3.3. Aloin degradation

As shown above, the aloins (A and B) undergo rapid decomposition in solution; the decomposition is known to be most rapid at basic pH values [14] but also in neutral or acidic solutions the degradation occurs in a short time.

Aqueous, aqueous–alcoholic (30%, v/v) and ethanolic (absolute) solutions of aloe were prepared, and an aloe solution was also added to different bitter beverage samples. From these samples, the degradation kinetics of aloin were obtained by plotting the decrease in the aloin peak area as a function of time. The results obtained are depicted in Fig. 5: after 10 days only 40% of the aloin added to the sample of alcoholic bitter beverage (pH 3.4) remains detectable by HPLC. It follows that, after a brief shelf-life of any aloe-containing solution, it will be impossible to detect the presence of aloin any longer.

3.4. Regulatory aspects

Aloin was included in the EEC list of twelve restricted compounds because of its possible adverse pharmacological effects on consumers, but comparable or even worse effects are attributable to other components of aloe [22,23]. The cathartic effects of aloe in humans were studied by Mapp and McCarthy [22] who stated that "it would appear preferable to replace aloes with aloin for human oral use, thus avoiding the use of resins, aloesin and other variable constituents which differed tremendously in the nearly 100 aloe species examined". That statement was made with reference to pharmaceutical preparations, where it does indeed make sense. In contrast, it does not make any sense to limit in beverages (MAC value 50 ppm) the concentration of a substance (such as aloin) which cannot actually be detected by analysis because of its instability in solution (see above).

In aloe-based beverages, other marker substances with a longer lifetime than that of aloin should therefore be used for monitoring that the concentration of aloe used (which involves the presence of many other pharmacologically active substances) falls in the range of "good manufacturing practice" and is safe for consumers. Chromatograms obtained from a sample of fernet are reported in Fig. 6. Several peaks eluted with the same retention times as those from the aloe solutions (e.g., peaks 3, 11, 27). When recorded, the absorbance spectra of peaks 3 and 11 strongly suggested the presence of aloesin and aloeresin A in the beverage analysed, indicating, as expected, that the beverage was an aloe-based product in which aloin itself was by now absent. We therefore believe that, at least as far as
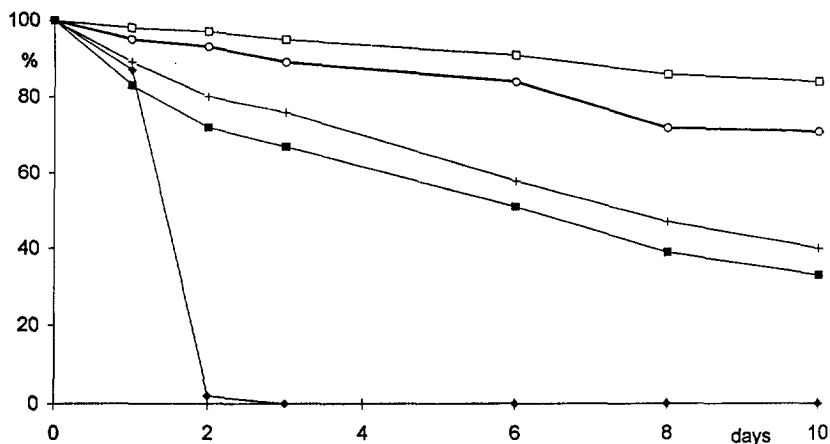

Fig. 5. Degradation kinetics of aloin solutions: percentages of aloin B peak area counts are reported versus shelf-life (days). □ = 100% ethanol at 20°C; ○ = 30% ethanol at 4°C; + = bitter sample at 20°C (pH 3.4); ■ = 30% ethanol at 20°C; ◆ = bitter sample at 20°C (pH 8.1).
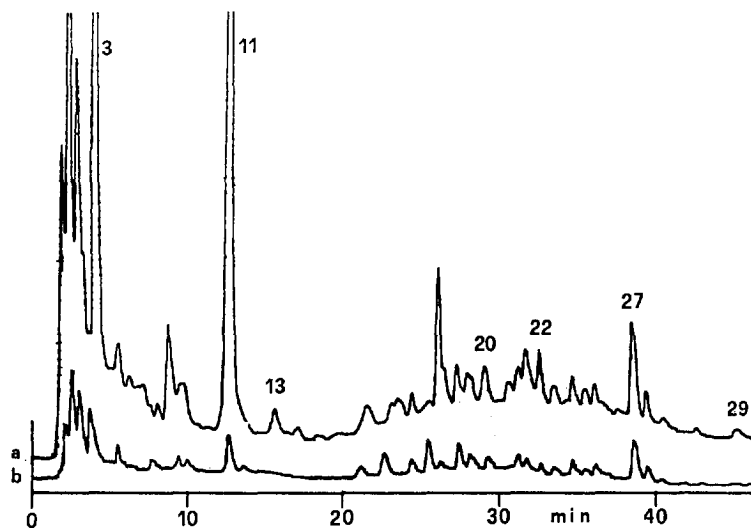
Fig. 6. Chromatograms of an aloe-based alcoholic beverage (fernet): (a) profile superimposed above, detection wavelength 220 nm; (b) profile below, detection wavelength 360 nm. Injection volume, 3 μl. Other chromatographic conditions and peak numbers as in Fig. 2.

beverages are concerned, the EEC list of restricted compounds should be revised and modified, excluding aloin and perhaps including aloeresin A and/or aloesin as aloe markers. Further research is needed, however, to confirm the identities of aloesin and aloeresin A, to improve their determination and to assess their lifetimes in beverages.

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Separation of planar organic contaminants by pyrenyl-silica high-performance liquid chromatography

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Abstract

Planar organic contaminants are known to induce specific biological activity (e.g. aryl hydrocarbon hydrolase and ethoxy resorufin-*o*-deethylase). An unequivocal identification and accurate measurement is essential in order to correlate the potential biological effect to these compounds. Pyrenyl-silica high-performance liquid chromatography allows an effective separation of these planar compounds from most biological co-extractants and from each other. This group of planar compounds includes specific chlorobiphenyls, polycyclic aromatic hydrocarbons, polychloro-dibenzo-*p*-dioxins, polychlorodibenzofurans and polychlorinated naphthalenes.

1. Introduction

The emphasis on the measurement of organic contaminants in marine environmental programmes in relation to observed biological effects has focused on those compounds which exhibit a relatively specific activity [1,2]. One such generic class of compounds is the toxic planar molecules which are known to induce liver microsomal enzyme activity. These compounds induce both aryl hydrocarbon hydrolase (AHH) and ethoxy resorufin-*o*-deethylase (EROD) and have a high affinity to the cytosolic receptor protein [3–6]. Both the toxic mechanism and the enzyme induction involve an initial binding of these contaminants to the same arylhydrocarbon (AH) receptor, and, not surprisingly, these compounds tend to have a similar spatial and structural chemistry [7].

The compound classes known to induce this type of biological activity are the polycyclic aromatic hydrocarbons (PAHs), the polychloro-dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), in particular the 2,3,7,8-substitution pattern, the non-*ortho* and mono-*ortho* chloro-biphenyls (CBs) and the polychlorinated naphthalenes (PCNs). Reports [8,9] on the determination of these compounds in environmental samples have tended to focus on the measurement of single compounds or compound classes while other materials present in the sample extract, including other planar compounds, are regarded as potential sources of interferences and are discarded.

When attempting to relate a specific biological effect, e.g. EROD, to a chemical insult, it is essential to measure all compounds that might have a significant contribution to the effect.

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When an accurate estimate of each individual compound is made, then it becomes possible to assess the relative toxic effect of each contaminant.

Although the final determination of each compound in each class must be specifically optimised, the isolation and separation of each group in a single sample is also necessary to obtain some assessment of the biological impact of these compounds as a whole. This holistic analysis may also be essential when the size of the sample, e.g. dab liver, is very limited and a separate analytical scheme is not an option. A low limit of detection $(1 \cdot 10^{-12}$ to $10^{-15})$ is required for such measurements so that a reliable determination can be made on a single animal. Where this low detection limit is unattainable for a single sample, it may be necessary to resort to the analysis of pooled samples [8,10]. With, perhaps, the exception of some of the PAHs, which tend to occur at relatively higher concentrations, the errors caused by recording false positive values from other interfering materials at the ultra-trace level continues to be a problem. All of these groups of compounds occur as complex mixtures which require careful separation from each other prior to the final determination.

The separation techniques to isolate planar CBs from other contaminants have been fully reviewed by de Voogt et al. [8] and Wells [10]. The initial methods used carbon or granular charcoal in various grades and particles sizes [11,12]. The carbon was used in milligram quantities as a free powder or adsorbed onto foam to offer a greater surface area [13]. More recently, silica has been coated with graphite as the basis of the commercially available high-performance liquid chromatography (HPLC) Hypercarb columns [14,15]. The separation is based upon the retention of the planar, or near planar, molecules by the graphitic surface of the adsorbent. Non-planar molecules are either unretained or have a limited retention, whilst molecules with a "flat" structure similar to tetrachlorodibenzo-*p*-dioxin (TCDD) interlock with the graphite surface and are only removed by backflushing with

a more polar solvent, like dichloromethane or toluene.

The development of the pyrenyl-silica HPLC column has made it possible to separate structurally similar molecules with different π-electron densities resulting from the spatial configuration of the aryl rings [16,17]. This stationary phase gives sufficient resolution between non-*ortho*, mono-*ortho* and the other *ortho*-chloro substituted CBs. Initially this type of column was developed to separate the toxic non-*ortho* CBs (CB 77, 126 and 169), which the carbon columns could achieve, and the mono-*ortho* CBs, which the carbon columns initially could not achieve. However, in addition to the separation of these toxic CBs, it is also possible to improve the resolution between other key CBs which may co-elute on the 5% phenylmethyl gas chromatography (GC) column. This technique, therefore, has the potential to remove a number of ambiguities that can exist in the final determination of CBs using GC [18,19]. Apart from the separation of different structural groups of CBs it is also possible to isolate the other planar contaminants which may co-elute or be compromised by other co-extracted materials.

In this paper we report on a number of key HPLC separations of CBs, and the extension of this technique to cover PCDDs, PCDFs, PCNs and PAHs using the pyrenyl-silica column prior to the final determination of these compounds by GC–electron capture detection (ECD) and GC–mass spectrometry (MS). The quantitative aspects of this technique and its application to the determination of specific CBs in biological matrices are given elsewhere [18,19].

2. Method

2.1. Chemicals

All solvents used were of the highest purity and were obtained as glass distilled grade from Rathburn Chemicals (Walkerburn, UK). The PAHs were obtained as individual pure, solid compounds from the European Union (EU)

Community Bureau of Reference, as were the CBs (CB 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 163, 170 and 180) [20], as either certified or well-characterised materials, which came also from Promochem (Wesel, Germany) (CB 44, 70, 77, 114, 126, 158, 169, 194). The PCDD and the PCDF standards were also obtained from Promochem via The Laboratory of the Government Chemist, London, UK. The PCN mixtures were obtained from the US Environmental Protection Agency. The internal standards (2,4-dichlorobenzyl alkyl ethers) were prepared and characterised in this laboratory [21]. All other materials used in the sample preparation have been reported elsewhere [22,23].

2.2. Liquid chromatography

The CBs were separated using a Cosmosil 5-PYE HPLC column [2-(1-pyrenyl)ethyl-dimethylsilylated silica gel], particle size 5 μm (Nacalai Tesque (Promochem)). The initial HPLC system used consisted of a Gilson 321 autosampler fitted with a 50-μl loop, a Gilson 302 pump and 401 diluter to dispense the sample into the loop. The fractions from the column were monitored with a Philips PU 4020 UV detector set at 254 nm and collected with a Gilson 202 fraction collector. The initial studies were conducted at room temperature (ca. 18–20°C) prior to using a column chiller. The injection and fraction cycle was controlled by the Gilson autosampler and the data collection was controlled by an Apple IIe microcomputer running Chromatochart software. This was superseded by a similar system using a Spectra Physics pump (P200) and autosampler (AS300). The temperature of the pyrenyl-silica column was regulated using a column chiller (Jones Chromatography 7955).

The cleaned-up samples from the alumina and silica [22,23] were concentrated to ca. 80 μl in Chromcol tapered vials and eluted with hexane at a flow-rate of 0.5 ml min^{-1}. Two internal standards, 2,4-dichlorobenzyl hexyl (D_6) and dodecahexyl (D_{16}) ether [24], were added (1 ml

of 1 μg ml^{-1}) to each fraction, the sample was reconstituted in iso-octane and the CB content determined by capillary GC.

2.3. Gas chromatography

The Varian 3500 GC and 8200 autosampler was fitted with a 50 m × 0.22 mm I.D. CPSil 8 CB fused-silica column and a ^{63}Ni electron capture detector. The samples (100 μl) were injected (1 μl) into a splitless injector at 270°C and chromatographed at 80°C for 1 min and at 3°C/min to 280°C using hydrogen as carrier gas at a linear velocity of 40 cm/s. The chromatograph and data collection was controlled a by microcomputer operating Minichrom (VG Data Systems).

3. Results

The k'-values obtained for each of the planar compounds injected into the pyrenyl-silica HPLC column are given in Table 1 and plotted on a log scale for each of the five groups (Fig. 1). These data were obtained by injecting solutions of the initial individual compounds sequentially to obtain the retention volume. The variability of the k'-values were $< \pm 1\%$ over a six-month period for pure calibration solutions and for well-prepared and cleaned-up samples, provided that the column temperature is kept constant. A considerably higher variability occurs in the presence of co-extracted materials such as traces of lipids from sediment or biological tissue. The retention variability was monitored by injecting a series of compounds and comparing the measured and expected k'-values. The normal effect of contamination by lipophilic material was a reduction in k'-value and an increase in variability caused by a partial coating of the pyrenyl-bonded phase, thus reducing the selectivity of the column.

The column performance was restored by flushing with ethyl acetate for a minimum of two hours (normally overnight) at a flow-rate of 0.5 ml min^{-1} followed by 20–30 column volumes of

Table 1
Capacity factors (k') for planar molecules on the pyrenyl HPLC column

Compond	k'	Compound	k'
PAHs		*PCBs*	
Pyrene	0.61	CB 28	0.42
Benzo[b]naphtho(2,1-d)thiophene	0.82	CB 52	0.42
Benzo[b]naphtho(1,2-d)thiophene	0.82	CB 101	0.45
Benzo[a]anthracene	0.91	CB 149	0.48
Chrysene	0.97	CB 153	0.49
Triphenylene	1.09	CB 138	0.63
Benzo[k]fluoranthene	1.29	CB 180	0.63
Benzo[b]fluoranthene	1.44	CB 118	0.70
Benzo[a]pyrene	1.56	CB 163	0.72
Benzo[e]pyrene	1.66	CB 128	0.73
Perylene	1.79	CB 105	0.80
Indeno(1,2,3-c,d)fluoranthene	2.05	CB 170	0.81
Indeno(1,2,3-c,d)pyrene	2.30	CB 156	1.00
Benzo(g,h,i)perylene	2.65	CB 77	1.20
		CB 126	1.74
		CB 169	2.22
PCDDs		*Halonaphthalenes*	
1,3,6,8 TCDD	3.31	DiCNs	0.37
1,2,3,4 TCDD	3.44	TriCNs	0.62
1,3,7,9 TCDD	3.48	TriCNS	0.74
1,2,3,7 TCDD	3.54	TriCNs	0.93
1,3,7,8 TCDD	3.61	TriCNs/TetraCNs	1.24
1,2,7,8 TCDD	3.67	1,2,3,4 TCN	1.25
1,2,3,8 TCDD	3.73	TetraCNs	1.36
1,2,8,9 TCDD	3.94	TetraCNs	1.77
2,3,7,8 TCDD	4.29	PentaCNs	2.47
1,2,8,7 TCDD	5.00	PentaCNs	2.64
1,2,6,7 TCDD	5.00	PentaCNs	2.92
1,2,3,4,7 PCDD	8.20	HexaCNs	3.21
1,2,3,7,8 PCDD	9.02	HeptaCNs	3.35
1,2,3,4,7,8 HCDD	19.27	HeptaCNs	3.76
1,2,4,6,7,8 HCDD	21.01	1,2,3,4,6,7,8,9 OCN	4.32
1,2,3,7,8,9 HCDD	21.01	HeptaCNs	5.05
1,2,3,4,6,7,8,9 OCDD	172.46	HexaCNs	5.96
		HexaCNs	6.46
PCDFs		HexaCNs	7.54
1,2,7,8 TCDF	3.36	HexaCNs	8.68
2,3,4,8 TCDF	3.91	HeptaCNs	9.31
2,3,7,8 TDCF	4.50	1,2,3,4,6,7,8,9, OFN	17.36
1,2,3,8,9 PCDF	8.28	2 Bromonaphthalene	0.39
1,2,3,7,8 PCDF	8.29	2 Bromoanthracene	0.38
2,3,4,7,8 PCDF	13.64	2,9 Dibromoanthracene	1.16
1,2,3,7,8,9 HCDF	16.88		
1,2,3,4,8,9 HCDF	18.89		
1,2,3,4,7,8 HCDF	21.29		
1,2,3,6,7,8 HCDF	21.76		
1,2,3,4,6,7,8 H7CDF	64.62		
1,2,3,4,6,7,8,9 OCDF	145.62		

According to Wells and Echarri [18,19].
Conditions: 150 × 0.46 mm I.D. column; column eluent: hexane, 1 ml/min; temperature 20°C.
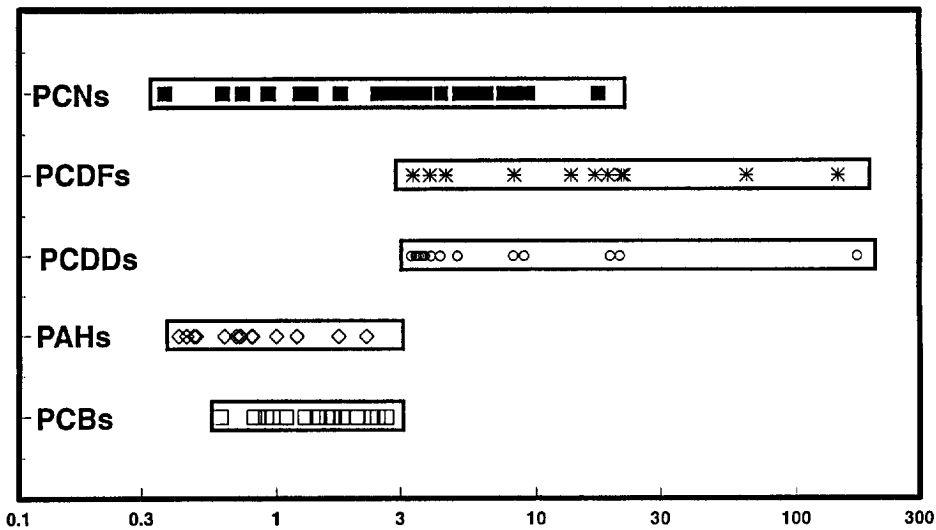
Fig. 1. Plot of log k' (capacity factor) for polychlorinated naphthalenes (PCNs), polychlorodibenzofurans (PCDFs), polychloro-dibenzo-p-dioxins (PCDDs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), obtained on the pyrenyl-silica PYE HPLC column, 150 × 0.46 mm I.D., using n-hexane as eluent at 1 ml min^{-1}.
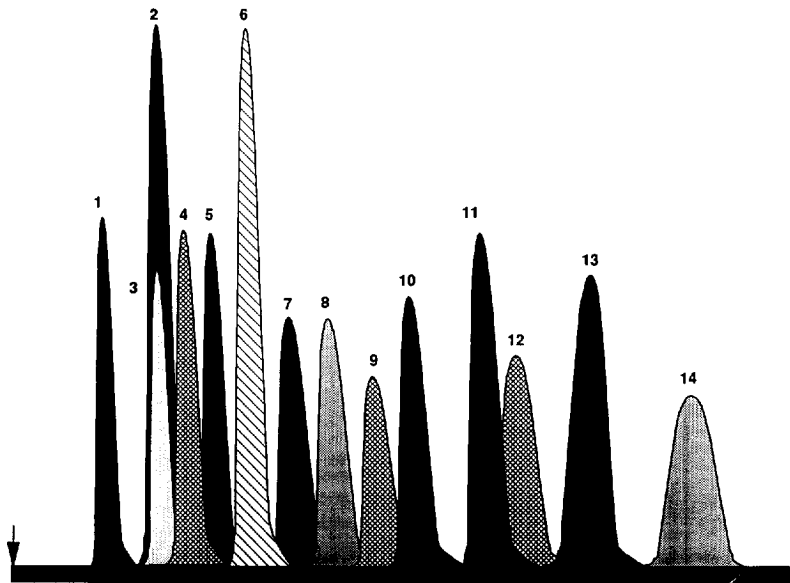

Fig. 2. Reconstructed chromatogram showing the separation of PAHs using the pyrenyl-silica PYE HPLC column, 150 × 0.46 mm I.D., with n-hexane as eluent at 1 ml min^{-1}. Peaks: 1 = pyrene, 2 = benzo[b]naphtho(2,1-d)thiophene, 3 = benzo[b]naphtho(1,2-d)thiophene, 4 = benzo[a]anthracene, 5 = chrysene, 6 = triphenylene, 7 = benzo[k]fluoranthene, 8 = benzo[b]fluoranthene, 9 = benzo[a]pyrene, 10 = benzo[e]pyrene, 11 = perylene, 12 = indeno(1,2,3-c,d)fluoranthene (internal standard), 13 = indeno(1,2,3-c,d)pyrene, 14 = benzo(g,h,i)perylene (see Table 1 for k'-values).

n-hexane to re-establish equilibrium. Using this method of periodic column restoration it has been possible to use the pyrenyl-silica column on a regular basis for some 3–4 years without replacement.

3.1. PAHs

The separation of planar and non-planar PAHs has been reported by Sanders et al. [25]. The PAHs included in this series of experiments were the less volatile >4 fused-ring compounds selected by the EC Measurement and Testing Programme (BCR) for the certification of environmental matrices. The pyrenyl-silica column is not only able to separate the aliphatic hydrocarbons

from the PAHs, but also to isolate some specific PAHs which are each difficult to separate by capillary GC. Two such cases are the separation of chrysene and triphenylene, and benzo[k]- and benzo[b]fluoranthene (Fig. 2). The clean-up of sediment extracts for specific PAH measurement can be highly labour intensive, with a number of separate steps to remove co-extracted materials (Fig. 3). The time required for this method was substantially reduced by transferring the extract to the pyrenyl-silica column after preliminary treatment through an alumina (5 g, 1% w/w water)–silica (5 g 12% w/w water)–copper column. This alternative sample preparation eliminated two additional time-consuming steps using the Sephadex LH20 (20 g) and a further silica column. There was also a considerable improvement in the peak shape of the PAHs in the subsequent GC separation when the extract was passed through the pyrenyl-silica column as a result of the PAHs being separated from other
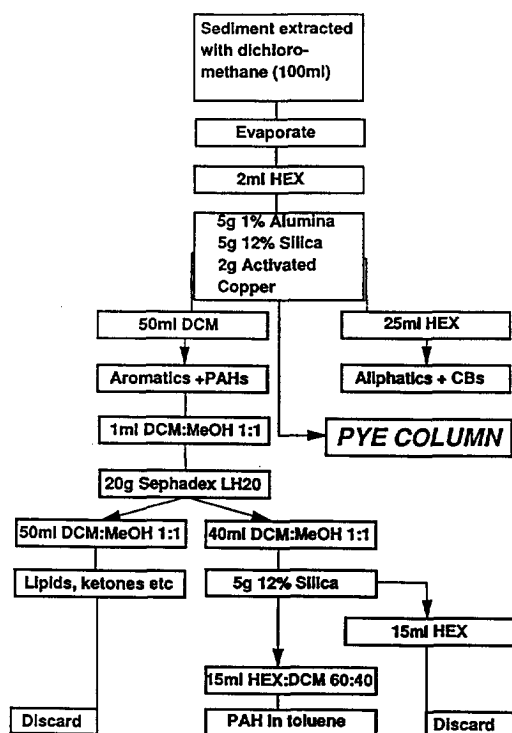

Fig. 3. Schematic diagram for the clean-up and isolation of PAHs from marine sediment. Scheme 1, on the left-hand side, shows the original method used in the SOAFD laboratory. Scheme 2, using the PYE column after alumina–silica clean-up is much more rapid and produces a cleaner extract for subsequent determination by GC or GC–MS.

Table 2
Capacity factors (k') for planar molecules on the pyrenyl HPLC column

Compound	k' at 0°C	k' at 25°C	Fraction (at 0°C)
CB3	1.44		I
CB52	1.58	0.53	I
CB101	1.66	0.59	I
CB149	1.68	0.53	I
CB153	1.77	0.69	I
CB70	1.79		I
CB180	1.87	0.88	I
CB138	2.02	0.84	I
CB128	2.11	0.96	I
CB118	2.16	0.96	I
CB163	2.18	0.96	II
CB114	2.20		II
CB170	2.30	1.10	II
CB105	2.39	1.17	II
CB156	2.78	1.41	II
CB157	2.88		II
CB77	3.28	1.74	III
HCB	3.62		III
CB126	4.76	2.52	III
CB169	5.65	3.24	III

Conditions: 250×0.46 mm I.D. column; column eluent: hexane, 1 ml/min.

co-extractants which affect the GC chromatog-
raphy (Fig. 4). This highly selective separation,
based on the spatial configuration of the de-
terminands, is ideally suited to a method which
subsequently uses a flame ionisation detector
(FID) as the final method of detection.

The one disadvantage was that the perform-
ance of the pyrenyl-silica column was degraded
after some 20–30 sediment samples, but this was
readily restored with ethyl acetate as described
earlier [18,19]. Even with this necessary LC
restoration step the method was more rapid and
produced a cleaner extract for the final GC
analysis than the conventional column clean-up.

3.2. Chlorobiphenyls

The pyrenyl-silica column was initially used to
separate the planar and non-planar CBs [16,17].
With some careful fractionation it was also
possible to isolate the non-*ortho* CBs, the mono-
ortho CBs and the di- and tri-*ortho* CBs from
each other [18,19]. In addition some other useful
separations have been possible, which further
improve the subsequent GC determination of
CB 138, which can be isolated from CB 163
[26,27]. This separation can be further enhanced
by careful control of the column temperature
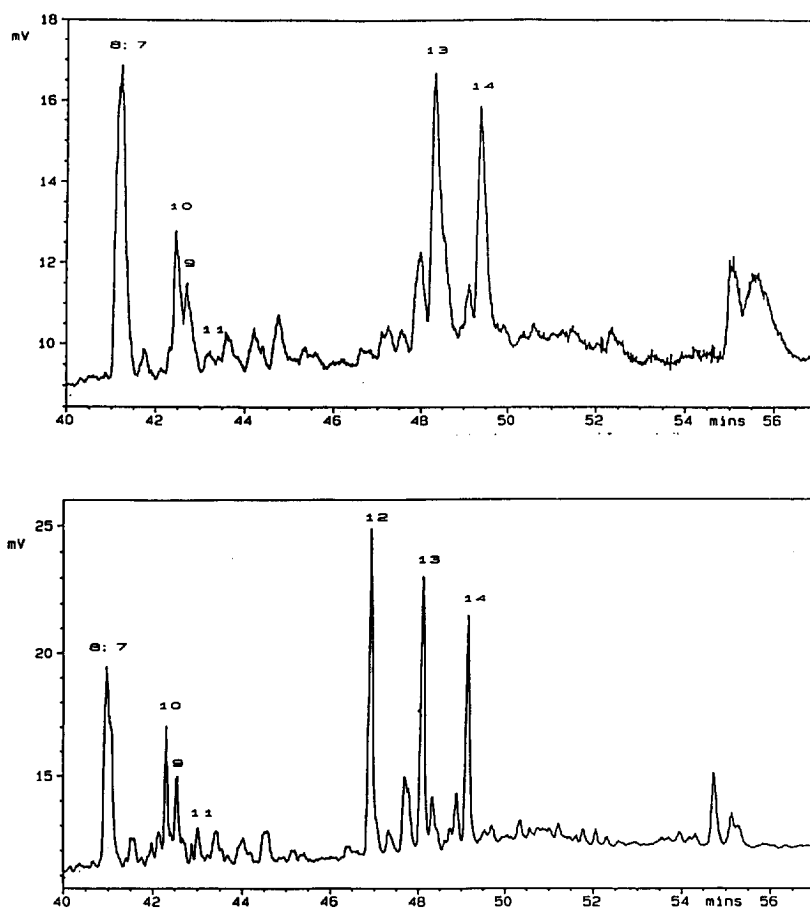(see later). Both of these CBs co-elute on most

Fig. 4. Comparison of the GC–FID chromatograms for PAHs in Firth of Clyde marine sediment cleaned-up using Scheme 1 (top)
and by Scheme 2 using the pyrenyl-silica column (bottom). See Fig. 3 for the clean-up scheme and Fig. 2 for identification of the
peak numbers.

commonly used GC phases, with the exception of the HT5 phase. The mono-*ortho* CB 105 is also separated from CB 132, which is difficult to resolve on a 5% phenyl methyl silicone phase [10]. Clearly, there will be no cutoff volume which will isolate all congeners in one or the other of the fractions. CB 180, for example, is split between fractions I and II when using the 150 mm column (Table 2), but can be isolated in one fraction using the longer (250 mm) column and a lower column temperature of 0°C rather than ambient (ca. 25°C). However, it is possible to adjust the separation of selected CBs on the basis of their spatial configuration into three fractions. The major advantage of such a method is that it isolates the non-*ortho* chloro CBs, which are present at significantly lower concentrations in environmental samples. This allows the fractions to be concentrated in a small

volume to obtain a higher level of sensitivity, particularly for biota where these toxic CBs are significantly metabolised [19]. In addition, some of the mono-*ortho* CBs are also difficult to determine using a single GC column [10]. In particular, CB 156 co-elutes with CB 171 and CB 202 on a 5% phenyl methyl silicone column and with other congeners if the GC column phase is changed. Ideally, it would be better if each of the key toxic CBs were separated on a single column, but, as yet, this seems unlikely. Currently there are two alternative methods. The first is multi-dimensional GC, which heart-cuts the unresolved peaks from one column onto a second column with a different phase which is then able to perform the more simple separations [28,29]. The second is an off-line separation using the pyrenyl-silica column or the PGC HPLC column [10]. In this study the CB
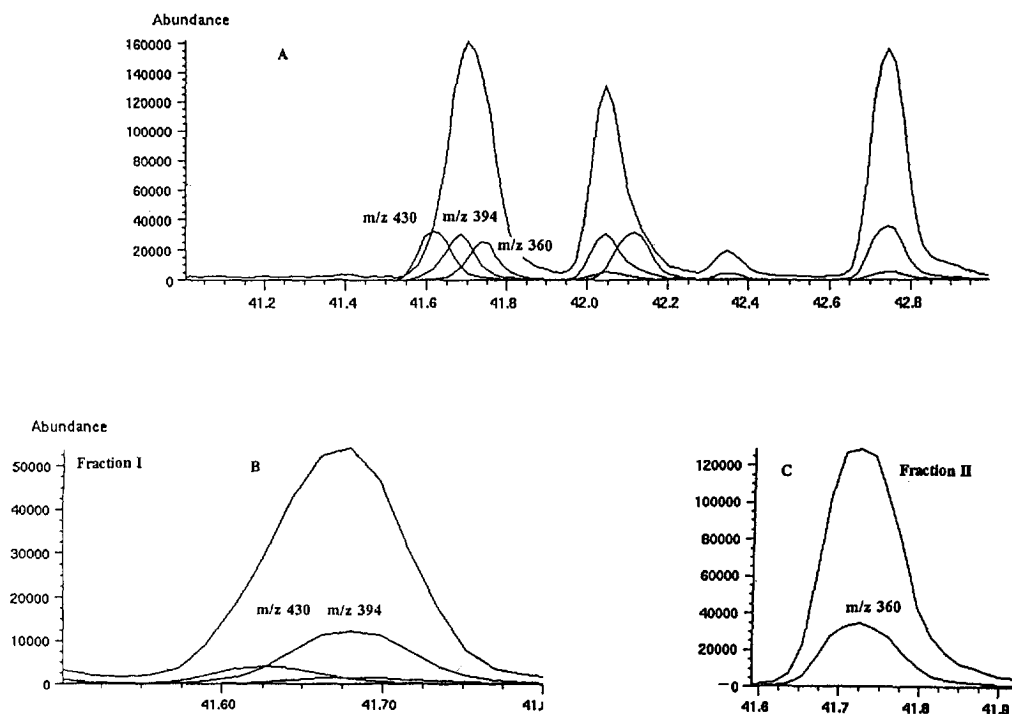

Fig. 5. (A) GC–MS total-ion and single-ion electron impact traces showing the separation of CB 156 (fraction II) from CB 171 and CB 202 (fraction I) using the pyrenyl-silica column. The upper GC–MS trace shows part of the composite mixture from the National Research Council of Canada (NRCC) with CB 156, CB 171 and CB 202 unresolved on a CP Sil 8 50 m column. The lower trace (B) for fraction I contains the CB 171 (*m/z* 394) and CB 202 (*m/z* 430) while the fraction II (C) only contains the CB 156 (*m/z* 360). (Time axis: min)

156 (fraction I) was separated from both CB 171 and CB 202 (fraction II) using the pyrenyl-silica column. This separation was confirmed using GC–MS in the single- and total-ion mode (Fig. 5). The three CBs were traced using m/z 360 for CB 156, m/z 394 for CB 171 and m/z 430 for CB 202. Care is required to fully separate these congeners, which are subsequently to be used to obtain the total burden of toxic CBs. False positive values from unresolved compounds can lead to a significant overestimation of those compounds and any subsequent calculation of toxic equivalent concentrations (TECs) [30].

3.3. PCDDs and PCDFs

Pyell and Garregues [31] have used the pyrenyl-silica column to separate some PCDDs by trapping on the column and backflushing. PCDDs and PCDFs (Cl > 4) can also be separated from both PAHs and CBs using the pyrenyl-silica column (Table 1, Fig. 6). The tetra CDDs and CDFs are isolated in a single fraction having a k' between 2.5 and 5.0. The remaining higher chlorinated PCDDs and PCDFs are eluted in hexane with a k' from 7.5 for the penta dioxins and furans up to k' 172 for the octachlorodibenzo-p-dioxin (OCDD). Some of the retention volumes are usually too excessive for normal isocratic analysis, both in terms of analytical time and the solvent volume of the final fraction. This may be overcome either by a stepwise or gradient elution using ethyl acetate or, less favourably, by backflushing the analytical columns following the elution of the tetrachloro dioxins and furans. Since most of the PCDDs and the PCDFs are subsequently determined by GC–MS in a single injection onto the chromatographic system, it is normally unnecessary to make a further group separation of these determinands. However, where additional group separation of the PCDDs and PCDFs is required, it is possible to obtain the necessary fractions from the pyrenyl-silica column.

3.4. PCNs

Polychlorinated naphthalenes (PCNs) are being used in industry and are present as planar environmental contaminants [32]. Many of these compounds exist as mixtures containing different degrees of chlorination, similar to the PCBs. One of the frequently used industrial formulations is the Halowax series with increasing degree of chlorination; HW 1010 (50% Cl), HW 1099 (52% Cl), HW 1013 (56% Cl) to HW 1014 (58% Cl). Mixtures of these PCNs were dissolved in iso-octane and chromatographed on the
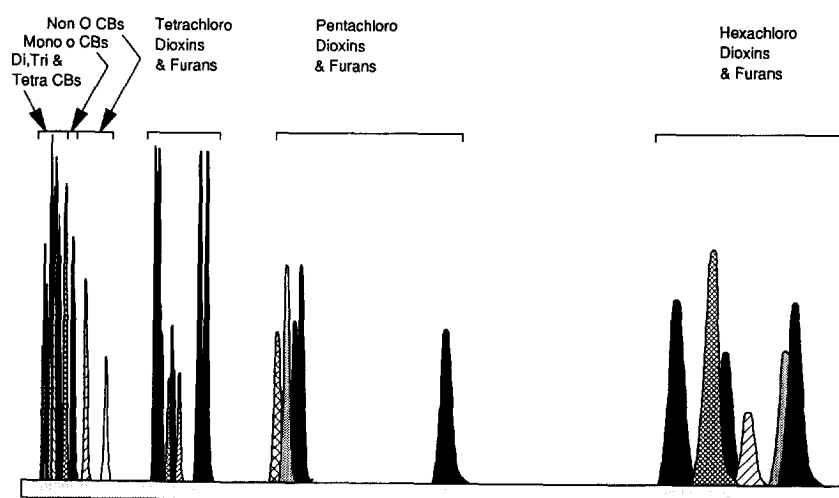

Fig. 6. Reconstructed chromatogram showing the separation of the PCDDs and PCDFs listed in Table 1 on the pyrenyl-silica pyrenyl-silica HPLC column, 150 × 0.46 mm I.D., using n-hexane as eluent at 1 ml min^{-1}. See Table 1 for k'-values.
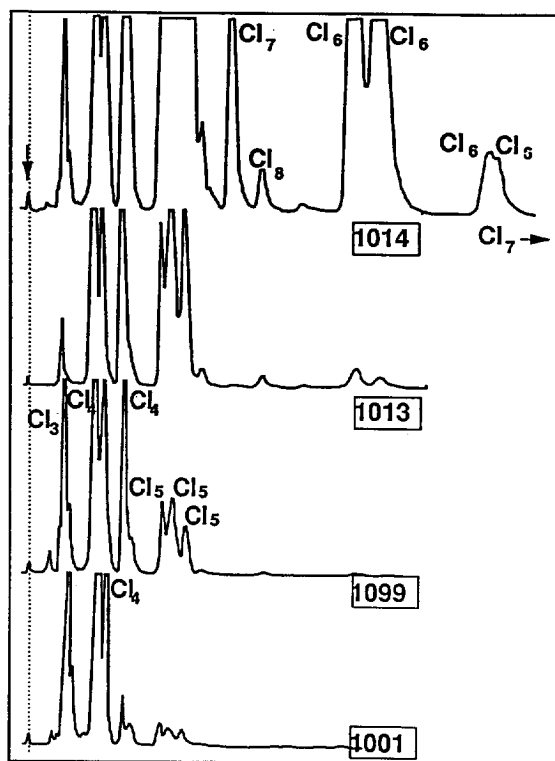
Fig. 7. HPLC chromatogram of the four Halowax (PCN) mixtures with increasing degree of chlorination: HW 1001 (50%), HW 1099 (52%), HW1013 (56%) and HW 1014 (56%). The level of chlorination found in each group was determined by collecting the fractions and re-examining by GC–MS. The separations were made using a 250 × 0.46 mm I.D. pyrenyl-silica HPLC column using *n*-hexane as eluent at 1 ml min^{-1}.

pyrenyl-silica column (Fig. 7). The k'-values for each of the major classes of chlorination are given in Table 1. It was clear from these chromatograms that the the penta- and hexachloronaphthalenes may co-elute in the same fraction as the non-*ortho* CBs (Fig. 1). A series of extracts including Halowax 1014 (Fig. 8, trace A), a cleaned-up extract of a cod liver (trace B) taken from the Firth of Clyde, West Scotland and a standard solution of the non-*ortho* CBs (trace C) were prepared to check these possible interferences. Each sample was injected onto the pyrenyl-silica column and the three fractions collected. Fraction III for each sample was analysed by GC and the chromatograms were compared (Fig. 8) using a 50 m × 0.22 mm CPSil 8 column, film thickness 0.25 μm. From these

chromatograms it can be seen that all three non-*ortho* CBs were well separated from the penta- and hexa-CNs which eluted in this fraction.

3.5. Effect of column temperature

The k'-values for eluants in HPLC are generally temperature dependent. Most reports on the temperature effects of separation have been related to the elevation of the column temperature and the use of column heating ovens. However, the separation of the planar compounds on the pyrenyl-silica column can be improved by reducing the column temperature with a solid-state Peltier heat pump. The Peltier system operates by applying a d.c. voltage to the heat sink, and by reversing the polarity it is possible to obtain a stable cooling effect to ca. 25°C below ambient. Improved separation of CBs at lower column temperatures (0°C) has been observed by Sanders et al. [25].

One critical separation on the pyrenyl-silica column is between CB 138 and CB 163 [18,19,32]. It is difficult to resolve these congeners on most GC phases [24,25], so it is necessary to make this separation prior to the final chromatographic determination. At HPLC column temperatures of around 25°C there is a 5% overlap of these two congeners on the pyrenyl-silica column [19], but when the column temperature is reduced it is possible to improve the resolution and to obtain two completely separate compounds. The resolution increases to a maximum around 0°C, after which there is no further improvement in column performance (Fig. 9). The k'-value continues to increase as the temperature is reduced, but the peak width of the determinands also increases, so the overall resolution begins to decline. A comparison of the k'-values at 25°C and at 0°C is given in Table 2.

4. Conclusions

The pyrenyl-silica column is able to isolate a wide range of planar compounds both from each other and from co-extracted interfering compounds. It is particularly effective in separating