Gas chromatographic determination of polycyclic aromatic compounds with fluorinated analogues as internal standards

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ABSTRACT

In the gas chromatographic determination of polycyclic aromatic compounds (PACs), internal standards (I.S.s) are frequently used in order to correct for losses and fluctuating experimental variables. It is shown that fluorinated PACs exhibit many advantages as I.S.s. Thus, for each parent compound, several standards can be obtained, which means that the optimum I.S. can be selected for each sample so as to avoid co-elution with other sample components. Another advantage is the possibility of adding I.S.s to the sample during different stages of the work-up with the result that the associated losses in each step can be measured in one determination. This work centred on fluorinated naphthalenes, biphenyls and phenanthrenes. Samples included a coal tar (SRM 1597) and a chimney ash.

INTRODUCTION

Owing to the widespread occurrence and well documented carcinogenic properties of many polycyclic aromatic compounds (PACs), this class of compounds has been the focus of much analytical work. Since sixteen polycyclic aromatic hydrocarbons (PAHs), a sub-group of the PACs, have been classified by the US Environmental Protection Agency (EPA) as priority pollutants, efficient and reliable analytical procedures are required for their determination. The complexity of most environmental samples frequently makes sample pretreatment necessary in order to remove matrix and interfering compounds before the analysis can be carried out. Liquid–liquid or solid-phase extractions and adsorption chromatography are typical first steps which can be followed by further clean-up steps if necessary. All such steps possess the inherent risk of leading to losses of all or some of the analytes and should be monitored for this possibility.

Chromatographic techniques are usually employed for the determination of PACs [1] as the large number of parent and substituted PACs present in most real samples makes an efficient separation imperative. Gas chromatography (GC) with highly efficient capillary columns, often coupled with selective detectors, and high-performance liquid chromatography (HPLC), which can be made considerably more powerful with a time-programmed fluorescence detector, are in routine use. Columns which separate all the EPA priority PAHs are commercially available for both techniques. For such determinations, standards must be used, either internal (I.S.) or external, depending on whether the standard compound is added to the sample or not.

Several demands must be fulfilled by com-

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pounds used as I.S.s in chromatography [2,3]: the I.S. and the analytes should have matching chemical and physical properties; the compound used as an I.S. must not be present in the sample; the I.S. should elute close to the analytes in question but resolved from them; the I.S. must not co-elute with any other detectable compound in the sample; the I.S. and analyte should show similar behaviour in the detector; and the I.S. should be obtainable in high purity and be chemically stable.

In many instances, aromatics which are not present in the sample have been used as the I.S. Isotopically labelled PACs have also become popular as I.S.s; several such compounds are commercially available. If a mass-selective detector is used, a PAC containing ^{13}C-labelled atoms can be used with advantage as it makes it

possible to use a compound that is present in the sample, thus in an ideal manner fulfilling the criterion that the I.S. should resemble the analytes as closely as possible. The chromatographic separation of the I.S. from the analyte is not of importance as the detector will record the I.S. and the analyte at two different m/z values.

Perdeuterated PACs have gained prominence for other GC detectors; however, the separation efficiency of the column must be high enough to separate the deuterated and the non-labelled compounds. An example where this is not the case is given in Fig. 1, from the area of polycyclic aromatic sulphur heterocycles (PASHs), which we have extensively investigated [4,5]. PASHs are frequently oxidized to the dioxides in order to separate them from the PAHs. Although perdeuterodibenzothiophene is sufficiently well
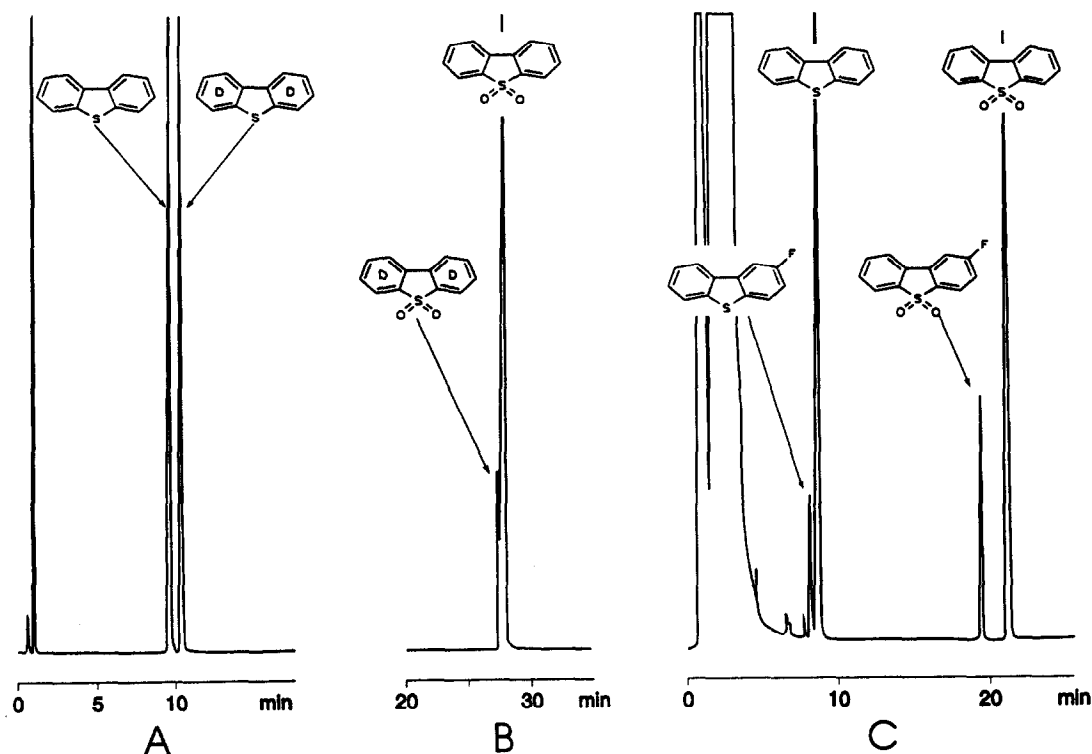

Fig. 1. Gas chromatographic separation of perdeuterodibenzothiophene, perdeuterodibenzothiophene dioxide, 2-fluorodibenzothiophene and 2-fluorodibenzothiophene dioxide from their parent compounds. GC conditions: (A) column, SB-Biphenyl-30, 25 m × 0.32 mm I.D., film thickness 0.25 μm; initial temperature, 100°C for 2 min, increased at 4°C/min; (B) same column as in (A) initial temperature 140°C for 2 min, increased at 4°C/min; (C) column, OV-1701, 25 m × 0.34 mm I.D., film thickness 0.10 μm; initial temperature, 150°C for 2 min, increased at 4°C/min.

separated from dibenzothiophene to be used as an I.S. (Fig. 1A), perdeuterodibenzothiophene dioxide is shown not to be sufficiently resolved from the non-labelled analogue (Fig. 1B). One solution to this separation problem might be to choose a different stationary phase, but this could lead to overlapping peaks in other areas of the chromatogram.

Despite the importance of I.S.s, the number of commercially available compounds suitable for such use is fairly limited. We speculated [6] that a series of benefits would be realizable if the number of potential I.S. could be increased considerably: for each parent compound several I.S.s can be made available; co-elution problems can be minimized, as a suitable I.S. can be chosen that elutes in an area of the chromatogram which is free of sample peaks; for recovery studies during work-up procedures, a different I.S. can be added to the sample before each step and all of them determined together at one time; and if the synthesis and the purification of the I.S. are simple enough, I.S.s for analytes that do not have isotopic analogues available commercially can be easily prepared.

Our first choice was fluorinated PACs, which to a first approximation fulfil the criteria listed earlier. The synthesis of fluorinated PACs has been extensively explored in recent years in connection with studies on the carcinogenicity of PACs and consequently suitable synthetic methods are available. As PACs possess several different carbon atoms that can be substituted, several fluorinated derivatives are possible for each parent. Further, it is conceivable to use di- and higher fluorinated compounds also, provided that they fulfil the other criteria.

The first example studied was that of 2-fluorodibenzothiophene (2-F-DBT) and its sulphone in order to see whether it might be a better choice than the deuterated analogues. As can be seen in Fig. 1C, 2-F-DBT shows about the same resolution from DBT as does perdeutero-DBT, but the resolution between the sulphones of 2-F-DBT and of DBT is much superior to that between the sulphones of perdeutero-DBT and DBT. This observation led to the following study which was undertaken in order to explore the gas chromatographic properties of fluorinated PACs

(F-PACs) and their use as I.S.s for the gas chromatographic determination of PACs.

EXPERIMENTAL

Chemicals were purchased from Fluka or Aldrich and Nanograde solvents from Promochem. The F-PACs were synthesized in a Wittig reaction from appropriately substituted fluorobenzylphosphonic acid diethyl esters with a fluorinated benzaldehyde [7]. The resulting stilbene was photocyclized to a phenanthrene [8]. As most of the compounds synthesized have not been described before, they were fully characterized by spectroscopic techniques. Those data will be published elsewhere. The compounds synthesized are listed in Table I and are denoted G–P.

Table II shows the GC conditions used for the various measurements. The columns are listed in Table III. In all instances hydrogen was used as the carrier gas. The temperature programme was as follows: 80°C for 2 min, increased at 4°C/min to 270°C and held at the final temperature for 5 min. Reference solutions were prepared in toluene at a concentration of 50 ng/μl. The retention indices were calculated according to ref. 9. As some F-PACs co-eluted with phenanthrene, their retention indices were measured using benzothiophene and dibenzothiophene as retention standards and then calculated with the equation

$$I(x) = \frac{t(x) - t(\mathrm{BT})}{t(\mathrm{DBT}) - t(\mathrm{BT})}$$
$$\cdot [i(\mathrm{DBT}) - i(\mathrm{BT})] + I(\mathrm{BT}) \quad (1)$$

where I = retention index, t = retention time, x = investigated compound, BT = benzothiophene and DBT = dibenzothiophene. The means of at least three injections are given in Table II. The standard deviations are given in the last column in Table II.

SRM 1597

An aliquot of a freshly opened ampoule of the National Institute of Standards and Technology (NIST) standard reference material SRM 1597 Coal Tar was weighed and a known amount of a

TABLE I

RETENTION INDICES OF SOME PACs AND F-PAHs ON VARIOUS STATIONARY PHASES

Columns as in Table III.

Peak	Compound	Formula	Retention index					Molar response[a]
			Column 1	Column 2	Column 3	Column 4	Column 5	
A	1-Fluoronaphthalene	$C_{10}H_7F$	200.11	200.19	199.50	197.62	198.43	1.03
B	Octafluoronaphthalene	$C_{10}F_8$	186.73	189.20	193.65	174.01	162.99	0.96
C	2-Fluorobiphenyl	$C_{12}H_9F$	232.03	231.69	227.97	228.76	226.44	1.24
D	4-Fluorobiphenyl	$C_{12}H_9F$	233.89	233.59	230.29	229.16	230.04	n.d.[b]
E	2,2'-Difluorobiphenyl	$C_{12}H_8F_2$	229.19	229.47	226.89	226.93	226.78	1.22
F	4,4'-Difluorobiphenyl	$C_{12}H_8F_2$	233.78	234.08	231.82	227.52	233.87	n.d.
G	1-Fluorophenanthrene	$C_{14}H_9F$	298.36	298.47	297.48	295.26	294.92	1.36
H	3-Fluorophenanthrene	$C_{14}H_9F$	297.01	297.18	297.45	293.84	296.87	1.41
I	1,2-Difluorophenanthrene	$C_{14}H_8F_2$	298.53	299.09	298.93	293.11	293.78	n.d.
K	1,3-Difluorophenanthrene	$C_{14}H_8F_2$	290.35	290.06	287.95	281.59	283.25	n.d.
L	1,6-Difluorophenanthrene	$C_{14}H_8F_2$	294.59	295.04	294.41	288.99	291.80	n.d.
M	2,4-Difluorophenanthrene	$C_{14}H_8F_2$	290.51	290.23	287.72	282.81	282.97	n.d.
N	3,6-Difluorophenanthrene	$C_{14}H_8F_2$	298.87	294.34	295.42	287.08	294.92	n.d.
O	2,4,6-Trifluorophenanthrene	$C_{14}H_7F_3$	288.88	288.87	287.38	277.79	280.76	1.37
P	1,3-Difluorobenzo[c]-phenanthrene	$C_{18}H_{10}F_2$	n.d.	n.d.	n.d.	370.67	n.d.	1.79
1	Naphthalene	$C_{10}H_8$	200.00	200.00	200.00	200.00	200.00	1.00
2	Benzothiophene (BT)	C_8H_6S	200.92	201.34	201.97	204.10	207.63	0.79
3	2-Methylnaphthalene	$C_{11}H_{10}$	n.d.	n.d.	n.d.	215.87	n.d.	1.10
4	1-Methylnaphthalene	$C_{11}H_{10}$	n.d.	n.d.	n.d.	220.67	n.d.	1.06
5	Biphenyl	$C_{12}H_{10}$	233.78	233.46	229.87	231.80	228.73	1.20
6	Acenaphthylene	$C_{12}H_8$	n.d.	249.19	n.d.	247.46	249.77	n.d.
7	Dibenzofuran (DBF)	$C_{12}H_8O$	n.d.	257.02	n.d.	254.77	256.99	1.12
8	Fluorene	$C_{13}H_{10}$	n.d.	268.37	n.d.	266.28	264.34	1.28
9	Dibenzothiophene	$C_{12}H_8S$	295.77	295.78	295.09	296.29	295.17	1.21
10	Phenanthrene	$C_{14}H_{10}$	300.00	300.00	300.00	300.00	300.00	1.32
11	Anthracene	$C_{14}H_{10}$	301.61	301.54	301.48	300.61	300.41	1.38
12	2-Methylphenanthrene	$C_{15}H_{12}$	n.d.	n.d.	n.d.	314.65	n.d.	1.52
13	4,5-Methylenephen-anthrene	$C_{15}H_{10}$	n.d.	n.d.	n.d.	320.88	n.d.	n.d.
14	1-Methylphenanthrene	$C_{15}H_{12}$	n.d.	n.d.	n.d.	319.96	n.d.	n.d.
15	Fluoranthene	$C_{16}H_{10}$	n.d.	n.d.	n.d.	341.58	n.d.	1.57
16	Pyrene	$C_{16}H_{10}$	n.d.	n.d.	n.d.	351.05	n.d.	n.d.
17	Benz[a]anthracene	$C_{18}H_{12}$	n.d.	n.d.	n.d.	395.63	n.d.	n.d.
18	Chrysene	$C_{18}H_{12}$	400.00	400.00	400.00	400.00	400.00	1.73

[a] Determined on column 4 (see Table III).
[b] n.d. = Not determined.

solution of the I.S. was added. Following dilution with toluene the solution was injected into the gas chromatograph five times. The analytes were quantified by comparison of the peak areas with that of the nearest eluting I.S. and final calculation by means of the following equation, using response factors determined previously:

$$w(x) = \frac{m(x)}{m(\text{SRM})}$$

$$= \frac{ar(x)}{ar(\text{I.S.})} \cdot \frac{rf(\text{I.S.})}{rf(x)} \cdot \frac{m(\text{I.S.})}{m(\text{SRM})} \qquad (2)$$

where w = concentration, m = mass, ar = peak

TABLE II

EXPERIMENTAL CONDITIONS FOR GC MEASUREMENTS AND STANDARD DEVIATIONS OF THE PARAMETERS MEASURED

Parameter	Apparatus	Injection technique	Column	Number of injections	Standard deviation
Retention index	Delsi Di200	Splitless	1[a]	3–6	≤0.08 i.u.
	Delsi Di200	Splitless	2[a]	3–6	≤0.12 i.u.
	HP 5890 II	On-column	3[a]	4–8	≤0.05 i.u.
	HP 5890 II	On-column	4[a]	4–8	≤0.05 i.u.
	Delsi Di200	Splitless	5[a]	3–6	≤0.12 i.u.
Response	HP 5890	On-column	4[a]	3	≤3%
	Carlo Erba FV 2300 AC	Splitless	BP-5, 25 m × 0.22 mm I.D., 0.25 μm	5	≤4%
Quantification, SRM	Carlo Erba FV 2300 AC	Splitless	BP-5, 25 m × 0.22 mm I.D., 0.25 μm	5	See Table IV
Quantification; chimney ash	Carlo Erba FV 2300 AC	Splitless	BP-5, 25 m × 0.22 mm I.D., 0.25 μm	3	See Table IV

[a] See Table III.

area, rf = response factor, x = analyte, I.S. = internal standard and SRM = SRM 1597.

Chimney ash

A flow-chart of the work-up procedure is shown in Fig. 2. A sample of 100 g of chimney ash from a paper- and wood-burning home furnace was ground in a mortar and known amounts of three I.S.s were added (see Fig. 2). After Soxhlet extraction with benzene (48 h) and

volume reduction by rotary evaporation, two additional I.S.s were added. After separation of the long-chain aliphatics and the PACs from polar compounds on an alumina column with benzene as the eluent, separation of the aliphatics and PACs was done by normal-phase HPLC on an aminopropyl-bonded 5-μm LiChrosorb NH₂ column (250 mm × 20 mm I.D.) with hexane as the eluent for the aliphatics and hexane–methylene chloride (96:4, v/v) for

TABLE III

PROPERTIES OF THE COLUMNS USED

| Column No.	Stationary phase	Polarity[a]	Length (m)	Inner diameter (mm)	Film thickness (μm)	Phase ratio, β
1	PB-1, polydimethylsiloxane	222	50	0.32	0.20	400
2	DB-5, methyl–phenylsilicone (95:5)	334	30	0.32	0.25	320
3	OV-1701, cyanopropyl–phenyl–methylsilicone (5:7:88)	789	30	0.20	0.15	330
4	DB-17, methyl–phenylsilicone (50:50)	884	30	0.32	0.25	320
5	DB-WAX 20M, polyethylene glycol	2308	30	0.32	0.25	320

[a] Sum of McReynolds constants.
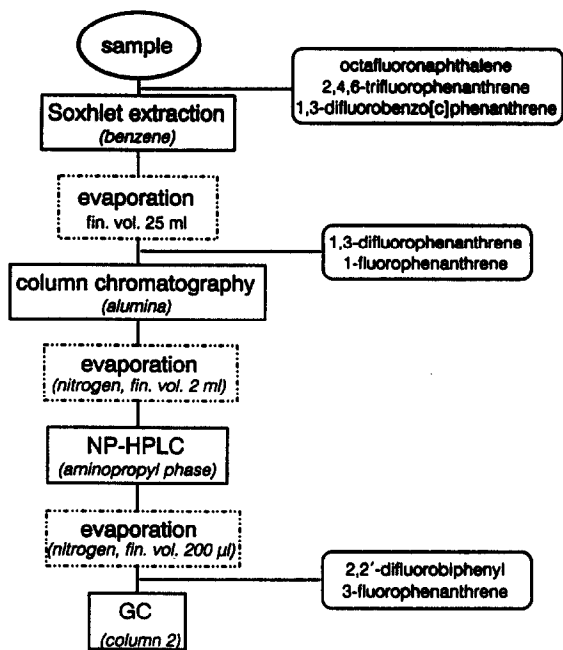
Fig. 2. Flow chart of the work-up procedure for chimney ash.

the PACs. After the final addition of two I.S.s, quantification was done by GC on a 0.25-μm DB-5 column (25 m $\times$ 0.22 mm I.D.).

RESULTS AND DISCUSSION

Several aromatic compounds containing two to four rings and one to three fluoro substituents were synthesized and investigated as potential I.S.s in the gas chromatographic determination of PACs in two samples. The F-PACs are listed in Table I together with the molar response factors (flame ionization detection) and the retention indices on five different stationary phases. Phases of different polarity were used so that the tabulated retention indices can serve as a guide to the selection of suitable fluorinated standards in other laboratories. The data in Table I show that many F-PACs are well resolved from the parent compounds.

As the fluorine atom is nearly as small as the hydrogen atom it does not cause much of a steric effect and therefore the causes responsible for

the differences in retention behaviour between the fluoroaromatics and their parent compounds must be electrostatic in nature. These interactions in gas chromatography with liquid stationary phases are known as Van der Waals interactions and include the interactions between permanent dipoles (Keesom forces), interactions between molecules with permanent dipoles and with induced dipoles (inductive interactions) and dispersive interactions between molecules without any dipole (London forces). In addition to these physical forces, chemical forces (donor-acceptor interactions, hydrogen bonding) also exist [10,11]. Fluorine substitution in general leads to a weaker interaction between a solute and the stationary phase and therefore to shorter retention times for the fluorinated aromatics, so that they elute ahead of their parent compounds. We therefore prefer to refer to retention decrements (not increments) when discussing the influence of the fluorine atom on the retention index. This effect is known for perfluorinated alkanes and alkenes also [12].

The strongly electron-withdrawing effect of fluorine, on the other hand, has consequences for the elution pattern of the F-PACs in that the changes in retention are particularly pronounced the more polar is the stationary phase.

As not all isomers of the mono- and disubstituted phenanthrenes were available, no attempt has so far been made towards an empirical correlation of the structure with retention. Some general conclusions can be drawn, however, from the results for the eight fluorophenanthrenes studied here. It is obvious that the position of the fluoro substituent has a strong effect on the retention, as can be seen by comparison of the data in Table I. The retention decrements are illustrated in Fig. 3.

As expected, the higher fluorinated derivatives show a larger decrement except for 1,2-difluorophenanthrene, which on some phases displays a surprisingly small decrement. This may be attributed to the so-called *ortho* effect, which is known to contribute to a longer retention time for *ortho*-substituted alkyl aromatics [13] and chlorobenzenes [14]. *Meta* substitution means a larger decrement than a simple addition of the decrements for the corresponding monosubsti-
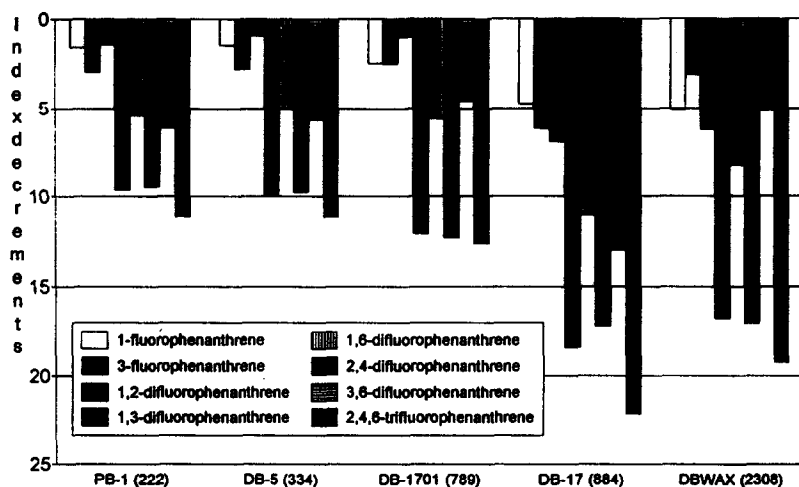
Fig. 3. Index decrements of eight fluorophenanthrenes on five different columns (see Table III).

tuted phenanthrenes would predict. Finally, it must be noted that the order of elution is dependent on the stationary phase. For instance, on methylphenylsilicone (column 4, DB-17), 1,6-difluorophenanthrene elutes earlier than the 3,6-derivative but on a polyethylene glycol phase (column 5, DB-WAX) the opposite is true. Several other such reversals can be seen through inspection of Fig. 3.

The chromatogram in Fig. 4A illustrates the resolution of all eight fluorophenanthrenes (peaks G–O) on DB-17. It is clear that all eight can be used simultaneously as I.S.s on this stationary phase provided that they do not co-elute with sample components. A difference in retention index of about 1 unit (Table I) means that the peaks are fully resolved.

The response of the flame ionization detector was investigated and the molar response calculated (Table I). The theoretical molar response was assumed to be unity for each carbon atom, independent of substitution [15]. Only dibenzofuran displays a lower than theoretical response, which is in line with previous data [16]. No such effect is seen for dibenzothiophene.

The results presented so far indicate that the F-PACs fulfil the criteria listed earlier for I.S.s. To test them under realistic conditions, they were used for the determination of PACs in two kinds of environmental samples.

SRM 1597

This sample consists of the aromatic fraction of a coal tar, dissolved in toluene. The concentrations of ten PAHs are certified by NIST and for eight more an orientation value is given. As only aromatic compounds are present in the solution supplied, no work-up is necessary. The gas chromatogram of SRM 1597 is shown in Fig. 4B, which illustrates how the selection of suitable I.S.s can be performed if a sample solution containing the aromatics to be determined is available. For the early-eluting analytes, 2-fluoro- and 2,2′-difluorobiphenyl (compounds C and E, respectively) were chosen because, as a comparison of Fig. 4A and B shows, they do not interfere with any peaks in the sample. As this is a sample with relatively few analyte peaks, almost any of the fluorinated phenanthrenes investigated here can be used without the problem of co-elution. Compounds G, H, K and O were selected. (In principle, only one of those standards would have been sufficient, but as will be shown in Fig. 5, the use of more than the theoretical minimum number of standards can be useful on occasion.) For the later eluting aromatics, 1,3-difluorobenzo[c]phenanthrene (compound P) was added. As shown in Fig. 3C, the analyte and standard peaks are well resolved from each other. For the calculation of the concentration of the analytes, the molar re-
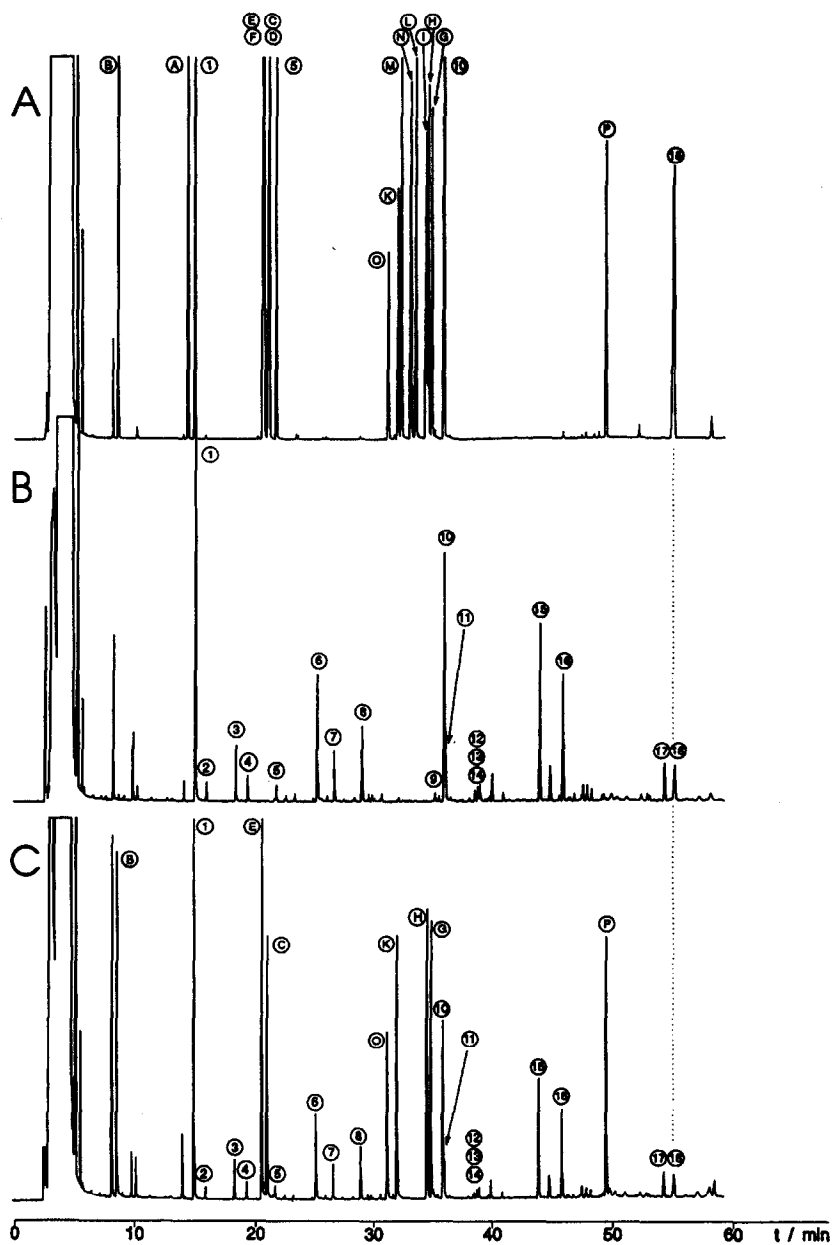
Fig. 4. Selection of the internal standards for the determination of PACs in SRM 1597. (A) Chromatogram of standard solution containing F-PAHs; (B) chromatogram of SRM 1597; (C) chromatogram of a solution containing SRM 1597 and the selected I.S. GC conditions: on-column injection on column 4, temperature programme as in text, flame ionization detection. Peak numbers as in Table IV.

sponse factors determined previously were used. In most instances, the results agree well with the certified concentrations, but there are four values that do not, namely those for biphenyl, dibenzofuran, dibenzothiophene and 4,5-methyl-

enephenanthrene (Table IV). For dibenzofuran and dibenzothiophene the NIST value was calculated using response factors of unity with respect to the nearest I.S. (acenaphthene and 1-methylphenanthrene, respectively), which is un-

TABLE IV

DETERMINATION OF PACs BY USE OF F-PAHs AS INTERNAL STANDARDS IN SRM 1597 AND CHIMNEY ASH

Peak	Compound	Certified value (μg/g)	Recalculated NIST value (see text)	Determined value[a] (μg/g)	Relative deviation from NIST value (%)	Chimney ash[a] (μg/g)
1	Naphthalene	1160 ± 50	–	1172 ± 14	1.0	
2	Benzothiophene	–	–	42.4 ± 1	–	
3	2-Methylnaphthalene[b]	97.1 ± 1.1	–	96 ± 5	–1.1	
4	1-Methylnaphthalene[b]	47.0 ± 0.6	–	47.8 ± 0.9	1.7	
5	Biphenyl[b]	27.0 ± 0.3	–	30 ± 1	11.1	
6	Acenaphthylene[b]	252 ± 1	–	256 ± 2	1.6	
7	Dibenzofuran[b]	88.9 ± 0.5	103.9	103 ± 1	–0.9	
8	Fluorene[b]	136 ± 1	–	133 ± 2	–2.2	
9	Dibenzothiophene[b,c]	23 ± 0.4	27.5	28 ± 1	3.6	
10	Phenanthrene	462 ± 3	–	460 ± 16	–0.4	41 ± 3
11	Anthracene	101 ± 2	–	106 ± 5	4.9	5 ± 1
12	2-Methylphenanthrene	–	–	17 ± 0.6	–	
13	4,5-Methylenephenanthrene[d]	51.3 ± 0.3	–	56 ± 5		
14	1-Methylphenanthrene	–	–	9.1 ± 0.5	–	
15	Fluoranthene	322 ± 4	–	329 ± 8	2.2	118 ± 5
16	Pyrene	235 ± 2	–	238 ± 7[e]	1.3	104 ± 3[e]
17	Benz[a]anthracene	98.6 ± 3.6	–	112 ± 8[f,g]		62 ± 3[f,g]
18	Chrysene + triphenylene	83.8	–	86 ± 6	2.6	97 ± 3

[a] Nearest I.S. was used for calculation.
[b] Compound quantified but not certified.
[c] Dibenzothiophene + naphtho[1,2-b]thiophene.
[d] Too high owing to co-elution with methylphenanthrene.
[e] Response factor of fluoranthene was used.
[f] Response factor of chrysene was used.
[g] Too high owing to insufficient separation from cyclopenta[cd]pyrene.

likely to apply. Under the conditions of the flame ionization detector used the molar response factors for those two compounds were 1.12 and 1.21, respectively. The recalculation of the NIST values using these two values gave the values listed in Table IV, which are in considerably better agreement with our values. For dibenzothiophene, it must additionally be noted that this compound co-elutes with naphtho[1,2-b]thiophene on non-polar stationary phases so that the integrated peak area yields the sum of those two compounds.

The SRM is a simple sample because no work-up is needed and the fairly high analyte concentrations and the small number of analytes present, allowing a wide and unproblematic choice of I.S.s. In principle, only one of those standards would have been sufficient, but as shown previ-

ously [6], the use of more than the theoretical minimum number of standards can be useful on occasion, especially in case of complex mixtures.

The PAC content of a chimney ash originating from an oil-heated furnace was determined, based on four different I.S.s. For 4,4′-difluorobiphenyl (compound F, Fig. 5B) all the values were always lower than if one of the other standards was used. A possible explanation for this observation is co-elution of standard F with a sample component. Indeed, when the temperature programme was changed, a previously hidden peak emerged from under the peak of the standard (Fig. 5). In such cases where the aromatic fraction is not available for screening for suitable "empty" spaces for standards (Fig. 4 shows a case where this is possible), the addition of several standards is recommended. The con-
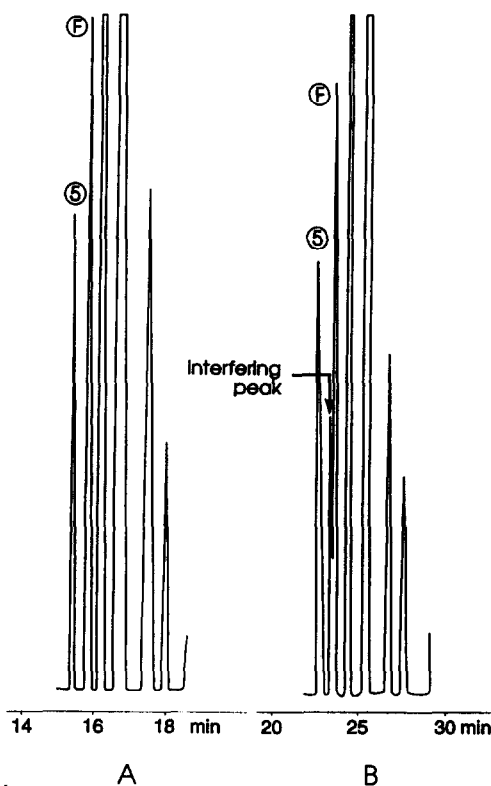
Fig. 5. Chromatogram of a chimney ash with two different temperature gradients: (A) 4 and (B) 2°C/min.

centration values obtained should be the same when calculated with any of the standards; if not, co-elution of the standard with the analyte might be the cause of the problem, invalidating the quantification. Such a co-elution would not be detectable with only one standard (*e.g.*, a per-deuterated derivative).

Chimney ash

A more complex case that requires several work-up steps before the analysis can be done was provided by a chimney ash taken from a furnace fuelled exclusively with paper and wood. In contrast to SRM 1597, the chromatogram of the aromatic fraction of this sample was not available beforehand so the selection of I.S.s had to be based on assumptions. As the PAC pattern is combustion derived, a more complex gas chromatogram than for the coal tar must be expected, making the selection of I.S.s somewhat delicate. As several work-up steps are

necessary, we took advantage of the possibility of multiple additions of I.S.s at different points of the work-up that is made available by the fluorinated PACs. The scheme is illustrated in Fig. 2 together with the relevant I.S.s. Three fluorinated I.S.s, one for each ring number, were added to the sample at the beginning of the work-up. Following the Soxhlet extraction with benzene and the reduction of the volume of the extract, two fluorinated phenanthrenes were added and finally, after two liquid chromatographic pretreatment steps, two more I.S.s were added just prior to the GC analysis.

A normal-phase liquid chromatographic separation of the aromatics and long-chain aliphatics, which adsorbed strongly enough to elute with the aromatic fraction from alumina, was necessary in order to obtain a clean gas chromatogram of the PACs.

The results for six PACs are provided in Table IV. They were calculated using the appropriate response factors and were corrected for losses, determined through the use of several I.S.s.

The calculation of the recovery of the initially added I.S., based on 2,2'-difluorobiphenyl and 3-fluorophenanthrene, shows that there is virtually no difference depending on which integration standards were used (Table V). This demonstrates that both of these standards elute in the gas chromatogram free of interferences from sample components. As the chromatogram without the I.S.s is not available, it is not self-evident that there is no co-elution of a sample component with the I.S.s. The data in Table V show that 2,4,6-trifluorophenanthrene, which was added initially to the sample, has the same recovery as the two fluorinated phenanthrenes which were added after the Soxhlet extraction and rotary evaporation (to a volume of 25 ml, Fig. 2). This means that in those early steps, no losses of any consequence occurred. The recovery of all fluorinated phenanthrenes of ca. 50% means that about half of the sample components in this volatility region are lost, probably during the reduction in volume using nitrogen. As expected, the more volatile standard compound octafluoronaphthalene was lost to a greater extent (60%) and the less volatile standard, 1,3-difluorobenzo[c]phenanthrene, to a lesser

TABLE V

RECOVERIES (%) OF THE INTERNAL STANDARD, CALCULATED USING 2,2'-DIFLUOROBIPHENYL AND 3-FLUOROPHENANTHRENE

Peak	Compound	2,2'-Difluorobiphenyl	3-Fluorophenanthrene	Mean
B	Octafluoronaphthalene	39	40	40
O	2,4,6-Trifluorophenanthrene	52	54	53
P	1,3-Difluorobenzo[c]phenanthrene	59	61	60
K	1,3-Difluorophenanthrene	49	50	50
G	1-Fluorophenanthrene	50	51	51

extent (40%) than the phenanthrenes. This hints at evaporative losses during steps involving a reduction in volume. The data also demonstrate how important it is to use standards in the appropriate volatility region for each analyte. It must be cautioned against the use of standards with a volatility that is much different from that of the analytes in question; in other words, the analyte and standard must elute fairly close to each other in the gas chromatogram (on non-polar stationary phases). The results obtained confirm the speculation that it is possible to determine the losses in every step of the work-up procedure in one run if a sufficient number of I.S.s are available.

CONCLUSIONS

The use of fluorinated compounds as standards for the determination of PACs has been demonstrated for two samples. Among the several advantages over the frequently used deuterated PACs is the possibility of using several standards for each ring number, thus allowing the determination of losses in serval work-up steps in one chromatogram. Another benefit is that if a standard by coincidence should co-elute with a sample component, this can easily be detected if multiple standards are used. The fact that the F-PAHs in general elute ahead of their parent compounds could be advantageous in the case of complex PAC patterns, when fractionation according to ring number is necessary: The F-PAHs would then elute in a region where no other components are present. Finally, if a chromatogram without standards is available, there is a wide choice of fluorinated compounds to choose from so that the possibility of co-elution can be avoided altogether.

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