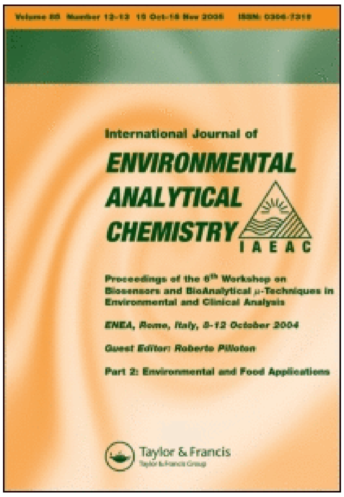

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Determination of Nanogram/Kilogram Levels of Polycyclic Aromatic Hydrocarbons in Foods by HPLC with Fluorescence Detection†

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Methodology was developed for the determination of eight polycyclic aromatic hydrocarbons (PAH) in five food categories including meat/fish, dried dairy products, cereals, leafy vegetables and vegetable/marine oils. The eight PAH were fluoranthene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene, 7, 12-dimethylbenzo[a]anthracene, dibenzo[ah]anthracene and dibenzo[ai]pyrene. Samples were digested with alcoholic KOH followed by partitioning into solvents such as cyclohexane or isooctane. Lipids and other interferences were removed by solvent partitioning with dimethylformamide or dimethylsulfoxide/water. Additional cleanup involving column chromatography on silica gel, Florisil or Sephadex LH-20 was employed as required. Reversed-phase chromatography with gradient elution and fluorescence detection was employed for the determinations. Confirmation was carried out by GC-MS/SIM. Detection limits ranged from 2–90 ng/kg depending on the PAH and food analyzed. Results of a small survey indicated that the meat/fish category had the highest levels (low μg/kg, on average) of the foods studied.

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INTRODUCTION

PAH are ubiquitous pollutants of the environment and occur in foodstuffs as a result of air, water and soil pollution (e.g. seafood, fruits and vegetables), curing smokes (e.g. smoked foods), modes of cooking (e.g. roasted and charcoal broiled foods), food processing (e.g. instant non-fat dried milk) and from endogenous sources.[1-6] To date twenty-two PAH have been identified in different foods and a number of them, e.g. benzo[a]pyrene (B[a]P), benzo[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), dibenzo[ah]anthracene (D[ah]A), dibenzo[ai]pyrene (D[ai]P), dimethylbenzo[a]anthracene (DMBA) have been found to be carcinogenic in experimental animals.

A large volume of data on PAH content in foods has been accumulated on a world-wide basis from work conducted mainly in Europe and the USA.[7-10] In comparison, background concentration data on PAH levels in foods in Canada are meagre. The only available data are from the studies by Genest and Smith[11] and Panalaks[12] on various smoked and charcoal broiled foods, by Dunn and Fee[13] on fish, mollusks and lobsters, and recently by Lawrence and Weber[14,15] on fish, shellfish, meat products, processed vegetables and dairy products from Canadian food retail outlets.

Various chromatographic separation procedures including column, paper, thin layer chromatography (TLC) and gas chromatography (GC) have been reported for the determination of PAH in foods. The technique of HPLC together with the highly selective and sensitive fluorescence detection is an effective tool for the separation and analysis of PAH. One of the most difficult aspects of PAH analysis is the resolution of the so-called "benzpyrene" fraction consisting of the 5-membered ring PAH including B[a]P, B[e]P, B[k]F, B[b]F and perylene. Another pair difficult to resolve is the 4-ring PAH isomers, B[a]A and chrysene. Efficient resolution of these PAH by HPLC has recently been demonstrated.[16] In addition to the improved resolution achievable by HPLC, the fluorescence characteristics of PAH compounds can be utilized for subpicogram detection.[17] At present, there are only a few published articles on the application of HPLC-fluorescence to PAH analysis in foods,[12,14,15,18] although extensive applications to the analysis of other environmental samples have been reported.

This work describes method development for the trace analysis of eight target PAH (three of which were not included in earlier surveys[14,15]) in foods using HPLC-fluorescence detection and capillary GC-MS/SIM (selected ion monitoring) confirmatory techniques followed by application of the methodology to the analysis of forty-eight selected market basket commodities for PAH content. The methodology was modified from earlier work[7,8,19,20] for extraction and cleanup. This along with the use of an HPLC column with appropriate selectivity enabled a 10–100 fold improvement in determination limits.

EXPERIMENTAL

Reagents

All solvents were distilled in glass or HPLC grade. The adsorbents, Florisil (60–100 mesh), silica gel (70–230 mesh) and Sephadex LH-20 were purchased from Floridin, EM Reagents and Pharmacia Canada Ltd., respectively.

The PAH standards were obtained from the Air Pollution Control Directorate, Department of the Environment, Ottawa, and National Bureau of Standards, Washington, and were used without further purification. Stock solutions were prepared by dissolving the compounds in cyclohexane at μg/mL concentrations. Appropriate dilutions with cyclohexane (followed by replacing the cyclohexane with methanol) were made so that the working concentrations of the standards were at the pg/μL level for chromatographic analysis.

High performance liquid chromatography (HPLC)-fluorescence detection

A Vydac 201TP (5 μm, 4.6 mm × 25 cm) reverse phase column with a C_{18} Ultrasphere ODS (5 μm) precolumn was used in a Beckman Model 420 system with Model 100A pumps and solvent programming using acetonitrile (CH_3CN) and water as mobile phase. A 50 μL loop was used for injection. A Schoeffel Model 970 fluorescence detector was used for detection and a Spectra-Physics SP 4100 was employed for data handling. HPLC/fluorescence conditions were as follows. Flow rate, 1.0 mL/min. Gradient program, 50% CH_3CN to

100% CH_3CN in 50 min; hold at 100% CH_3CN for 15 min; 100% CH_3CN to 50% CH_3CN in 10 min. Fluorescence wavelengths were 280 nm (ex) and >389 nm (em).

Gas chromatography-mass spectrometry/selected ion monitoring (GC-MS/SIM)

A Hewlett-Packard Model 5790A GC with a Hewlett–Packard Model 5970A mass selective detector, and Hewlett–Packard Model 2671G printer was used for capillary GC-MS/SIM work. An SPB-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film) was used with helium as the carrier gas. Injection was made with the column at 50°C isothermal for 5 min followed by programming the oven temperature from 50° to 300°C at 10°C/min and held isothermally until all components were eluted. An on-column injection technique with an injection port temperature of 250°C was employed. The injection volume was 1.0 μL. The MS/SIM parameters used were as follows: electron multiplier voltage, 2800 V; SIM window, 0.1 amu; dwell time, 100 m sec. In the SIM mode, the ions monitored were m/e 202, 228, 252, 256, 278 and 302 which correspond respectively to the masses of the molecular ions of fluoranthene, B[a]A, B[k]F/B[b]F/B[a]P, DMBA, D[ah]A and D[ai]P.

Extraction and cleanup

All commercial food samples were purchased from retail outlets and fast food chains in the metropolitan area of Toronto. The samples after collection were processed as quickly as possible, and processing was done by pulverizing/comminuting and mixing weighed amounts using a Waring blender. The homogenates were stored in a deep freeze (−20°C) until extracted and analyzed. With charbroiled hamburgers from fast food chains, only the meat patties without buns and garnishes were sampled.

1. Meat/Fish group

A 200 g quantity of sample was saponified with 33.7 g of KOH in 300 mL of H_2O/methanol (1:9 v/v) for 4 hours under reflux. The mixture was cooled, diluted with 400 mL of H_2O/methanol (20%

v/v), and extracted twice with 800 mL cyclohexane. The cyclohexane was washed with 400 mL of methanol/water (50% v/v) and then further washed with 400 mL of distilled water. The cyclohexane extracts were combined, concentrated to 30 mL, and then partitioned with 60 mL of DMF/H_2O (9:1 v/v). The cyclohexane layer was discarded, and the DMF/H_2O layer was extracted with H_2O/cyclohexane (1:1 v/v). After the separation of two layers, the cyclohexane layer containing the PAH was collected, filtered through anhydrous Na_2SO_4 and concentrated to a small volume on a rotary evaporator at 30°C.

The concentrated extract was applied to the top of 5 g 15% H_2O (by weight) deactivated silica gel in a column and eluted with 100 mL cyclohexane. The cyclohexane eluate was collected and concentrated in a rotary evaporator at 35°C to about 1 mL. Further cleanup of the cyclohexane extract concentrate was performed by fractionation on a Sephadex LH-20 (10 g, 25–100 μm) using 200 mL isopropanol as eluent. The fraction that eluted between 38 and 190 mL was collected, concentrated to near dryness on a rotary evaporator at 30°C and then to dryness in a stream of nitrogen. The residue was dissolved in 5 mL of methanol prior to HPLC-fluorescence analysis. For GC-MS/SIM work, a 3 mL aliquot was concentrated to 0.5 mL for analysis.

2. *Cereal/Dairy products*

A 100 g quantity of sample was mixed in a separatory funnel with 400 mL distilled water, 120 mL 28.6% NaOH, 75 mL acetone, and 120 mL ethanol. The resulting slurry was then extracted first with 200 mL isooctane, and then twice with 100 mL isooctane. The aqueous phase was discarded, and the combined isooctane extracts were washed first with 3 × 250 mL of ethanol-acetone-water (24:15:100 v/v) followed by 2 × 250 mL of warm water (~50°–60°C), filtered through anhydrous sodium sulfate and concentrated to a small volume on a rotary evaporator at 35°C.

The concentrated extract was then added to the top of a Florisil column (60 g absorbent 60–100 mesh, 2.5% water deactivated) and eluted with 175 mL benzene. The eluate was collected and concentrated in a rotary evaporator at 35°C to near dryness. The residue was dissolved in 200 mL isooctane, and the isooctane solution after being washed with 3 × 100 mL H_3PO_4 was partitioned with 50 mL

DMSO. The DMSO layer was collected, and the isooctane layer was extracted with 2 × 50 mL DMSO. The combined DMSO extracts were partitioned three times with 25 mL portions of isooctane which were then discarded. The DMSO was diluted with 300 mL of H_2O and then back extracted twice with 50 mL portions of isooctane. The isooctane extracts were combined, washed with 2 × 200 mL H_2O, filtered through anhydrous sodium sulfate, and concentrated to near dryness on a rotary evaporator at 30°C and then to dryness in a stream of nitrogen. The residue was dissolved in 5.0 mL of methanol prior to HPLC-fluorescence analysis. For GC-MS/SIM work, a 3 mL aliquot was concentrated to 0.5 mL in methanol prior to analysis.

3. *Leafy vegetables*

A 100 g quantity of sample was saponified exactly as described for the meat/fish samples. For cleanup, the concentrated extract was applied to the top of a 60 g, 15% H_2O (by weight) deactivated Florisil column (60–100 mesh) and eluted with 200 mL cyclohexane. The eluate was collected and concentrated to near dryness on a rotary evaporator at 30°C and then to dryness in a stream of nitrogen. The residue was dissolved in 2 mL of methanol prior to HPLC-fluorescence analysis.

4. *Vegetable/Marine oils*

A 100 g quantity of vegetable/marine oil dissolved in 500 mL cyclohexane was subjected to liquid-liquid partition in 500 mL DMF/water (9:1 v/v). To the DMF layer, 500 mL H_2O was added and the mixture was re-extracted with 500 mL cyclohexane. The cyclohexane layer was collected, washed with 2 × 100 mL H_2O, filtered through anhydrous Na_2SO_4 and concentrated to a small volume on a rotary evaporator at 30°C. The cleanup by silica gel and Sephadex LH-20 column chromatography was carried out exactly as described for the meat/fish group.

RESULTS AND DISCUSSION

HPLC-Fluorescence analysis

A number of C_{18} stationary phases have been shown to provide different selectivities and separation efficiencies for PAH. The differences in selectivity have been partly attributed to the surface of the C_{18} layer.[21-23] The organic layer chemically attached to the silica surface can be monomeric or polymeric, depending on the silane and reaction conditions used in the derivatization step. Columns containing C_{18} monomeric materials generally show higher efficiencies than those packed with polymeric materials. But columns containing C_{18} polymeric materials are known to exhibit better selectivity for PAH isomers.

In the present study, a C_{18} microparticulate column with a polymeric layer, namely, Vydac 210TP was evaluated for the separation of the eight PAH by HPLC-fluorescence. Figure 1 illustrates a typical separation. It is seen in the figure that the column gives baseline separation of the "benzpyrene" fraction consisting of B[b]F, B[k]F and B[a]P, thus substantiating the work of Bartle *et al.*[16] Also, it gives good separation of DMBA from other PAH. Studies with C_{18} monomeric packings (e.g. Zorbax ODS) have shown that

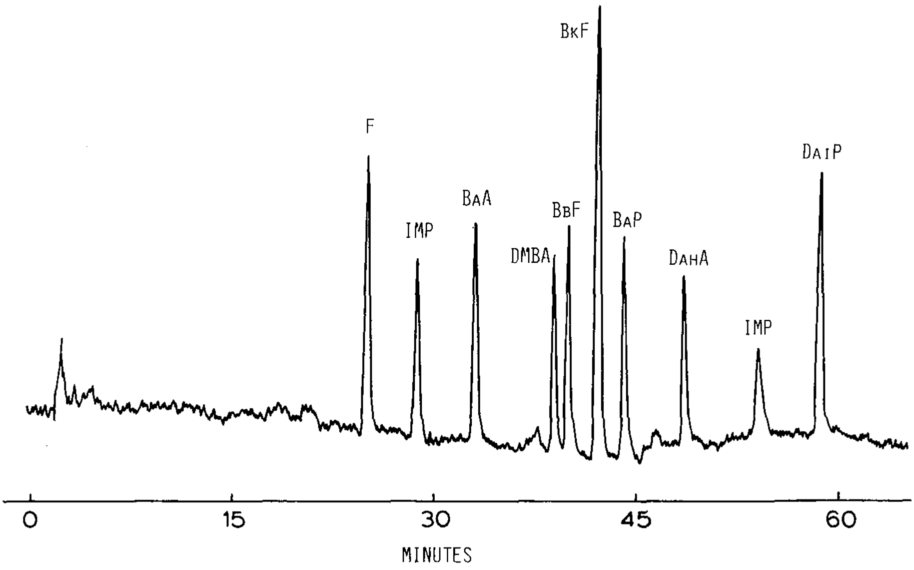

FIGURE 1 HPLC/fluorescence chromatogram of the eight PAH standards. Conditions as described in the text. IMP = impurity. 125 pg injected of each except B[b]F (130 pg) and D[ai]P (2750 pg).

the column fails to resolve either chrysene from B[a]A, or B[k]F from B[b]F in the multicomponent mixture. Attempts at complete resolution of the PAH by other C_{18} reverse phase columns, (e.g. Ultrasphere ODS) were also unsuccessful. Ultrasphere ODS gave good separation of B[b]F, B[k]F and B[a]P, but failed to give adequate resolution of DMBA and B[a]P. Also, the order of elution of PAH with this column was found to be quite different from that observed in Vydac 201TP column. For example, the DMBA in the Ultrasphere ODS eluted immediately after B[a]P, whereas it was found to elute before B[b]F on the Vydac column.

The excitation and emission wavelengths were compromise wavelengths selected so that all eight PAH would be adequately detected. Application of similar excitation and emission conditions to the detection of PAH in HPLC has been reported earlier.[17] Calibration plots for the PAH were found to be linear at the concentrations studied which ranged from 1 to 250 pg injected of each PAH in the mixture (D[ai]P 200–300 pg).

The limits of detection of the PAH analyzed are shown in Table 1. This represents the amount of standard material to produce a response of 1 cm which is roughly twice the background noise. The actual method sensitivity in the analysis of food sample, however, depended on the amount of sample material injected, the background noise (interferences) and the recovery throughout the

TABLE I
Detection limits of eight PAH by HPLC/fluorescence and GC-MS/SIM analysis

	Limits of detection, Pg.	
PAH	HPLC-Fluor.	GC-MS/SIM
F	18.4	30
B[a]A	13.6	30
DMBA	12.0	30
B[b]F	12.3	30
B[k]F	4.7	
B[a]P	12.5	60
D[ah]A	15.6	60
D[ai]P	264.4	300

method. Thus, for example, assuming 100% recovery, a 50 μL injection of the extract of a 40 g food sample concentrated to 1 mL in methanol gave method sensitivities as follows (calculated from the limits of detection listed in Table 1, and expressed as μg/kg): F (0.008), B[a]A (0.005), DMBA (0.006), B[k]F (0.005), B[b]F (0.002), B[a]P (0.005), D[ah]A (0.006) and D[ai]P (0.090).

The precision of the HPLC-fluorescence technique was studied by multiple injections of 50 μL samples of the eight PAH on a single day. The retention times, peak areas and peak heights of each were found to be highly reproducible. Similar repeatability was also observed when injecting a PAH mixture at three day intervals under identical chromatographic conditions. This is shown in Table 2.

GC-MS/SIM analysis

The GC-MS/SIM profile (SIM data acquisition and reconstructed chromatogram) of the eight PAH mixture is presented in Figure 2. Under the GC-MS conditions used, the fused silica SPB-5 capillary column failed to resolve B[k]F and B[b]F, although other PAH were well resolved. Therefore, the response indicated on the reconstructed ion chromatogram for ion 252 has been interpreted as representing B[b]F and/or B[k]F.

The GC-MS/SIM detection limits of the PAH are listed in Table 1. The detection limits of most PAH are in the order of 30 to 60 pg with higher levels (300 pg) for D[ai]P.

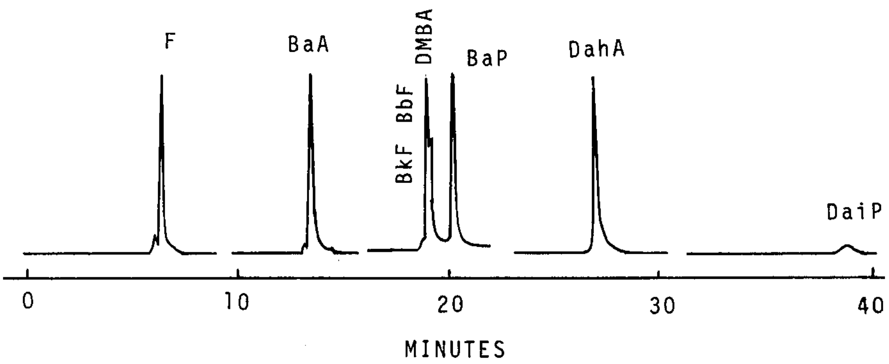

FIGURE 2 GC-MS profile of the 8 PAH standards. Chromatogram represents the sum of the monitored ions. Conditions as described in the text.

TABLE II

Repeatability by HPLC/fluorescence for the 8 target PAH

	Run I			Run II[a]			Run III[b]		
PAH	Area	Peak Ht. (cm)	Ret. Time (min)	Area	Peak Ht. (cm)	Ret. Time (min)	Area	Peak Ht. (cm)	Ret. Time (min)
F	10,223	4.5	25.80	10,583	4.6	25.11	10,206	4.6	25.07
B[a]A	8,465	4.0	33.77	8,733	3.9	33.26	8,291	3.7	32.20
DMBA	9,436	4.5	39.52	11,006	5.1	39.28	8,845	4.2	39.23
B[b]F	8,931	4.1	40.48	8,971	4.0	40.38	8,597	4.0	40.16
B[k]F	25,382	11.2	42.66	25,453	11.0	42.73	24,815	11.0	42.48
B[a]P	8,897	4.0	44.58	9,181	4.1	44.69	8,362	4.1	44.44
D[ah]A	6,585	3.3	48.97	6,641	3.3	49.16	6,305	3.3	48.87
D[ai]P	10, 449	4.7	58.90	10,270	4.5	59.55	10,074	4.6	59.02

[a] Three days after Run I.

[b] Six days after Run I.

Recovery studies

Recoveries through the various procedural steps were determined by spiking both method blanks (no sample matrix) and control blanks (food samples with minimum or no background) at different levels of PAH. Control blanks for method validation studies consisted of unprocessed fresh Ocean Perch representing foods in the meat/fish group and a coffee whitener and a bran cereal representative of the dairy and cereal groups, respectively.

No background PAH were observed in the method blanks when analyzed by HPLC-fluorescence, and only traces of fluoranthene and B[a]A were detected by GC-MS/SIM analysis. Typical recoveries are shown in Table 3. The Ocean Perch (control blank) sample gave average recoveries of most PAH ($>62\%$) at the three levels of spiking. However, D[ah]A and D[ai]P both gave low recoveries at the $25\,\mu g/kg$ level. Also, it was not readily apparent why the recoveries of the latter PAH increased as the spiking level decreased, since no D[ai]P or potentially interferring coextractive was observed in the sample blank.

A bran cereal blank spiked at a $25\,\mu g/kg$ level gave recoveries for B[b]F and B[a]P of 62.7% and 34.2%, respectively. A powdered

TABLE III

Per cent recoveries of PAH through the extraction/cleanup procedure for the meat/fish group

	Method blank level, (μg/kg)	Ocean Perch level, (μg/kg)		
PAH	20.0	1.0	5.0	25.0
Fluoranthene	89.8	—[a]	—	66.7
B[a]A	81.3	70.7	50.8	69.8
DMBA	73.4	—	—	51.3
B[b]F	93.0	49.6	57.5	79.0
B[k]F	98.1	—	—	70.7
B[a]P	100.0	81.2	92.5	92.3
D[ah]A	98.0	—	—	45.8
D[ai]P	94.6	78.6	56.4	20.6

Background PAH levels (μg/kg): Fluoranthene (0.23), B[a]A (0.02), DMBA (0.00), B[b]F (0.007), B[k]F (0.004), B[a]P (0.005), D[ah]A (0.00) and D[ai]P (0.00).

[a] = Not analysed.

milk product blank spiked at the 25 μg/kg level for the two PAH gave recoveries greater than 98%. Since the extraction method for these two foods was virtually the same, it appears that the bran exerts a matrix effect on the recovery of B[a]P.

Analysis of samples

The food samples analyzed for PAH include both domestic and imported items, and were selected from the five food groups as described in the Experimental. Eight samples (4 from the meat/fish group, 2 from the vegetable/marine oils, and 1 each from the cereal and dairy group) were analyzed by GC-MS/SIM to confirm the HPLC-fluorescence results. The results were not corrected for recoveries.

Figure 3 shows HPLC-fluorescence chromatograms for several products in the meat/fish group. It can be seen that numerous peaks are present in addition to the target compounds. Since these unknown peaks pass through the same extraction and cleanup procedures as the known standards and also since they are detectable by fluorescence, it is likely that the unknowns are related polycyclic aromatics. Figure 4 shows a similar picture for the vegetable products. The spinach chromatogram was typical of the leafy vegetables. This class proved to contain the lowest levels of PAH in the foods analyzed. In fact none of the target compounds were detected in any of the lettuce, chard or cabbage analyzed. One spinach sample contained 0.032 μg/kg of fluoranthene. These results are similar to results found earlier[15] and seem to indicate that leafy vegetables are not a significant source of PAH for humans although the potential for higher PAH levels in such crops grown in heavily industrialized areas has been demonstrated.[24]

Tables 4 and 5 list survey results for various seafood and cereal products. The seafood contained significantly higher levels of PAH than the cereals group. Also the charbroiled hamburgers from fast food outlets (Table 6) had PAH levels comparable to the seafood group. However, because of their greater consumption, charboiled meats would be expected to contribute more to the total intake of PAH by the majority of the population.

Table 7 (and Figure 4) presents results from powdered dairy products. It can be seen that low μg/kg levels of PAH can be

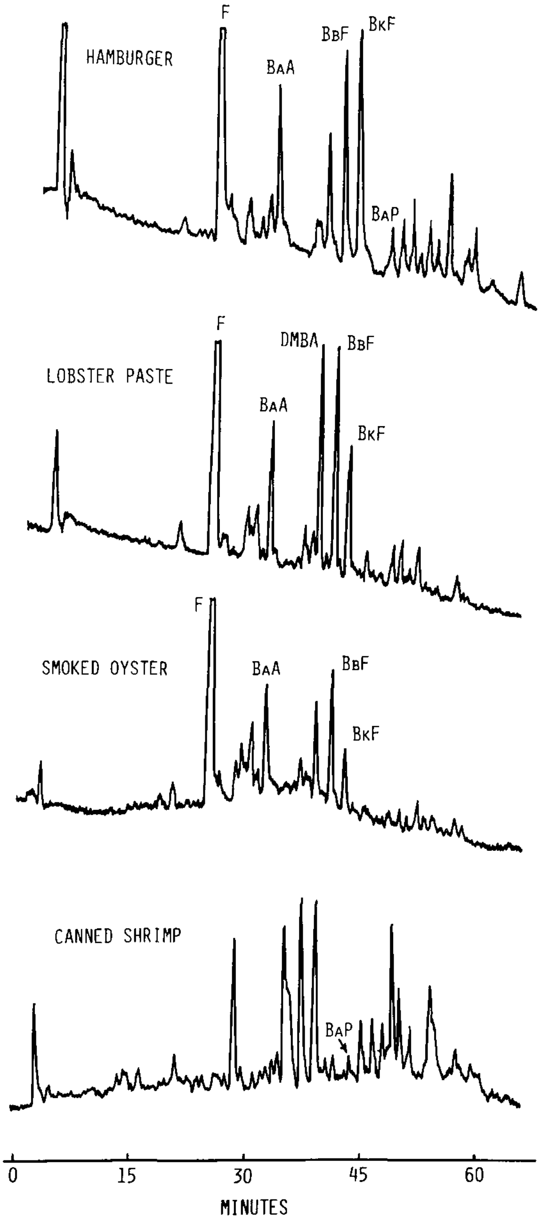

FIGURE 3 HPLC/fluorescence chromatograms obtained for various seafood and meat products. Equivalent quantities of sample injected: hamburger, 80 mg; lobster paste, 40 mg; smoked oyster, 40 mg; shrimp 80 mg. Conditions as described in the text. Identified peaks were confirmed by GC-MS/SIM.

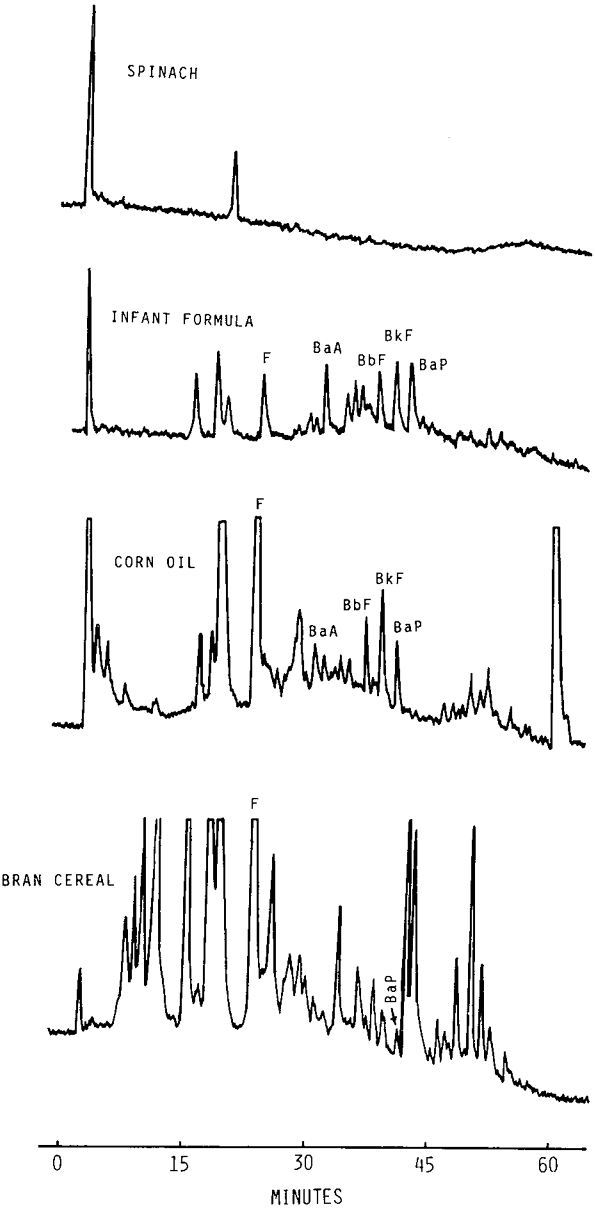

FIGURE 4 HPLC/fluorescence chromatograms obtained for various vegetable products. Equivalent quantities of sample injected: spinach, 5 g; infant formula, 100 mg; corn oil, 4 g; bran cereal, 1 g. Conditions as described in the text. Identified peaks were confirmed by GC-MS/SIM.

TABLE IV
PAH in seafoods ($\mu g/kg$)

PAH	Lobster Paste[a]	Lobster paste	Lobster paste	Lobster meat	Smoked mussels	Mussels in brine	Smoked herring	Smoked herring[a]	Smoked oysters[a]	Unsmoked oysters	Smoked oysters	Smoked oysters	Frozen shrimp	Frozen shrimp	Canned shrimp	Canned shrimp[a]
F	32.5	1.32 (1.02)	0.70	7.4	4.6 (2.8)	6.1	174 (156)	191	39.4	28.5	24.7 (13.4)	67.5	0.63	0.53 (0.23)	0.41	ND[b]
B[a]A	4.8	0.19 (0.12)	0.10	3.0	1.31 (0.60)	2.9	ND (ND)	ND	4.5	2.0	3.1 (4.1)	27.3	0.025	0.02 (0.02)	0.09	1.81
DMBA	1.2	ND (ND)	ND	0.71	ND (ND)	0.81	15.9 (10.3)	11.6	ND	ND	ND (ND)	6.6	ND	ND (ND)	ND	ND
B[b]F	7.4	0.17 (0.11)	0.08	2.8	1.26 (0.61)	3.2	ND (ND)	ND	5.2	4.4	2.2 (2.4)	15.1	ND	0.02 (0.01)	0.09	1.8
B[k]F	3.4	0.036 (0.042)	0.06	1.2	0.67 (0.34)	1.5	ND (ND)	ND	1.9	1.6	1.2 (1.3)	8.7	ND	0.01 (0.02)	0.05	1.2
B[a]P	4.6	0.083 (0.093)	0.06	2.6	0.76 (0.81)	1.4	ND (ND)	ND	2.8	0.79	2.3 (2.4)	13.3	ND	0.03 (0.02)	0.18	3.8
D[ah]A	ND[b]	ND (ND)	ND	0.12	ND (ND)	ND	ND (ND)	ND	ND[b]	ND	ND (ND)	0.63	ND	ND (ND)	ND	ND
D[ai]P	ND	ND (ND)	ND	ND	ND (ND)	ND	ND (ND)	ND	ND[b]	ND	ND (ND)	ND	ND	ND (ND)	ND	ND[b]

[a] Analyzed by both HPLC-fluorescence and GC-MS/SIM.

[b] Trace detected in GC-MS/SIM.

( ) = Results in brackets from duplicate analysis.

ND = Not detected; HPLC-fluorescence method sensitivity ($\mu g/kg$): F (0.008); B[a]A (0.005); DMBA (0.006); B[b]F (0.005); B[k]F (0.002); B[a]P (0.005); D[ah]A (0.006) and D[ai]P (0.090).

TABLE V

PAH in cereals (μg/kg)

	Bran cereals						Puffed cereals			
PAH	A	B	C	D	E	F	Wheat[a]	Rice	Oats	Corn
F	0.46	1.8 (1.4)	1.4	1.3	1.5 (1.4)	1.6	3.2	1.2 (0.76)	1.9	1.2
B[a]A	0.07	0.16 (0.33)	ND	0.11	0.02 (0.17)	0.06	ND	ND (0.02)	0.16	ND
DMBA	0.04	ND (ND)	ND	ND	ND (ND)	ND	ND	ND (ND)	ND	ND
B[b]F	0.06	ND (0.04)	ND	0.63	0.09 (0.03)	0.11	0.09	ND (0.02)	0.079	0.05
B[k]F	0.03	0.04 (0.03)	ND	0.06	0.03 (0.03)	0.05	0.04	ND (ND)	ND	0.02
B[a]P	ND	ND (ND)	0.02	0.07	0.03 (0.05)	0.11	0.04	ND (ND)	ND	0.03
D[ah]A	ND	ND (ND)	ND	ND	ND (ND)	ND	ND	ND (ND)	ND	ND
D[ai]P	ND	ND (ND)	ND	ND	ND (ND)	ND	ND	ND (ND)	ND	ND

[a] Analyzed by both HPLC-fluorescence and GC-MS/SIM.

( ) = Results in brackets from duplicate analysis.

ND = Not detected; HPLC-fluorescence method sensitivity (μg/kg): F (0.007); B[a]A (0.004); DMBA (0.005); B[b]F (0.004); B[k]F (0.002); B[a]P (0.004); D[ah]A (0.005) and D[ai]P (0.072).

TABLE VI

PAH in charbroiled hamburgers (μg/kg)

PAH	A	B[a]	C	D
F	6.2 (7.0)	15.4	14.7	0.73
B[a]A	2.7 (3.4)	4.9	5.8	0.29
DMBA	1.5 (2.2)	ND[b]	1.7	0.09
B[b]F	1.9 (2.5)	2.1	3.6	0.23
B[k]F	0.96 (3.3)	1.2	2.1	0.10
D[ah]A	1.2 (1.5)	ND[b]	1.2	0.07
D[ai]P	ND (ND)	ND[b]	ND	ND

[a] Analyzed by both HPLC-fluorescence and GC-MS/SIM.

[b] Trace detected in GC-MS/SIM.

( ) = Results in brackets from duplicate analysis.

ND = Not detected; HPLC-fluorescence method sensitivity shown in Table 4.

TABLE VII

PAH in powdered dairy products (μg/kg)

	Infant formula		Powdered milk		Coffee whitener	
PAH	A[a]	B	A	B	A	B
F	1.4	8.2	1.0 (0.75)	1.2	0.34	0.88
B[a]A	1.2	3.9	0.01 (ND)	ND	0.10	0.10
DMBA	ND	ND	ND (ND)	ND	ND	ND
B[b]F	0.86	1.6	0.03 (0.02)	0.03	0.16	0.05
B[k]F	0.33	0.97	ND (ND)	ND	0.14	0.03
B[a]P	1.2	0.04	ND (ND)	ND	ND	0.12
D[ah]A	ND[b]	ND	ND (ND)	ND	ND	ND
D[ai]P	ND	ND	ND (ND)	ND	ND	ND

[a] Analyzed by both HPLC-fluorescence and GC-MS/SIM.

[b] Trace detected in GC-MS/SIM.

( ) = Results in brackets from duplicate analysis.

ND = Not detected; HPLC-fluorescence method sensitivity shown in Table 5.

detected in some of the products. These are similar to results published earlier[15] and appear to be related to the drying process employed.[6]

The six oils analyzed (corn, soya, rapeseed, sunflower, peanut, cod liver) and a beef oil margarine sample contained levels generally less than 1 μg/kg total target PAH. These levels are much lower than those found elsewhere.[25,26]

CONCLUSION

The use of HPLC/fluorescence for the determination of PAH is probably the most cost effective means of detecting low levels of these substances in foods. The extraction and cleanup procedures described in this work along with the proper selection of reversed-phase column can lead to the quantitation of ng/kg levels of PAH. Gas chromatography combined with mass spectrometry has been shown to be equally as sensitive and is a very useful means of confirming results obtained by HPLC.

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