Considerable toxicity is also attributed to some mono-*ortho*-chlorinated PCBs, especially IUPAC Nos. 105 and 156. Based on toxic and biochemical potencies, the relative toxicity of PCB congeners compared with 2,3,7,8-TCDD may be expressed as toxicity equivalence factors (TEFs). The total "dioxin-like" toxicity of a mixture of PCBs may be calculated as the summation of the concentration of individual toxic congeners times their TEF and it is used in environmental studies [5]. Therefore, to cope with the new requirements of monitoring programmes there is a need for the development of analytical methods for the isolation of the different groups of PCBs. In this sense, for the isolation of the previously mentioned seven PCB congeners there is an extensive literature of analytical methods mainly based on sulphuric acid clean-up, sorption

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Correspondence to: D. Barceló, Environmental Chemistry Department, CID-CSIC, c/Jordi Girona 18, 08034 Barcelona, Spain.

column chromatography with Florisil and silica-alumina [3], gel permeation chromatography [6] and normal-phase high-performance liquid chromatography (LC) [7]. However, for the isolation of the more toxic PCBs, only a few methods have been developed, basically using activated charcoal columns [8–11] and recently developed methods involving 2-(1-pyrenyl)ethyldimethylsilylated silica LC column [12] and Florisil column chromatography [13].

The separation techniques for isolating coplanar PCBs have been reviewed recently [3,8]. Initial methods of isolation used carbon or granular charcoal in various grades and particle sizes. More recently, silica has been coated with graphite as the basis of the commercially available LC Hypercarb column. The separation is based on the retention of planar or near planar molecules by the graphitic surface of the adsorbent. Non-planar molecules are either unretained or have a limited retention, whereas non-*ortho*-substituted PCBs can assume a coplanar configuration more readily than the *ortho*-substituted isomers, allowing a stronger interaction with the planar porous graphitic carbon structure. In general, the existing methods for the isolation of planar PCBs congeners from other PCB congeners have been criticized because large volumes of eluent, *e.g.*, over 1000 ml [14], per sample are needed. In other instances, the use of highly carcinogenic solvents is involved (*e.g.*, benzene) [9,15,16] or the need for multiple solvents, *e.g.*, methylene chlorine, cyclohexane and toluene [17] and light petroleum and toluene [18].

This paper report on the isolation of coplanar PCB congeners from environmental samples with the use of sulphuric acid treatment and a Hypercarb LC column. Further confirmation is achieved by using capillary GC columns of different polarity and/or GC–MS in the electron impact (EI) and selected ion monitoring (SIM) mode.

EXPERIMENTAL

Reagents and apparatus

All solvents (*n*-hexane, dichloromethane and diethyl ether) were of pesticide grade from Merck (Darmstadt, Germany). Alumina, Florisil and silica gel were also obtained from Merck. Analytical-reagent grade individual PCB components (Promochem, Wesel, Germany) with IUPAC numbers are shown in Table 1. For substitution patterns of the different PCBs, see ref. 3.

An eluent flow-rate of 3 ml/min of *n*-hexane was delivered by a Model 64 high-pressure pump (Knauer, Bad-Homburg, Germany) coupled with a UV detector set at 254 nm (Knauer). Standard solutions and biota samples containing coplanar PCBs were injected via a 50- and 400-μl loop, respectively (Rheodyne, Cotati, CA, USA). A Hypercarb (Shandon Scientific, Warrington, UK) porous graphitic carbon (7-μm particle size) LC column (50 × 4.7 mm I.D.) was used. The material has a surface area of about 150 m^2/g, a mean pore volume of 2.0 cm^3/g and a particle porosity of 70%.

Samples of 2 μl were injected in the splitless mode (gas hold-up time = 35 s) with a Hewlett-Packard (Palo Alto, CA, USA) Model 7673A automatic sampler in a Hewlett-Packard Model 5890 capillary gas chromatograph equipped with a ^{63}Ni electron-capture detector at 310°C. The columns used were CP-Sil-8-CB (Chrompack, Middelburg, Netherlands) and DB-5, both containing 5% phenyl–95% methylpolysiloxane, DB-17 containing 50% phenyl–50% methylpolysiloxine and DB-225 containing 50% cyanopropyl–50% phenylpolysiloxane, all from J and W Scientific (Folsom, CA, USA). The chromatographic conditions for each column used are given in the figure legends.

A Hewlett-Packard Model 5995 GC–MS instrument interfaced to a Model 59970C data system was used. The DB-225 GC column was used for GC–MS confirmation of coplanar PCB congeners, with direct introduction into the ion source and with the temperature programmes indicated in the figure legend. Helium was used as the carrier gas (30 cm/s). The ion source and the analyser were maintained at 200 and 230°C, respectively. EI mass spectra were obtained at 70 eV.

Sample work-up

The protocols that can be used for the isolation of coplanar PCBs from other organic compounds are shown in Fig. 1. The general analytical scheme shown is much broader than the results indicated in this paper. A major point of this paper is that using a common method (method A) that permits the determination of a broad range of pollutants such as PCBs, DDTs, hydrocarbons and organochlorine

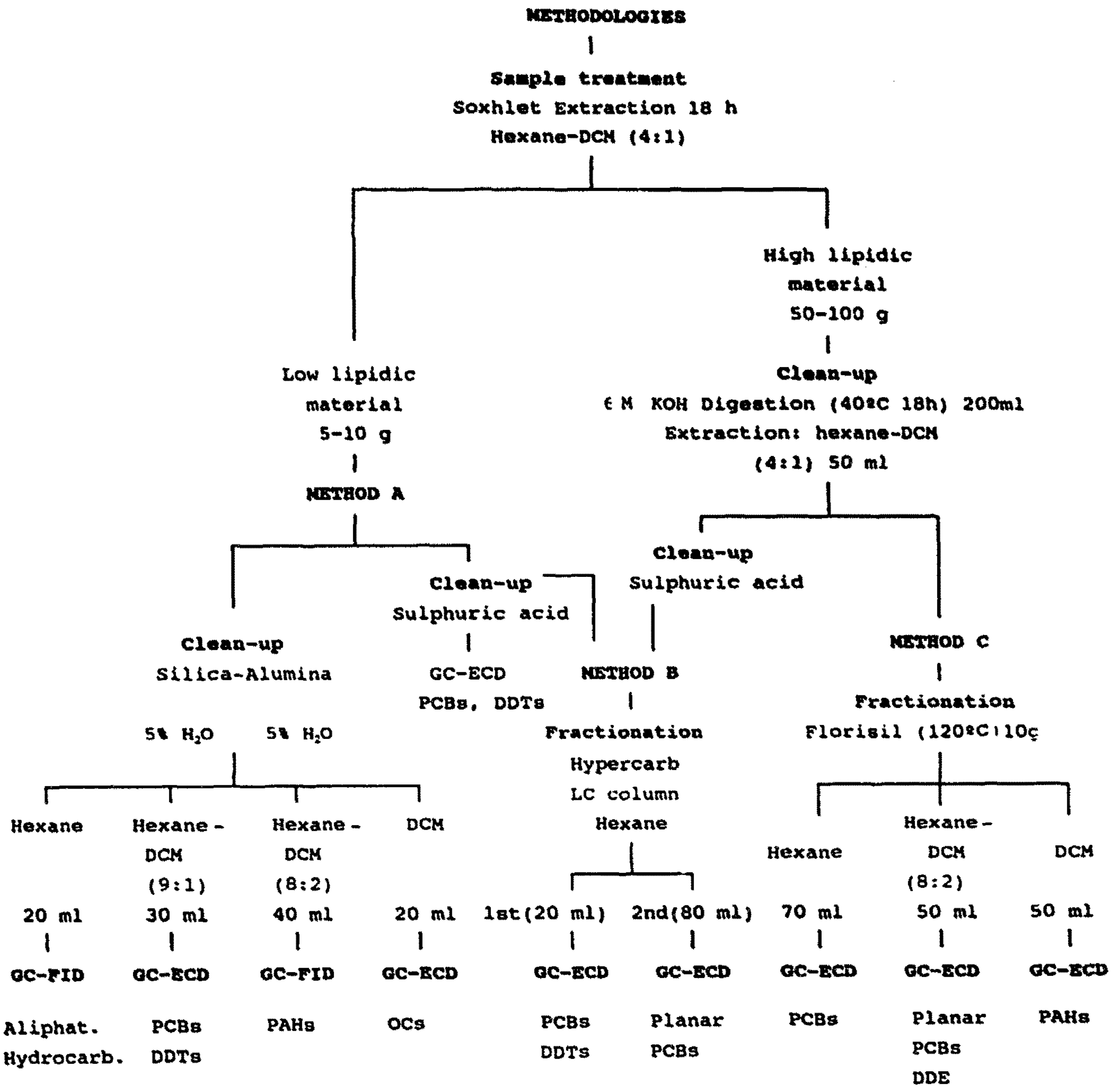

Fig. 1. Analytical methodology used for the isolation of coplanar PCBS from other PCBs and several organic micropollutants. PAH = polycyclic aromatic hydrocarbons; OC = organochlorine pesticides; DDTs = *pp*′-DDT + *pp*′-DDE + *pp*′-DDD; DCM = dichloromethane.

pesticides (OCs), we can easily include in the protocol method B, which is a "daughter" method from method A. Method B should permit the isolation of coplanar PCBs, and also of all the other pollutants. This indicates that although coplanar PCBs are relevant from the point of view of toxicity and can be found in biota samples, we should also not forget the other major organic pollutants that are currently determined in biota samples within the different monitoring programmes.

For the recovery studies, spiking of the samples was carried out prior to the extraction by careful mixing of the standard solution (10 ml of a solution of 50 or 200 ng/ml for coplanar or conventional PCBs) with the sample to be analysed in the Soxhlet apparatus, before extraction and fractionation.

After extraction was completed, the organic extract was dissolved in *n*-hexane (10 ml) and cleaned up by vigorous shaking with 3 ml of sulphuric acid for about 3 min. This extract obtained is injected into a Hypercarb LC column (*ca.* 200 μl) using *n*-hexane as eluent at 3 ml/min. In this way, the coplanars PCBs (IUPAC Nos. 81, 77, 126 and 169) are collected in the second LC fraction between 25 and 110 ml. Subsequently, the different solutions collected were evaporated just to dryness and the residues were dissolved in 250 μl of isooctane and were then ready to be injected into the GC–ECD and/or GC–MS systems for quantification.

Quantitative analysis

External calibration was used with the mixed PCB congeners containing 40–200 pg/μl in isooctane, injecting the samples and standards with the automatic sampler.

The linearity of the detector was determined by injecting a series of PCB congeners at ten different concentrations and plotting the ratio of the peak-height response to the mass of the determinand against the mass injected to determine the true linear range for the determinand. The system was linear between 80 to 400 pg injected.

RESULTS AND DISCUSSION

Studies on recoveries

The recoveries and relative standard deviations (R.S.D.s) obtained for a variety of PCBs obtained using the different methods are indicated in Table I. Most of the PCBs can be easily separated using DB-5 or CP-Sil-8 CB apolar capillary columns. However, the whole separation of all the group of PCBs is not possible even using these stationary phases in a 30- or 50-m column, which are the most widely used for monitoring PCBs in environmental samples. Concurrent analysis with a more polar column has been suggested [19,20]. This is certainly required for the complete separation of congeners 149–118 and 138–163 (IUPAC numbers). In addition, the polar DB-17 GC column also improves the separation between PCBs 153 and 105. Co-elution problems between certain congeners, such as PCBs 138 and 163, were found during a certification exercise, where most of the laboratories were using DB-5-type columns; the two congeners were not resolved, thus making it impossible to certify the PCB 138 congener. Consequently, certification of PCB 138 was indicated as the total amount of PCB 138 + 163 [4]. The separation of this particular mixture was until now only known to be possible either on a very polar SP-2330 (biscyanopropylsiloxane) column [19] and recently on a polar FFAP (polyethylene glycol terephthalate) column [20]. Here we recommend for the first time a DB-17 capillary column for a complete separation of PCB pairs that usually co-elute using DB-5-type columns.

Fig. 2 shows GC–ECD traces of a fish sample, *Sardina pilchardus*, using these two columns. When we compared the columns for a variety of PCBs, we found that the complete separation of PCBs 118 and 149 is only achieved by using the more polar DB-17 column. This result shows that in order to avoid the problems encountered and described above, monitoring programmes involving analysis for a variety of PCBs should include the use of two GC columns of different polarity.

A typical LC separation using the Hypercarb column corresponding to a mixture of PCBs and the coplanar PCBs Nos. 81, 77, 126 and 169 is shown in Fig. 3. In Fig. 4 the GC–ECD traces of (A) the total sulphuric acid extract obtained with method A, (B) the injection of the first fraction and (C) the injection of the second fraction are shown. The GC–ECD traces in Fig. 4A and B are almost identical, which makes it impossible to distinguish the coplanar PCBs. This indicates that (i) when using conventional clean-up procedures with sulphuric acid the toxic PCB congeners cannot be analysed directly, as their peaks are confused with the predominant peaks of other PCBs, and (ii) the amount of toxic coplanar PCB congeners is small compared with the total amount of PCBs and they can only be distinguished on injecting the second fraction (Fig. 4C). Good recoveries are obtained for the conventional PCBs and DDTs obtained in the first fraction, as pointed out by other workers when using carbon column fractionation [11].

Fig. 5 shows the GC–ECD traces of an extract of

TABLE I

AVERAGE RECOVERIES VALUES AND RELATIVE STANDARD DEVIATIONS OF PCB CONGENERS USING THE DIFFERENT METHODS REPORTED IN FIG. 1

Concentrations of PCB congeners varied between 50 and 200 ng/g (n = 5). n.d. = Not detected (below 1 ng/g).

PCB IUPAC No.	Method A				Method C			
	Silica–alumina		Hypercarb column, method B via method A		Hexane		Hexane–CH_2Cl_2	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
28	77	4	80	2	77	7	n.d.	–
44	87	4	86	2	83	5	n.d.	–
52	84	5	85	10	64	9	10	50
61	87	6	85	9	76	9	n.d.	–
77	n.d.	–	92	7	n.d.	–	97	2
81	n.d.	–	94	6	n.d.	–	102	10
101	80	5	97	6	61	9	2	50
105	90	7	93	6	66	8	13	50
118	87	8	89	4	102	5	n.d.	–
126	n.d.	–	90	8	n.d.		96	7
128	97	3	96	9	105	4	n.d.	–
138	85	5	90	6	72	7	4	25
149	90	5	96	5	92	4	n.d.	–
153	90	3	101	6	90	7	1	50
156	91	7	89	9	84	7	3	30
163	85	5	90	5	88	4	n.d.	–
169	n.d.	–	97	7	n.d.	–	95	7
170	93	4	97	9	94	6	n.d.	
180	84	7	99	8	88	4	n.d.	
187	90	5	104	6	88	4	n.d.	

Scomber scombrus spiked at 50 ng/g with coplanar PCBs and 200 ng/g with PCBs obtained using method C. Fig. 5A and B show the profiles obtained after injecting (A) the n-hexane and (B) the n-hexane–dichloromethane fractions. Although the recoveries of the coplanar PCBs 77, 126 and 169 were very high, two problems were encountered: first, coelution of PCB 81 with DDE, and second, the separation of all the PCB congeners is incomplete, so PCBs 105 and 156 are obtained in both the first and second fractions. In addition, other congeners, which are not coplanar, are also obtained in this second fraction, as can be seen in Table II. This indicates that this method does not offer a complete separation of coplanar PCBs from conventional PCBs as does the Hypercarb LC column. In any event, we can agree with a recent paper [13] that recommended the use of Florisil column chromatography for the isolation of coplanar PCBs 77, 126 and 169 without determining PCB 81, which is the one that causes co-elution problems with DDE using this method.

GC–MS confirmation

The use of GC–MS for confirmation purposes in PCB analysis has been pointed out by several workers [9,13,21], who indicated that there is a need to avoid problems related to cross-contamination. The determination of coplanar congeners of PCBs depends on the level of the other co-extractants and it will depend on the biota sample studied. Thus, confirmation by GC–MS is necessary, as there are always several interfering peaks in the GC–ECD traces [9]. Rigorous protocols such as for PCDDs have

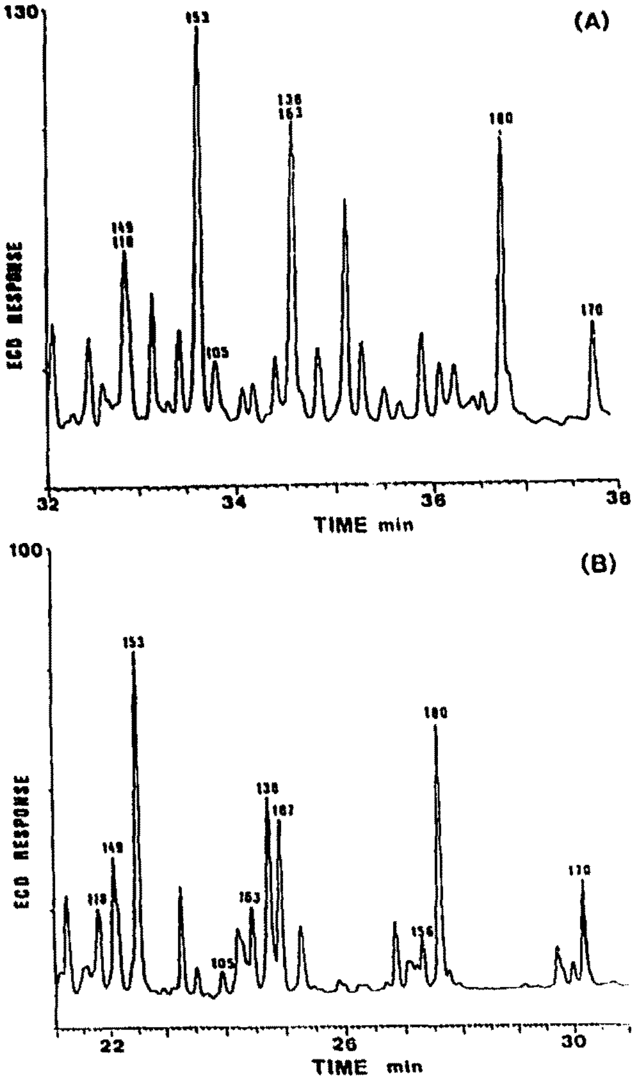

Fig. 2. GC–ECD traces of an extract of biota sample of *Sardina pilchardus* obtained using method A with only sulphuric acid clean-up and with (A) a 30 m × 0.25 mm I.D. 0.25-μm DB-5 capillary column, temperature programmed from 80 to 290°C at 6°C/min, the final temperature being maintained for 10 min, helium as carrier gas at a flow velocity of 30 cm/s, high-purity nitrogen as make-up gas at a flow-rate of 60 ml/min and an injector temperature of 270°C, and (B) a 0.25-μm DB-17 column (30 m × 0.25 mm I.D.) with a temperature programme identical with that for the DB-5 column. Concentrations IUPAC Nos. 118, 149, 153, 138, 187, 180 and 170 were 0.1, 0.2, 4.6, 3.7, 3.5, 1.8 and 0.1, ng/g wet tissue, respectively.

also recently been developed for more toxic PCBs involving isotope dilution GC with high-resolution MS [16].

The main ions used for confirmation purposes in GC–MS with SIM are indicated in Table II. $[M]^+$

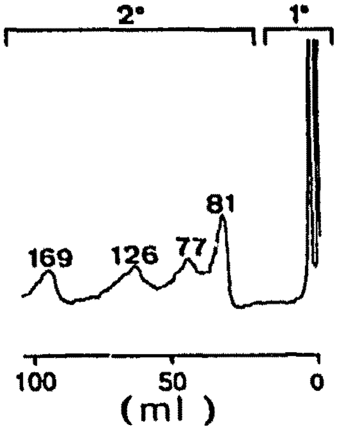

Fig. 3. LC–UV traces of a standard mixture containing 50 ng/ml of several PCBs in an LC Hypercarb column (volume injected, 400 μl). Fractions obtained: (1) corresponding to common PCBs and (2) coplanar congeners of PCBs. UV detection at 254 nm; eluent, *n*-hexane at a flow-rate of 3 ml/min.

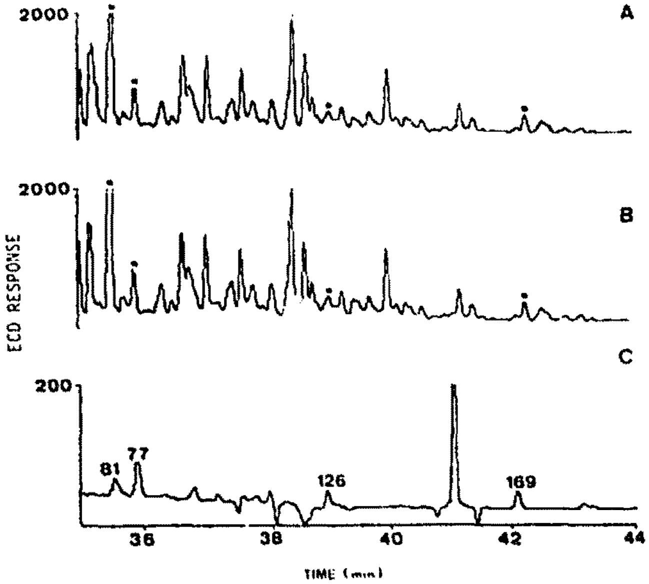

Fig. 4. GC–ECD traces of an extract of herring oil obtained after sulphuric acid clean-up treatment using method A, and before and after passing the sample extract through the Hypercarb column. A capillary column of 50 m × 0.12 mm I.D. coated with 0.25-μm chemically immobilized CP-Sil-8 CB was temperature programmed as for the DB-5 column in Fig. 2. The GC–ECD traces correspond to the injection of (A) the extract obtained after clean-up with sulphuric acid, (B) after collecting the first fraction and (C) after collecting the second fraction of Hypercarb column. Amount of co-planar PCBs congeners in the sample were 2, 3, 2 and 2 ng/g of PCBs No. 81, 77, 126 and 169, respectively.

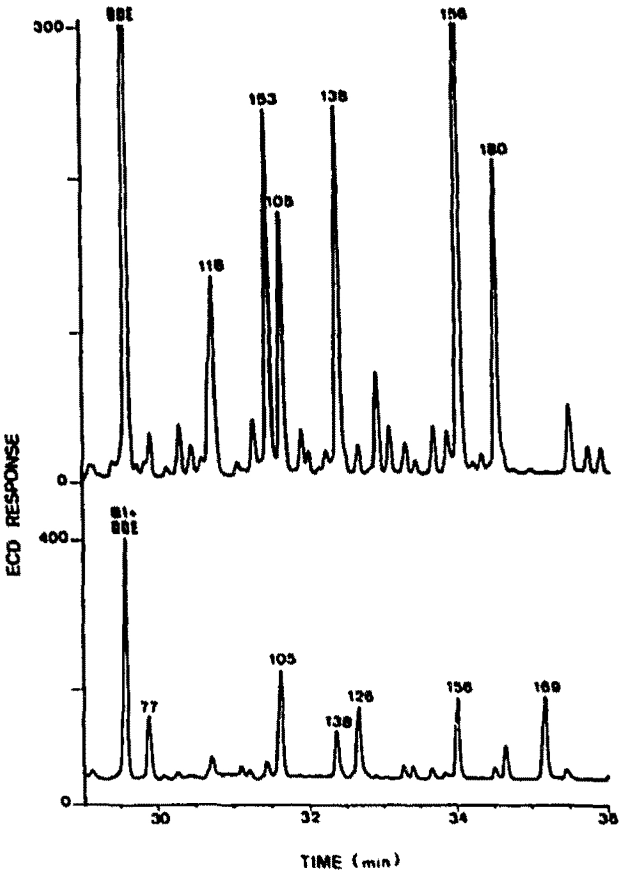

Fig. 5. GC–ECD traces of an extract of *Scomber scombrus* spiked at 50 ng/g with coplanar PCBs and at 200 ng/g with the other PCB congeners obtained using method C (A) and (B) show the profiles obtained after injecting (A) the *n*-hexane and (B) the *n*-hexane–dichloromethane fractions. A DB-5 capillary column was used under the experimental conditions given in Fig. 2.

TABLE II

RETENTION TIMES AND MAIN IONS OF COPLANAR PCBs CONGENERS IN SEVERAL GC COLUMNS

IUPAC No.	Retention time (min)			Main ions (*m/z*)
	DB-5	DB-17	DB-225	
81	35.5	30.0	22.5	290, 292
77	35.9	30.4	24.4	290, 292
126	39.0	32.9	31.5	324, 326
169	42.1	35.1	39.2	360, 362

and $[M + 2]^+$ cluster ions were used as the identification criteria together with the retention times of the pure standards. Fig. 6 shows the GC–MS traces of the fractions obtained in the Hypercarb fractionation procedure (Fig. 4B and C) of a herring fish oil. The retention times of the suspected peaks of the four coplanar PCBs (Fig. 6B) match well with the peaks of an authentic mixture of these congeners (Fig. 6A). Moreover, their relative abundances parallel those of the original chromatogram (Fig. 4C). The higher noise level exhibited by this fraction in the GC–MS analyses is consistent with the very low concentrations of the coplanar PCBs with respect to other components. In fact, coplanar PCB congeners usually occur at concentration levels 10–50 times lower than those of the conventional PCBs. The use of GC–MS with SIM enabled a limit of detection of less than 1 ng/g in the sample analysed to be achieved.

The fraction corresponding to the other PCBs (Fig. 6C) also shows many more peaks in the GC–MS traces than the fraction of coplanar PCB congeners, although the GC–MS analysis was carried out using SIM. This is a common feature when analysing PCB mixtures as all the PCBs with an identical number of chlorines will exhibit similar diagnostic ions, so PCBs with four, five and six chlorines

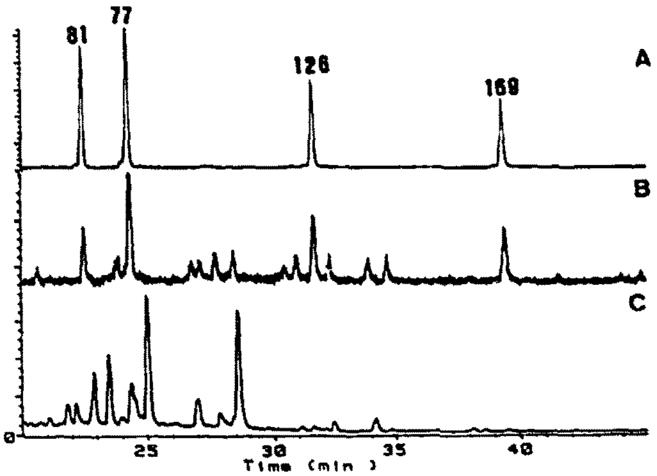

Fig. 6. GC–MS with SIM of (A) standard sample of PCBs 81, 77, 126 and 169, (B) the extract of the same herring oil corresponding to Fig. 4C and (C) that corresponding to Fig. 4B. A 0.25-μm DB-225 capillary column (15 m × 0.15 mm I.D.) was used. The injector temperature was kept at 240°C and the column temperature was programmed from 90 to 150°C at 10°C/min and from 150 to 240°C at 1.5°C/min.

in their structure will exhibit the same diagnostic ions as the coplanar PCB congeners monitored in the samples shown in Fig. 6.

The different coplanar PCB congeners shown in Figs. 4 and 6 were determined in concentrated fish oil. However, when their determination must be carried out in conventional biota tissues, some sample requirements must be considered. The most important is that the amount should be increased *ca.* tenfold to produce an ideal ECD response [22]. This is indicated in the analytical scheme in Fig. 1, with the recommendation of using a larger amount of sample for the isolation of coplanar PCBs.

The method reported involving Hypercarb fractionation provides good recoveries of coplanar congeners of PCBs 81, 77, 126 and 169 and offers a complete separation from the conventional PCBs and other micropollutants, such as organochlorine pesticides. This method can be easily coupled to existing clean-up procedures in most environmental laboratories currently analysing PCB congeners and other organic micropollutants in biota samples. A major criticism of the proposed method may be the re-use of the Hypercarb column in the analysis of large numbers of biota samples. The column used in this work has been used for *ca.* 100 analyses with no problems with its re-use. After each analysis, the column was washed with *n*-hexane and dichloromethane for the elimination of possible interferences. Such a washing step will help in removing possible dioxins, as the same column can be used for isolation of these compounds but eluting with more polar solvents. Although it is partly true that the present method is expensive, as the column contains 7-μm particles with a cost of *ca.* US $1000, *versus* the lower cost of using other particle sizes of 40–100 μm in disposable cartridges, so far we have been able to use the Hypercarb column for 100 analyses, with a column-cost per analysis of US $10, which is about 2–3 time more expensive than using cartridges.

CONCLUSIONS

A general analytical scheme (methods A and B in Fig. 1) that facilitates the isolation of coplanar PCB congeners Nos. 81, 77, 126 and 169 from sixteen other PCBs and several organic micropollutants such as organochlorine pesticides and hydrocarbons has been reported. Concerning the determination of conventional PCB congeners, it has been shown for the first time that a DB-17 capillary column offers a complete separation of PCB congeners 149–118 and 138–163, which cannot be achieved with the DB-5 common.

A two-step clean-up method with sulphuric acid and LC fractionation with Hypercarb has been proposed for the determination of coplanar PCB congeners (IUPAC Nos. 81, 77, 126 and 169). It uses *n*-hexane as the eluent in a small volume (100 ml) and it is safer, as confirmation by GC–MS with SIM is guaranteed. Their determination is feasible at low levels of 1 ng/g in wet tissue. The proposed method is superior to Florisil column chromatography, which showed interferences from other PCBs and organochlorine pesticides, such as DDE.

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