Journal of Chromatography, 456 (1988) 155–164
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 850

SELECTIVE ENRICHMENT PROCEDURES FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS AND POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL SAMPLES BY GEL PERMEATION CHROMATOGRAPHY

P. FERNÁNDEZ, C. PORTE, D. BARCELÓ*, J. M. BAYONA and J. ALBAIGÉS
Environmental Chemistry Department, CID-CSIC, C/Jorge Girona Salgado 18-26, 08034 Barcelona (Spain)

SUMMARY

An improved two-step clean up procedure involving alumina–silica column chromatography and gel permeation chromatography (GPC) of air particulate matter (NBS SRM 1648) and river sediment extracts and a GPC clean up procedure for marine biota samples are described for the determination of polycyclic aromatic hydrocarbons with two to five rings and selected polychlorinated biphenyl congeners, respectively. Bio-Beads SX-12 and SX-3 were used as packing materials. The recoveries obtained varied from 52 to 78% depending on the compound. Quantitative data for NBS SRM 1648 were comparable with those described previously for this sample.

INTRODUCTION

Environmental matrices contain many compounds interfering in the determination of xenobiotics. Consequently, extended time consuming clean up procedures, involving laborious fractionation steps, are required. The isolation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from environmental samples has been performed by liquid–liquid partitioning, by adsorption chromatography using silica gel, alumina, silica–alumina or Florisil, and by high-performance liquid chromatography[1–3]. Gel permeation chromatography (GPC) is an alternative technique to remove the higher-molecular-weight coextractive lipids which so often interfere in the gas chromatographic (GC) analyses of organophosphorus and organochlorinated pesticides, PCBs and PAHs[4–9].

The separation mechanisms in GPC involve adsorption, partition and size exclusion. The predominance of one mechanism over the others is largely determined by the choice of mobile phase and packing pore size. In the case of GPC packings with large pore sizes (1000–2000 molecular weight exclusion), size exclusion and adsorption occurs in the presence of poorly solvating mobile phases. This will be the case when using Bio-Beads SX-3 for analyzing PCBs with different eluting solvents such as cyclohexane, dichloromethane–hexane, dichloromethane–cyclohexane, toluene–ethyl acetate and ethyl acetate–cyclohexane[5,8,9]. On the other hand, when smaller pore sizes (400 molecular weight exclusion) are used in combination with highly polar solvents,

0021-9673/88/$03.50

such as tetrahydrofuran (THF), dimethylaniline (DMA) or dimethylformamide (DMF), only size exclusion predominates[10]. Other examples in the literature include multistep analytical approaches with silica–alumina column chromatography and GPC with Sephadex LH-20[11] and Bio-Beads SX-12[12] to isolate PAHs from selected environmental samples.

The objective of the present study is to test the validation of GPC for selective trace enrichment of PCBs and PAHs in different environmental matrices: air particulate, river sediment and biota. We report the use of two different GPC packing materials Bio-Beads SX-3 and SX-12 for isolation of seven PCB individual congeners (I.U.P.A.C. Nos. 28, 52, 101, 118, 153, 138 and 180) from biota samples and PAHs and sulphur-containing compounds from air particulate matter and river sediments, respectively. A reference material (NBS SRM 1648) was subjected to the same fractionation procedure for PAHs in order to validate the quantitative data.

EXPERIMENTAL

Materials

The solvents, ethyl acetate and cyclohexane, were pesticide grade (SDS, Peypin, France) and THF was HPLC grade (Shepshed, U.K.). Isooctane, dichloromethane and methanol were distilled from glass before use. Alumina and silica gel (70–230 mesh) were supplied by E. Merck (Darmstadt, F.R.G.). Analytical reagent grade PCB individual components (Promochem, Wesel, F.R.G.) were used: I.U.P.A.C. numbers 28, 2,4,4′-trichlorobiphenyl; 52, 2,2′,5,5′-tetrachlorobiphenyl; 101, 2,2′,4,5,5′-pentachlorobiphenyl; 118, 2,3′,4,4′,5-pentachlorobiphenyl; 138, 2,2′,3,4,4′,5′-hexachlorobiphenyl; 153, 2,2′,4,4′,5,5′-hexachlorobiphenyl and 180, 2,2′,3,4,4′,5,5′-heptachlorobiphenyl.

2,3-Dimethylnaphthalene and benzo[*a*]pyrene were obtained from Fluka (Buchs, Switzerland) and chrysene from Scharlau (Barcelona, Spain). Alkyldibenzothiophene isomers were kindly provided by Professor Milton L. Lee (Brigham Young University, Provo, UT, U.S.A.). Other standards were available in our laboratory.

Sample preparation for PCBs

An homogenized mixture of fish tissue (1–2 g) was mixed with 20–30 g of anhydrous sodium sulphate and extracted for 18 h with ethyl acetate in a soxhlet apparatus. The ethyl acetate extract (100 ml) was evaporated just to dryness and the residue was dissolved in 50–100 μl of ethyl acetate–cyclohexane (1:1). Afterwards the fish extracts were injected onto the GPC column using the same eluent.

Sample preparation for PAHs

Air particulate. A 2-g amount of NBS Substance Reference Material 1648 was soxhlet extracted for 48 h using methanol–benzene (1:3) The extracts were concentrated to near dryness in a rotary evaporator, reconstituted up to 1 ml and adsorbed onto 1 g of alumina. The solvent was evaporated under a gentle stream of nitrogen and the mixture was transferred to the top of a glass column (25 cm × 0.9 cm I.D.), slurry packed with 8 g of silica gel and 7 g of neutral alumina (top), previously deactivated with 5% of water. The PAH fraction (AS-3) was eluted with 40 ml of 20% dichloromethane in hexane as described previously[2].

Sediment. A river sediment sample (Besós) was collected with a Van Veen dredge, wrapped in aluminium foil and frozen at −20°C until analysis. The sampling site was located 2 km upstream from the river mouth. A 20-g amount of freeze-dried sediment was extracted by sonication using dichloromethane–methanol (2:1). Sample fractionation was performed as described for air particulate.

Chromatographic analysis

GPC. Eluent delivery was provided by a 64 high-pressure pump (Knauer, Bad Homburg, F.R.G.) coupled with a Vari-chrom UV–VIS detector (Varian, Sunnyvale, CA, U.S.A.) at 254 nm. Samples were injected via a 50-μl loop from Rheodyne (Cotati, CA, U.S.A.). Stainless-steel columns (450 mm × 10 mm I.D.) (Tracer Analítica, Barcelona, Spain) packed with Bio-Beads SX-3 and SX-12 (mesh size 200–400) (Bio-Rad Labs., Richmond, CA, U.S.A.) were used. For the PCB samples, the column was placed in a jacket and thermostatted at 40°C, The eluting solvents pumped, respectively, at 0.5 and 1.0 ml min^{-1} were ethyl acetate–cyclohexane (1:1) for PCBs and THF for PAHs. Five fractions (G1–G5) were collected for PAHs.

GC. GPC fractions were evaporated to dryness, dissolved in isooctane and injected on to a 6000 Vega series gas chromatograph (Carlo Erba, Milan, Italy) equipped with flame ionization (FID), flame photometric (FPD) and electron-capture (ECD) detection. A 50 m × 0.25 mm I.D. CP-Sil 5 CB (Chrompack, Middelburg, The Netherlands) and a 25 m × 0.32 mm MPMS (Alltech, Deerfield, IL, U.S.A.) fused-silica capillary column were used. Hydrogen was the carrier gas at 50 cm s^{-1}. The injector and detector temperatures were held at 300 and 330°C, respectively. The former column was programmed from 60 to 300°C at 6°C min^{-1}, the latter at 15°C min^{-1} to 150°C and then to 270°C at 4°C min^{-1}, keeping the final temperature for 20 min. Quantitation was performed using phenanthrene, chrysene and benzo[*ghi*]-perylene as external standards.

GC–mass spectrometric (MS) analyses were carried out in 5995 instrument interfaced to a 9825A data system (Hewlett-Packard, Palo Alto, CA, U.S.A.). Helium was used as the carrier gas (30 cm s^{-1}). A 25 m × 0.25 mm I.D. CP-Sil 5 CB (Chrompack) fused-silica column was introduced directly into the ion source. Other chromatographic conditions were identical to those described for the GC analyses. The ion source and the analyzer were held at 200 and 230°C, respectively. Spectra were obtained by elctron impact at 70 eV at a scan speed of 0.86 scans s^{-1}.

RESULTS AND DISCUSSION

GPC optimization

PAHs. PAHs were isolated using Bio-Beads SX-12 as the packing material. The column efficiency was determined at different flow-rates (0.25–2 ml min^{-1}) for coronene, the optimum value being 2990 plates m^{-1} at 0.5 ml min^{-1}. The separation mechanism of PAHs under these conditions can be inferred from the fact that the elution volume increases from coronene to naphthalene, thus indicating a predominance of size exclusion. Further, it should be noticed that coronene is coeluted with chrysene indicating that catacondensed structures are more retained than pericondensed ones (Table I). Klimisch and Reese[13] studied the elution behaviour of PAHs in the THF/styrene–divinylbenzene system (Bio-Beads SX-8). They noted that molecular

TABLE I

CHROMATOGRAPHIC CHARACTERIZATION OF STANDARD COMPOUNDS

Column packing	*Compound*	*Spike level**	*Elution volume** (ml)*	*Recovery (%)****
Bio-Beads SX-12	Naphthalene	18.8	26–30	81
	Anthracene	16.6	24–28	88
	Chrysene	11.5	20–24	72
	Benzo[*a*]pyrene	5.0	20–24	70
	Coronene	50.8	18–22	63
Bio-Beads SX-3	PCB No. 28	2.8	22–34	52
	PCB No. 52	2.5	22–34	54
	PCB No. 101	2.3	22–34	73
	PCB No. 118	2.0	22–34	78
	PCB No. 153	1.7	22–34	78
	PCB No. 138	2.0	22–34	78
	PCB No. 180	1.2	22–34	78

* Spike level was in μg for PAHs and in ng for PCBs.
** Measured at a flow-rate of 1 ml min^{-1}.
*** Mean of two determinations.

sieving effects predominated for alkanes and catacondensed PAHs, while pericondensed PAHs were eluted strictly according to adsorption principles. The different behaviour between this chromatographic system and the one used in this work can be basically ascribed to the difference in pore sizes between the columns, enabling adsorption of the larger pore size material.

Recoveries of PAHs having two to seven aromatic rings are listed in Table I. Apparently they decrease as the molecular weight increases, ranging from 63 to 88% at the μg level. Giger and Shaffner[11] reported different results on Sephadex LH-20, eluting with benzene–methanol (1:1), with recoveries exceeding 100%. At present, there is no explanation for these features.

It should be mentioned that Bio-Beads SX-12 could not be used as GPC column packing for isolating PCBs because of the molecular weight exclusion limit (400 daltons).

PCBs. A standard sample containing seven PCB congeners (I.U.P.A.C. Nos. 28, 52, 101, 118, 153, 138 and 180) was analyzed using Bio-Beads SX-3 as the GPC column packing with a molecular weight exclusion limit of 2000 daltons and the experimental conditions indicated above. The collection time was from 22 to 34 min at 1.0 ml min^{-1}. The mean recovery varied from 52 to 78% while the relative standard deviation (R.S.D.) varied from 12 to 15 ($n = 10$) as shown in Table I. Although PCB congeners 28 and 52 exhibited recovery values between 50 and 60%, these are in the range previously reported in interlaboratory studies[4]. Another factor that needs to be taken into consideration is that previously published recovery values using Florisil or silica–alumina column chromatography were referred to total PCBs as, *i.e.*, Aroclor 1254, with values up to 80%[2] and often being lower than in GPC[8]. In addition, using the same experimental GPC conditions for clean up of PCBs, a mixture of PAHs was injected onto the GPC apparatus and exhibited a collection time between 30 and 40 min which interfered with PCBs, using dichloromethane–cyclohexane as previously

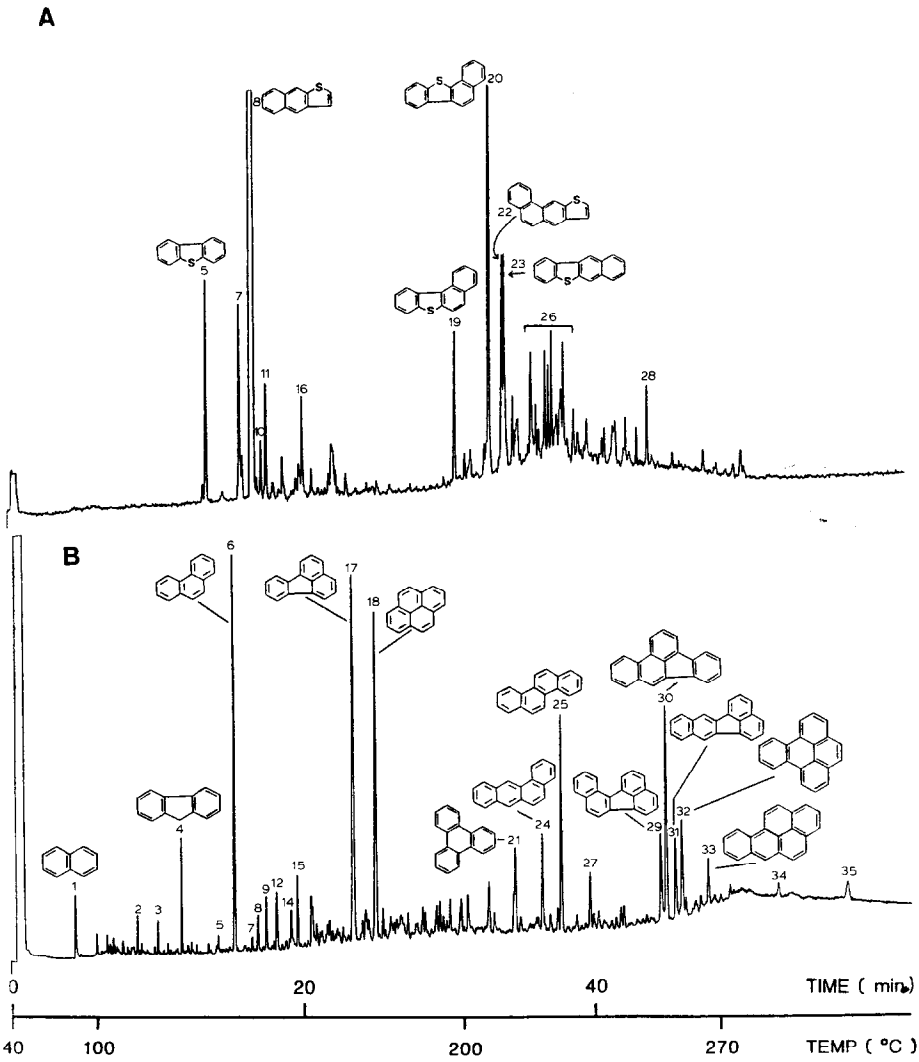

Fig. 1. Gas chromatograms of fraction G-5 from NBS SRM 1648; (A) FPD and (B) FID traces. A 25 m × 0.32 mm I.D. MPMS liquid crystal fused-silica column was used. Compound identification as in Table II.

reported[6]. Therefore the use of Bio-Beads SX-12 offers a better solution for clean up of PAHs, as reported in the present paper.

Environmental matrices

Air particulate sample. Fig. 1 shows the FID and FPD chromatograms of PAH fraction G-5 obtained from an air particulate (NBS SRM 1648). The compound identification and quantitation are given in Table II. Several isomeric pairs that cannot be resolved on isotropic stationary phases were successfully resolved (methyldibenzothiophenes, triphenylene/chrysene, benzofluoranthene isomers). The quantitative data obtained (Table II) are in accord with those reported for this sample[14] except for

TABLE II

PAHs AND S-PACs IDENTIFIED IN NBS SRM 1648

Peak No.	*MW*	*Concentration** ($\mu g\ g^{-1}$)	*Identification*
1	128	0.63	Naphthalene
2	168	0.26	Dibenzofuran
3	172	0.21	C_1-substituted dibenzofuran
4	166	0.75	Fluorene
5	184	0.29	Dibenzothiophene
6	178	4.16	Phenanthrene
7	198	a	4-Dibenzothiophene
8	184	1.40	Napho[2,3-*b*]thiophene
9	192	0.46	9-Methylphenanthrene
10	198	a	2-Methyldibenzothiophene
11	198	a	3-Methyldibenzothiophene
12	192	0.70	1-Methylphenanthrene
13	198	a	1-Methyldibenzothiophene
14	192	0.29	2-Methylphenanthrene
15	204	0.69	2-Phenylnaphthalene
16	208	a	Phenanthro[4,5-*bcd*]thiophene
17	202	5.65	Fluoranthene
18	202	4.63	Pyrene
19	234	a	Benzonaphtho[1,2-*d*]thiophene
20	234	0.54	Benzonaphtho[2,1-*d*]thiophene
21	228	1.20	Triphenylene
22	234	a	Phenanthro[1,2-*b*]thiophene
23	234	a	Benzo[*b*]naphtho[2,3-*d*]thiophene
24	228	1.32	Benz[*a*]anthracene
25	228	3.03	Chrysene
26	248	a	C_1-substituted 234
27	240	0.72	11H-Benz[*bc*]aceantrylene
28	258	a	Chryseno[4,5-*bcd*]thiophene
29	252	1.19	Benzo[*j*]fluoranthene
30	252	3.30	Benz[*e*]acephenanthrylene
31	252	1.14	Benzo[*k*]fluoranthene
32	252	1.32	Benzo[*e*]pyrene
33	252	0.65	Benzo[*a*]pyrene
34	276	a	Indeno[1,2,3-*cd*]pyrene
35	276	a	Benzo[*ghi*]perylene

* Concentrations below 0.15 $\mu g\ g^{-1}$ are indicated with a.

high-molecular-weight components which presented lower values. These results are consistent with the lower recovery data obtained for higher-molecular-weight standard compounds (Table I).

River sediment. The Bio-Beads SX-12 fractionation was evaluated for the corresponding PAH fraction isolated by silica–alumina column chromatography from a sediment river extract (AS-3). This fraction was further fractionated into five subfractions (G-1 to G-5). Fig. 2 shows the reconstructed total ion current of G-2 to G-5 and compound identifications are listed at Table III. Fraction G-1 contained high-molecular-weight components not amenable to recovery under the analysis conditions. In G-2 were identified alkyl and steryl wax esters, with molecular weights

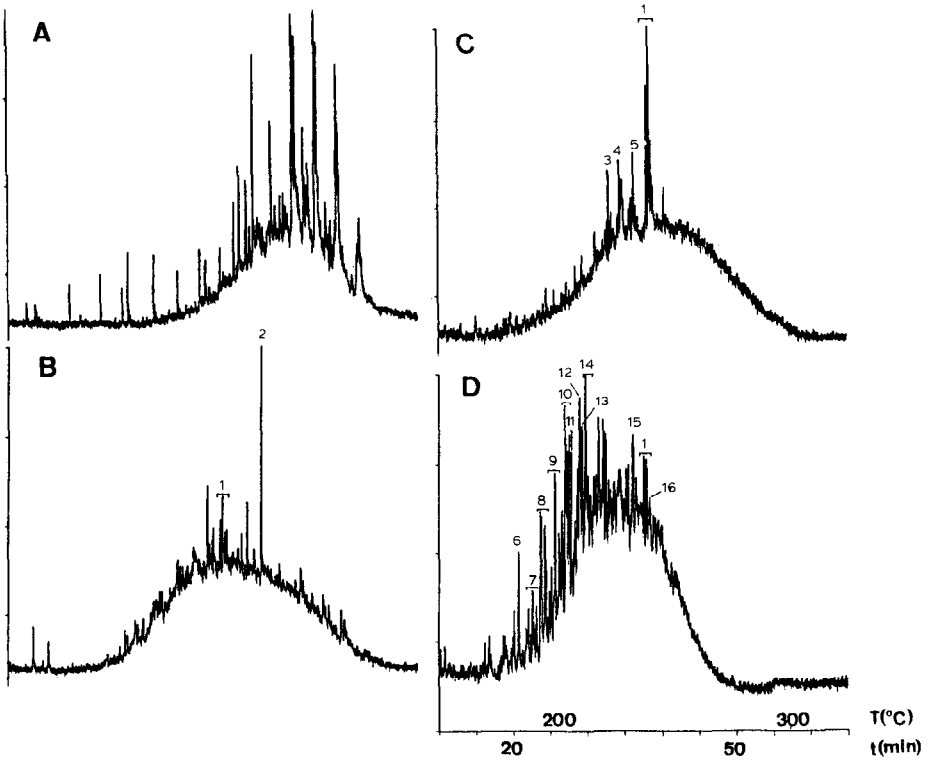

Fig. 2. Total ion current of GPC fractions (A) G-2 (13.2–15.4 ml), (B) G-3 (15.4–17.4 ml), (C) G-4 (17.4–19 ml) and (D) G-5 (19–27.5 ml) eluted with THF at 0.5 ml min^{-1} from fraction AS-3 of a Besós river sediment extract. A 25 m × 0.25 mm I.D. CP-Sil 5 CB fused-silica capillary column was used. Compound identification as in Table III.

TABLE III

COMPOUNDS IDENTIFIED IN A FRACTION FROM BESÓS RIVER SEDIMENT SUBFRACTIONATED BY GPC

Peak No.	*GPC fraction*	*MW*	*Identification*
1	3—5	312	Polystyrene trimer isomers
2	3	410	Squalene
3	4	272	Unknown (*m/z:* 181, 167)
4	4	286	Unknown (*m/z:* 181, 167)
5	4	300	Unknown (*m/z:* 181, 167)
6	5	178	Phenanthrene
7	5	198	C_1-substituted dibenzothiophenes
8	5	192	C_1-substituted phenanthrenes
9	5	212	C_2-substituted dibenzothiophenes
10	5	206	C_2-substituted phenanthrene
11	5	202	Fluoranthene
12	5	226	C_3-substituted dibenzothiophenes
13	5	202	Pyrene
14	5	226	C_3-substituted dibenzothiophenes
15	5	228	Chrysene
16	5	242	C_1-substituted chrysene

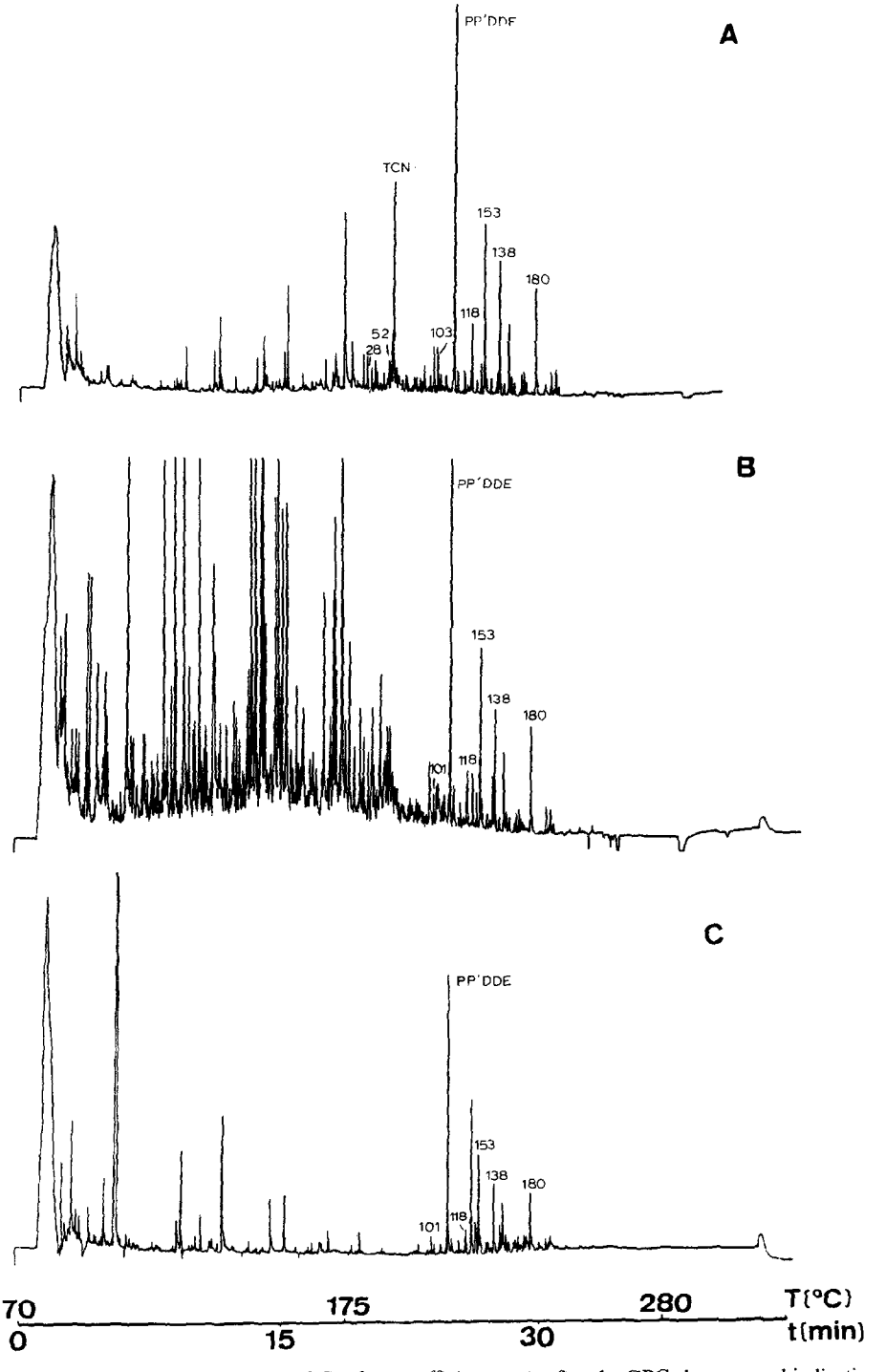

Fig. 3. GC–ECD chromatogram of *Gambussia affinis* extracts after the GPC clean up and indicating the individual PCB congeners. (A) Typical crude extract with the internal standard 1,2,3,4-tetrachloronaphthalene (TCN), (B) Complex crude extract using the same procedure as in (A). (C) Crude extract from (B) with further clean up using sulphuric acid. A 50 m × 0.25 mm I.D. CP-Sil 5 CB column was used.

ranging from *m/z* 424 to 508, whereas in fractions G-3 and G-4 an isomeric mixture of polystyrene trimer isomers in addition to squalene (major component in fraction G-3) were detected. PAHs were eluted entirely in fraction G-5, exhibiting a characteristic profile of fossil hydrocarbons with a great predominance of alkylated over parent components. Sulphur containing PAHs (S-PAC) (C_1-substituted dibenzothiophene isomers) in a relative high concentration with respect to the neutral PAHs were detected for this sample. It should be noted that no interferences were found in the PAHs containing fraction (G-5), although this chemical class was in relatively low abundance.

Biota. GPC in combination with GC–ECD analysis was applied to different biota samples of the mosquito fish *Gambussia affinis* collected during different periods in 1987 at the rice fields of the Ebro Delta (South Barcelona). A typical chromatogram of a fish extract is shown in Fig. 3A. When crude extracts were injected chromatograms as in Fig. 3B were obtained. These profiles can be attributed either to seasonal changes of the samples or to the size and type of sample, exhibiting in some cases higher lipid contents. For these complex mixtures a further clean up is needed for analyzing PCBs with the use of 1–2 ml of sulphuric acid. However, it should be mentioned that the quantitative results for the individual PCB congeners shown in Fig. 3B and 3C are the same. Any difference will be for the compounds eluted before PCBs which are not stable to the treatment with sulphuric acid.

In conclusion, the determination of two- to five-ring PAHs and PCBs from complex environmental samples was successfully achieved by GPC. In this sense, GPC is a good alternative to classical clean up methods for PAHs (*e.g.* saponification), because no destruction of the more polar PAHs occurs. As regards the determination of PCBs from marine biota samples, it has been demonstrated that the technique used in this paper offers valuable clean up and cannot be underestimated *versus* normal phase liquid chromatography fractionation[15].

ACKNOWLEDGEMENTS

Two of us (P.F. and C.P.) were recipients of postgraduate fellowships from Ministerio de Educacion y Ciencia. Financial support of this research was provided by Consejo Superior de Investigaciones Científicas and Comisión Asesora de Investigación Científica y Técnica. J.M.B. is indebted to M.L. Lee for a postdoctoral stage at the Brigham Young University (Provo, UT, U.S.A.).

REFERENCES

1 P. De Voogt, J. C. Klamer and H. Govers, *J. Chromatogr.*, 363 (1986) 407.
2 M. Aceves, J. Grimalt, J. Albaigés, F. Broto, L. Comellas and M. Gassiot, *J. Chromatogr.*, 436 (1988) 503.
3 B. P. Dunn, in A. Bjorseth (Editor), *Handbook of Polycyclic Aromatic Hydrocarbons*, Marcel Dekker, New York and Basel, 1983, pp. 439–458.
4 A. L. Alford-Stevens, J. W. Eichelberger and W. L. Budde, *Environ. Sci. Technol.*, 22 (1988) 304.
5 A. H. Roos, A. J. Van Munsteren, F. M. Nab and L. G. M. Tuinstra, *Anal. Chim. Acta*, 196 (1987) 95.
6 J. F. Lawrence, *Int. J. Environ. Anal. Chem.*, 29 (1987) 289.
7 M. J. Shepherd, in J. Gilbert (Editors), *Analysis of Food Contaminants*, Elsevier, Amsterdam, 1985, pp. 1–72.
8 M. D. Ericksson, *Analytical Chemistry of PCBs*, Butterworth, Stonehaus, MA, 1986, pp. 149–153.

9 L. G. M. Tuinstra, J. J. M. Driessen, H. J. Keukens, T. J. Van Munsteren, A. H. Roos and W. A. Traag, *Int. J. Environ. Anal. Chem.*, 14 (1983) 147.
10 M. L. Lee, M. V. Novotny and K. D. Bartle, in *Analytical Chemistry of Polycyclic Aromatic Compounds*, Academic Press, New York, 1981, pp. 143–152.
11 W. Giger and C. Schaffner, *Anal. Chem.*, 50 (1978) 243.
12 D. L. Vassilaros, P. W. Stoker, G. M. Booth and M. L. Lee, *Anal. Chem.*, 54 (1982) 106.
13 H. J. Klimisch and D. Reese, *J. Chromatogr.*, 67 (1972) 299.
14 S. A. Wise, B. A. Benner, S. N. Chesler, L. R. Hilpert, C. R. Vogt and W. E. May, *Anal. Chem.*, 58 (1986) 3067.
15 G. Petrick, D. E. Schulz and J. C. Duinker, *J. Chromatogr.*, 435 (1987) 241.