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The Identification of High Molecular Weight Polynuclear Aromatic Hydrocarbons in a Biologically Active Fraction of Cigarette Smoke Condensate*

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INTRODUCTION

Recent work in this laboratory on the fractionation of cigarette smoke condensate (CSC) for bioassay by mouseback testing has resulted in the isolation of two highly refined polynuclear aromatic hydrocarbon (PAH) subfractions: F-20 and F-55 (Fig. 1) (1—5). Fraction F-20 contained only 0.4% of the weight of the crude condensate but accounted for virtually all of the tumor-initiating activity of CSC (6) and "promoted" the development of more tumors than did any previously tested, neutral fraction (7). Using the gel filtration (GF) chromatographic behavior of benzo(a)pyrene on Bio-Beads SX-2, fraction F-20 was further separated into two subfractions F-54 and F-55 (Fig. 2) (5). Fraction F-55 represented only 15% of F-20 or only 0.05% of CSC and, more importantly, was almost as tumorigenic as was F-20 (8). In previous work, we isolated the PAH in F-20 and unambiguously identified the broad spectrum of PAH ranging from indene to indeno(1,2,3-cd)pyrene (9). This paper reports the results of our isolation and identification of the PAH in the more refined and active F-55 fraction. In effect, GF chromatography on SX-2 resulted in the concentration of the high molecular weight (MW) PAH into a single fraction. We found that F-55 contained only compounds larger than fluoranthene. Thus, the gel filtration step which converted F-20 to F-55 removed all of the low molecular weight PAH and resulted in a concentrated fraction of high molecular weight PAH compounds with considerable biological activity.

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Figure 1. PAH separation scheme for cigarette smoke condensate (CSC).

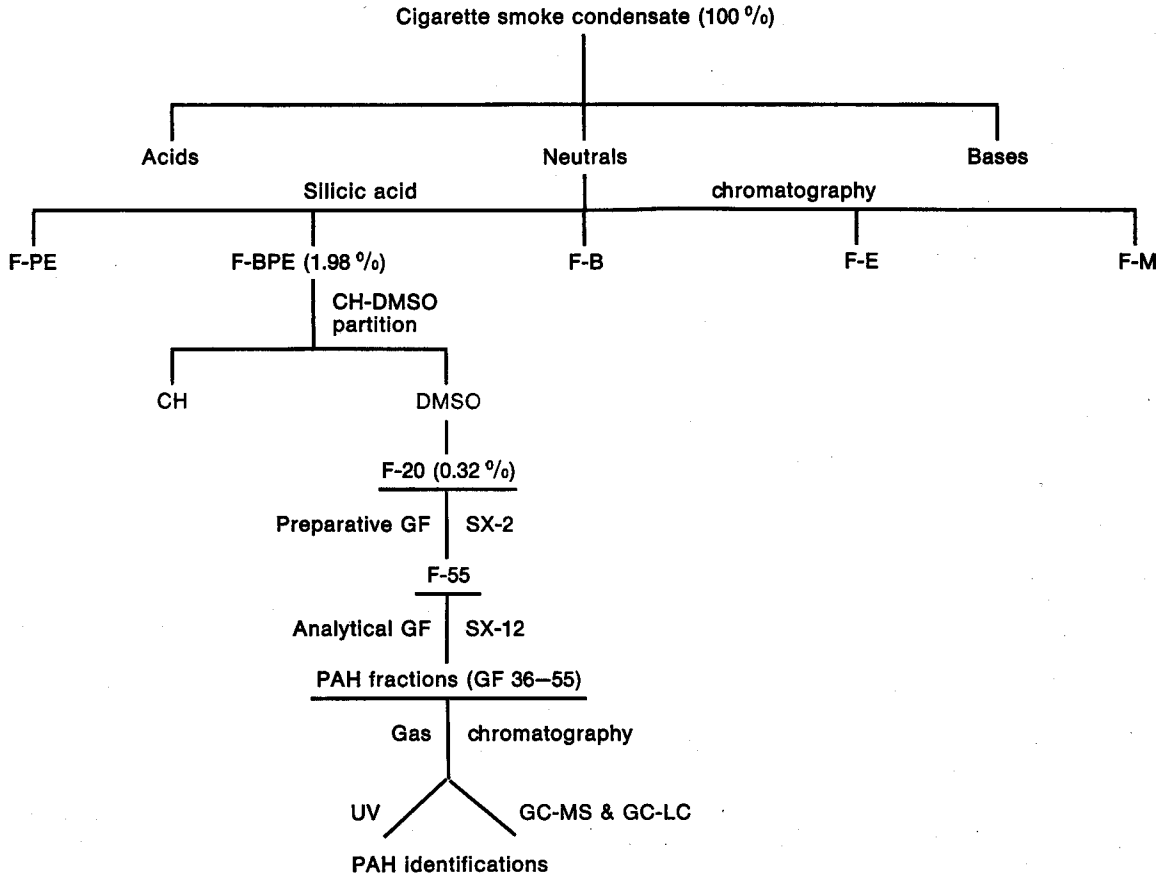
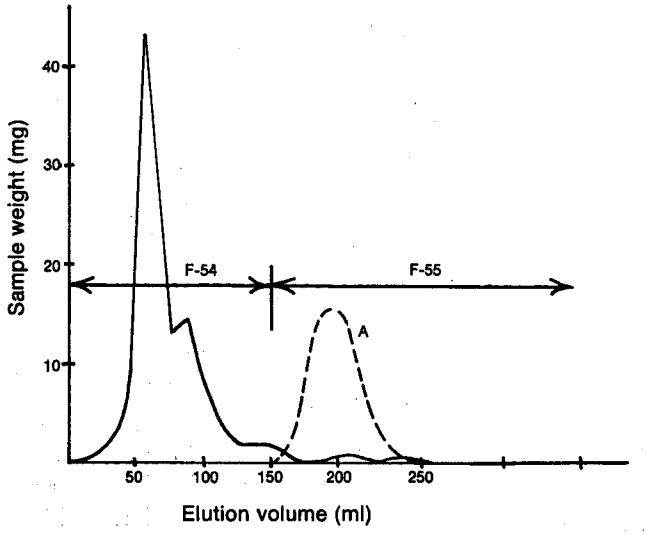


Figure 2. Separation of F-20 on Bio-Beads SX-2 into F-54 and F-55. Curve A – Elution of benzo(a)pyrene.



EXPERIMENTAL

Fractionation of Cigarette Smoke Condensate (CSC)

All solvents used were Burdick and Jackson* distilled-in-glass grade. The CSC was prepared in 1.0 kg batches at the Roswell Park Memorial Institute and shipped to us as previously described (10). A total of three 1 kg batches were fractionated. Although details for the isolation of the PAH concentrate, fraction F-55, have been reported elsewhere (5), the general fractionation scheme is outlined in Figure 1. In brief, CSC neutrals were isolated by consecutive acid and base extractions and then chromatographed on silicic acid. The 25 % benzene : petroleum ether eluate (fraction F-BPE) was partitioned between cyclohexane (CH) and dimethylsulfoxide (DMSO) to yield F-20. Fractionation of 3 kg of CSC yielded 9.6 g of F-20. Preparative GF of F-20 on Bio-Beads SX-2 in acetone gave 1.0 g of F-55 (Fig. 2). This highly concentrated PAH fraction was then separated by analytical GF on Bio-Beads SX-12 in benzene on a four-column gel system similar to that described previously (11). The columns (1.25 cm × 109 cm) were connected in series and the slurry was packed in benzene with a total of 200 g of Bio-Beads SX-12 (dry weight). Samples (0.25 g/ml of F-55 per run) were placed on the columns with a 1 ml injection loop and pumped through the columns at a flow rate of 120 ml/h. Column effluent was monitored at 280 nm with a Chromatronix Model 230 UV detector, equipped with a flow cell, and 8 ml fractions were collected. Elution of UV-absorbing material began with GF fraction 24 and continued up to GF fraction 55. By the use of standard 2,3,5-trimethylnaphthalene, the beginning of the PAH elution was found to be GF fraction 36. Four separate runs were required to fractionate the entire sample of F-55. Fractions with the same number, from each run, were combined for subsequent GC. The reproducibility of the described gel system allowed such combinations. The amount of material in latter fractions was increased by combining GF fractions 50 and 51 and GF fractions 52 to 55 inclusive.

Gas Chromatography (GC)

GF fractions 45, 46, 47, 48, 49, 50–51, and 52–55 were subjected to analytical GC analysis on a Hewlett-Packard Model 5750 gas chromatograph equipped with a 15'×1/8" stainless steel column packed with 5 % Dexsil 300 GC on 100/120 mesh Chromosorb W-AW (temperature program: 200–325 °C at 2°/min, after an initial hold at 200 °C for 5 min; 48 ml/min He; injection temperature, 290 °C; flame detector, 350 °C). A Varian Model 485 electronic integrator was used to determine the areas of GC peaks.

A Hewlett-Packard Model 5750 gas chromatograph equipped with a thermal conductivity (TC) detector was used for preparative GC of the above GF fractions. Preparative GC conditions were identical to those for analytical GC. PAH were collected at the exit port of the TC detector in glass capillary tubing (TC oven temperature, 350 °C). Whenever possible, samples were collected during the upslope, top, and downslope of GC peaks to give three cuts of a single peak. The GF fraction number and the corresponding number of preparative collection cuts, respectively, were: GF fraction 45, 120; GF fraction 46, 73; GF fraction 47, 91; GF fraction 48, 68; GF fraction 49, 65; GF fraction 50–51, 76; GF fraction 52–55, 36 (total samples, 529).

Ultraviolet Spectral Data of Preparative GC Samples

The glass capillary tubes containing the PAH from the above preparative GC runs were rinsed into 0.9 ml cuvettes with 95 % ethanol. Ultraviolet (UV) spectra were obtained with a Beckman Acta C III spectrophotometer.

High-Pressure Liquid Chromatography (HPLC)

A DuPont 830 liquid chromatograph equipped with a 25 cm × 2.4 mm DuPont Zorbax ODS column was used to separate the individual components of the preparative GC cuts. A linear solvent gradient of 3 %/min, ranging from 65 % CH_3OH/H_2O to 85 % CH_3OH/H_2O, was employed. The initial flow rate was 0.5 ml/min. However, this decreased to about 0.3 ml/min during the course of this work, possibly due to column compression or blockage. Increased retention times for components did not affect their separation. A total of 156 preparative GC cuts were selected for analysis by HPLC. This quantity represented 51 of the 59 distinct GC peaks in GF fractions 45 to 55. The preparative GC cuts were concentrated in tapered test tubes to about 5 µl (in EtOH) and injected into the liquid chromatograph with anthracene as an internal standard. Elution of the samples was monitored at 254 nm, and separated components were collected in 4 ml vials. UV spectra of the separated components were obtained in CH_3OH (generally 85 % CH_3OH). Eluates corresponding to definable peaks or portions of peaks were collected for each run and over 1500 UV spectra were recorded.

GC-Mass Spectral Data

A Varian Model 1400 GC instrument was used in conjunction with a DuPont 21–492 mass spectrometer. The gas chromatograph was equipped with a 10'×1/8" stainless steel column packed with 5 % Dexsil 300 GC on 100/120 Chromosorb W-AW (injection temperature, 290 °C; flame detector, 350 °C; and 20 ml/min He). GF fractions 45, 46, 47, 48, 49, 50–51, and 52–55 were chromatographed by the use of a temperature program of 2°/min from 200 to 325 °C.

* Reference to a company or product name does not imply approval or recommendation by the U.S. Department of Agriculture.

Mass spectral (MS) analyses of the GC effluents were performed as follows. The effluent was split with a 1:1 splitter, one half going to the flame ionization detector of the gas chromatograph and the other half to the source area of the mass spectrometer. Before MS analysis, a jet separator at 300 °C was used to strip helium from the GC effluent. Mass spectra of effluent GC peaks were obtained at a scan rate of 10 s/mass decade, a minimal resolution of 1000, and an ionization potential of 70 eV. Mass spectra were taken as often as possible during the elution time of a GC peak to determine mass integrity. The spectra were recorded by a high-speed recording oscillograph and/or an AEI DS-30 computerized data system. MS data were analyzed by both manual and computer-aided techniques. HPLC-separated components of doubtful identity were submitted to probe MS analyses after evaporation of the solvent.

Quantitation of
Selected High Molecular Weight PAH in CSC

The amounts of several high molecular weight PAH in CSC were quantitated by our recently developed accelerated PAH analysis method (11, 12). Three batches of 270 Kentucky 1R1 Reference cigarettes were smoked and the smoke was collected in dry-ice traps. CSC from each batch was treated as follows. The CSC was rinsed into a 1000 ml separatory funnel with benzene, methanol, and ether (100 ml of each) and washed with H_2O. The organic solubles were reduced in volume, chromatographed on silicic acid, and eluted first with petroleum ether followed by 25% benzene:petroleum ether. The second eluate was evaporated and the residue chromatographed on a four-column Bio-Beads SX-12 system in benzene. The beginning of the elution of benzo(a)pyrene (BaP) was used to determine the start of the elution of larger PAH. The combined GF fractions from all three GF runs were pooled and analyzed by GC. After application of detector response corrections, the CSC levels of PAH larger than BaP were determined relative to the known [2.4 μg/100 cigarettes (11)] BeP/BaP levels (Table 3).

RESULTS AND DISCUSSION

The PAH-containing GF fractions (41–55) were subjected to analytical GC on Dexsil 300 GC. The GC runs showed definite changing profiles (Figs. 3–5). GF fractions 41–44 contained only small amounts of the same PAH occurring predominantly in GF-45. Subsequently, each GF fraction (45, 46, 47, 48, 49, 50–51, and 52–55) was submitted to preparative GC to give cuts representing single peaks or portions of peaks. UV spectra were obtained for each preparative GC cut. Where possible, multiple cuts were taken of peaks for determination of peak integrity. MS data were obtained on each GF fraction by GC-MS techniques. The UV

Figure 3. Gas chromatograms of PAH constituents in GF fractions 45 and 46.

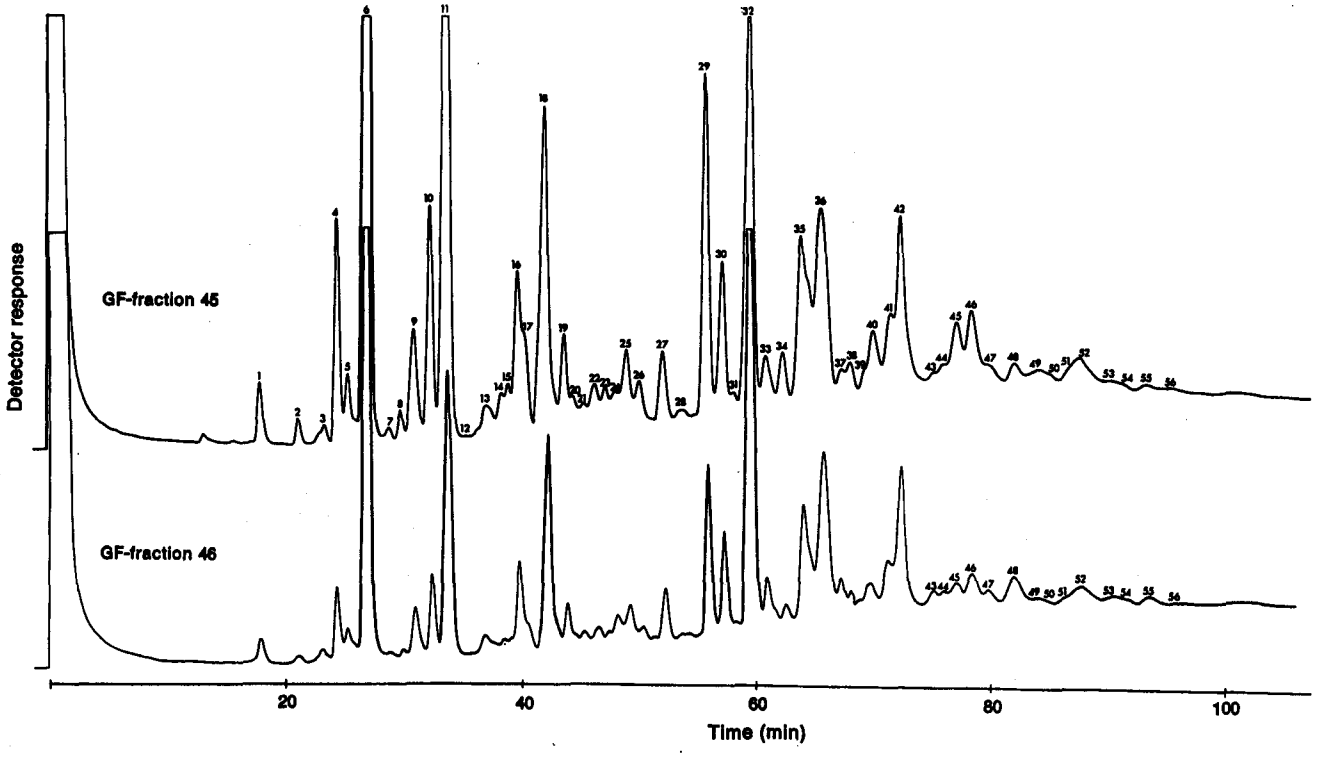


Figure 4. Gas chromatograms of PAH constituents in GF fractions 47 and 48.

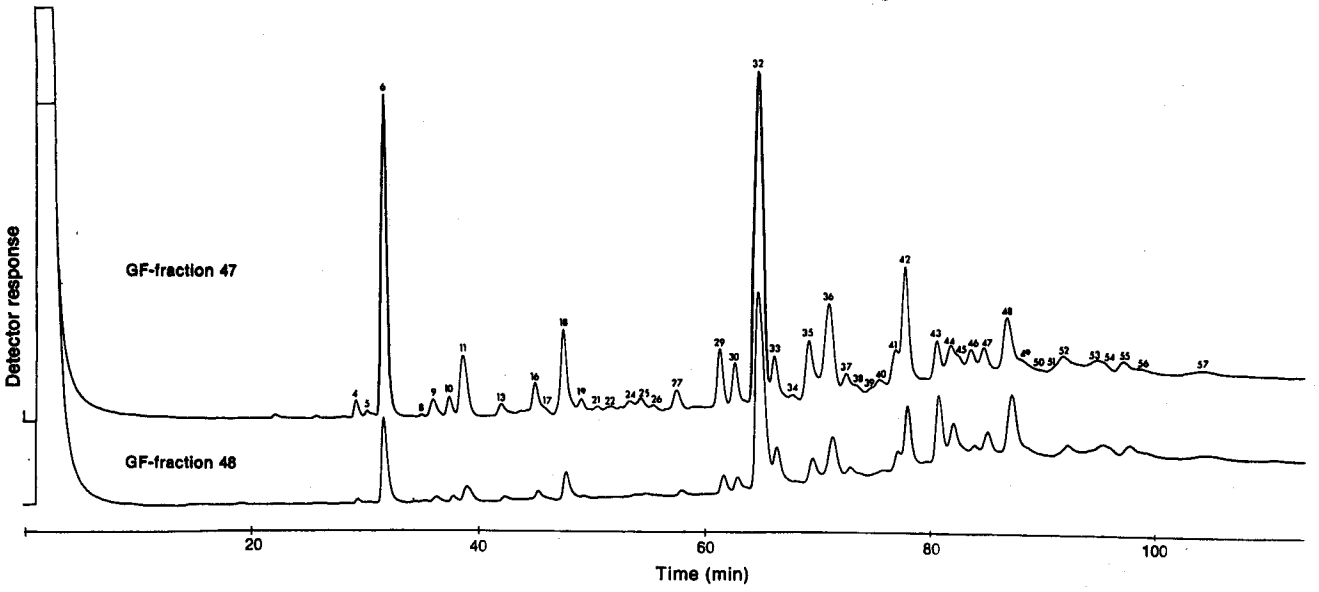
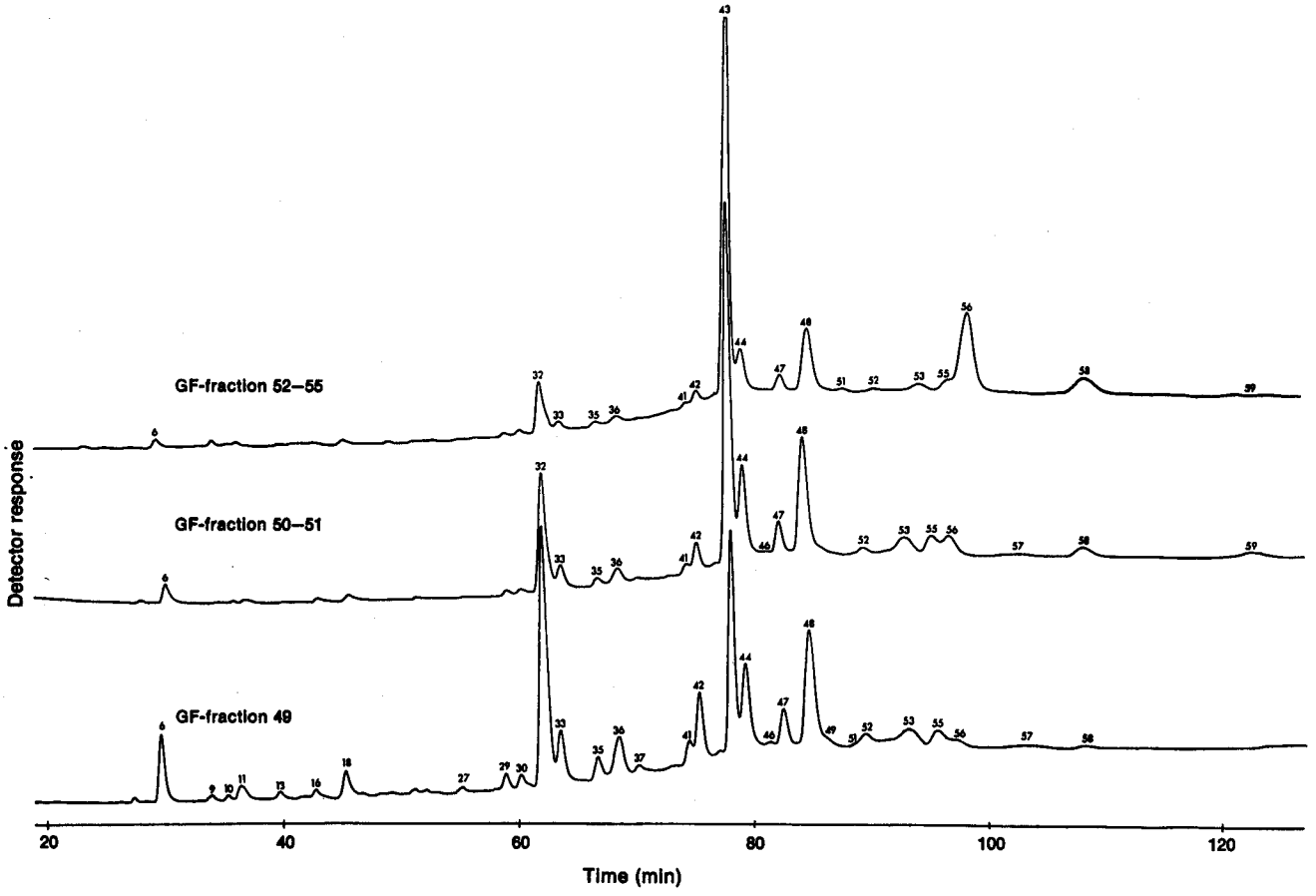


Figure 5. Gas chromatograms of PAH constituents in GF fractions 49, 50–51 combined, and 52–55 combined.



and MS data showed that most GC peaks were complex mixtures. For unambiguous identification, further separation of the components in each peak was necessary. HPLC on Zorbax ODS proved to be an excellent tool in effecting the necessary separations. Individual preparative GC cuts were chromatographed by HPLC, and UV spectra were obtained on the separated components. In this manner, 51 of the 59 GC peaks in GF fractions 45–55 were analyzed by HPLC.

The results of the identification and quantitation of the components in GF fractions 45–55 are given in Table 1. The corresponding gas chromatograms are

given in Figures 3–5. Peaks are tabulated in order of relative retention time (RRT) with peak number 32 (BaP/BeP) being assigned an RRT value of 1.000. (Peaks having the same RRT are given the same number in all tables and chromatograms.) Although individual peak percentages will depend on total GC volatiles of a gel fraction, they are strongly indicative of the concentration of components. Where possible, the major component is indicated in multiple component peaks. Applying parent PAH detector response values, we calculated that at least 80% of the material in combined GF fractions 45–55 was GC volatile. The data in Table 1 show that 90–95% of these GC volatiles were identified. Thus, at least 72% of the components in the F-55 PAH fraction have now been identified unambiguously.

The basis for identification of most of the compounds in Table 1 is given in Table 2, including the results of the HPLC separations. Usually PAH compounds, whose GC RRT and/or UV spectra are available in the literature, have not been included in Table 2. However, several compounds of this type were included to indicate their HPLC order of elution, given in terms of the RRT. For the most part, the individual RRT can be compared from run to run to indicate order of elution of constituents. However, as work progressed, the RRT of some GC peak components increased, probably due to blockage and the use of the constant pressure pump. Although the separating ability of the HPLC was not affected, a constant flow pump would have produced more consistent RRT. Although analysis time was long by conventional HPLC standards, it proved to be best for preparative separations. Efforts to decrease retention time by increasing the methanol concentration of the mobile phase also decreased resolution. Thus, the use of a 3%/min gradient from 85% to 95% CH_3OH/H_2O halved the RRT of compounds but also compressed the peaks and gave ambiguous UV spectra for collected peaks.

Unambiguous identifications were made by correlation of all the data from the GF, GC, and HPLC separations with the corresponding UV and MS information. The unique properties of the gel columns in retaining and concentrating the high molecular weight PAH were used to advantage in the final purification step of F-55. The two separation characteristics of the gels have been described (13). Briefly, PAH are separated from interfering material by an absorption-type mechanism and are eluted from the gel columns after the impurities and in order of increasing ring number. Thus, PAH larger than fluoranthene were found in GF fractions 45–55. An additional mechanism of separation also occurs in that methyl PAH eluted from the gels before their parents. An increase of methyl substitution results in earlier elutions relative to parent PAH. These two phenomena are well illustrated by the relative GC peak changes in the gas chromatograms of GF fractions 45–55 (Figs. 3–5) and in the data in Table 1. The dimethyl derivatives of pyrene, chrysene, benzofluoranthene, benzo(a)pyrene, benzo(e)pyrene, indenopyrene, and benzo(ghi)perylene were eluted in earlier gel fractions than their monomethyl analogs, which in turn eluted earlier than the parent compounds. (For brevity, indeno(1,2,3-cd)pyrene will be termed indenopyrene and indeno(1',2',3'-3,4)fluoranthene, indenofluoranthene.)

For HPLC on Zorbax ODS, the trend of elution was unsubstituted PAH, followed by monomethyl-, dimethyl-, and trimethyl-substituted PAH. Because of the large number of compounds that co-elute in each gel fraction, it would have been futile to attempt HPLC analysis of the whole gel fraction. Consequently, preparative GC was used to resolve the GF fractions into "peaks" that were collected and then subjected to HPLC. Since PAH differ in their absorption coefficients at 254 nm, the heights of peaks in the HPLC chromatograms, unlike those for GC may not be indicative of the relative concentrations. These separations, in conjunction with the data in Table 2, demonstrated that most of the GC peaks contained multiple components.

Table 1. Composition of gel filtration fractions 45 to 55.

Peak No.	Compound[a]	RRT[b]	Gel fraction							Criteria of identification		
			45	46	47	48	49	50+51	52-55	GC-RT[d]	UV[e]	MS[f]
			Percent composition[c]									
1	4,5-Methylenephenanthrene	0.362	0.82	0.26	0.21	0.07	–	–	–	+	+	+
2	Unidentified	0.415	0.36	0.15	0.12	0.05	–	–	–		+	+
3	Dimethylphenanthrene	0.456	0.19[g]	0.08[g]	<0.05[g]	–	–	–	–		+	+
4	Fluoranthene	0.472	3.03	1.57	0.64	0.16	0.37	0.24	<0.05	+	+	+

Table 1 (cont'd.).

Peak No.	Compound[a]	RRT[b]	Gel fraction: 45	46	47	48	49	50+51	52-55	Criteria of identification: GC-RT[d]	UV[e]	MS[f]
			Percent composition[c]									
5	Acephenanthrylene	0.487	1.16[g] M	0.56[g] M	0.08[g] M	<0.05[g] M	–	–	–		+	+
6	Pyrene	0.514	19.36	22.05	16.45	10.34	5.50	1.71	<0.05	+	+	+
7	Unidentified	0.540	0.12	<0.05	–	–	–	–	–		+	+
8	8-Methylfluoranthene	0.558	0.36	0.07	0.16	<0.05	–	–	–		+ (17)	+
9	1-Methylfluoranthene	0.575	2.06 M	1.20 M	0.78 M	0.16 M	0.42 M	–	–		+ (17)	+
	2-Methylfluoranthene										+ (17)	+
	2,3-Benzofluorene									+	+	+
	3,4-Benzofluorene									+	+	+
10	2-Methylpyrene	0.597	3.42	1.72	0.80	0.26	0.39	0.14	<0.05	+	+	+
11	1-Methylpyrene	0.618	13.20 M	8.00 m	4.08 m	2.31 m	1.05 m	0.47 m	<0.05 m	+	+	+
	4-Methylpyrene		M	M	M	M	M	M	M	+	+	+
12	Methyl-2,3-benzofluorene	0.639	<0.05	–	–	–	–	–	–		+	+
	Dimethylpyrene										+	+
	Dimethylfluoranthene										+	+
13	Methylbenzofluorenes	0.666	0.11[h] M	0.34[h] M	0.43[h] M	0.66 M	0.14 M	<0.05 M	–		+	+
	Dimethylpyrenes		m	m	m	m	m	–			+	+
	Dimethylfluoranthenes		m	t	–	–	–	–			+	+
14	Dimethylpyrene	0.689	0.32 M	<0.05 M	–	–	–	–	–		+	+
	Benzo(c)phenanthrene		t	t						+	+	+
15	Dimethylpyrene	0.698	0.41 M	<0.05 M	–	–	–	–	–		+	+
16	Benzo(ghi)fluoranthene	0.708	2.12 M	1.78 M	1.25 M	0.38 M	0.10 M	0.30 M	0.46 M	+	+	+
	Dimethylpyrenes		M	M	M	m	–	–	–		+	+
17	Dimethylpyrene	0.719	1.11	0.46	0.15	<0.05	–	–	–		+	+

Table 1. Composition of gel filtration fractions 45 to 55 (cont'd.).

Peak No.	Compound[a]	RRT[b]	Gel fraction 45	46	47	48	49	50+51	52-55	Criteria of identification GC-RT[d]	UV[e]	MS[f]
			Percent composition[c]									
18		0.744	7.51	6.54	5.33	3.54	2.17	0.65	<0.05			
	1,2-Benzanthracene			m	m	m	m	m	–	+	+	+
	Chrysene			M	M	M	M	M	M	+	+	+
	Triphenylene			M	M	M	M	M	M	+	+	+
	Cyclopenta(cd)pyrene		m	m	t	–	–	–	–		+ (18)	+
19		0.768	1.30[h]	0.69[h]	0.29[h]	0.31[h]	<0.05[h]	–	–			
	Methyl-2,3-benzofluorene										+	+
	3,4-Dimethylenepyrene		M	M	M	M	M				+ (18)	+
	Dimethylpyrenes										+	+
20		0.777	0.32[g]	0.13[g]	<0.05[g]	–	–	–	–			
	Trimethylpyrenes										+	+
21		0.790	0.38[h]	0.11[h]	0.08[h]	<0.05[h]	–	–	–			
	Dimethylbenzofluorene										+	+
	Methyl-3,4-dimethylenepyrene		M	M	M	M					+	+
	Trimethylpyrene										+	+
22		0.807	0.71[h]	0.14[h]	<0.05[h]	–	–	–	–			
	Methyl-1,2-benzanthracene										+	+
	Dimethylbenzofluorene		M	m	–						+	+
	Methylbenzo(ghi)fluoranthene		M	M	M						+	+
	Trimethylpyrenes		t	t	t						+	+
23		0.820	0.48	<0.05	–	–	–	–	–			
	3-Methylchrysene									+	+	+
	Methyltriphenylene										+	+
	2-Methylchrysene									+	+	+
	Trimethylpyrenes		t	t							+	+
24		0.829	0.98[g]	0.52[g]	0.32[g]	0.32[g]	0.28[g]	–	–			
	4-Methylchrysene									+	+	+
	Methyl-1,2-benzanthracenes										+	+
	Trimethylpyrene		t	t	t	–	–				+	+
25		0.845	1.54[g]	0.67[g]	0.32[g]	0.30[g]	0.23[g]	–	–			
	4,5-Methylenetriphenylene										+	+
	1-Methylchrysene									+	+	+
	6-Methylchrysene									+	+	+
	Methyl-1,2-benzanthracene		t	t	–	–	–				+	+
	Trimethylpyrene		t	t	–	–	–				+	+
26		0.862	0.38[g]	0.26[g]	<0.05[g]	–	–	–	–			
	Dimethylchrysene		t	t	–						+	+
	Dimethyl-1,2-benzanthracene		t	t	–						+	+
	3,4-Trimethylenepyrene		M	M	M						+ (19)	+
27		0.890	1.35[g]	1.38[g]	1.01[g]	0.68[g]	0.27[g]	–	–			
	4,5-Methylenechrysene				t	t	t				+	+
	Dimethyltriphenylene										+	+
	Dimethylbenzo(ghi)fluoranthene										+	+
	Dimethyl-1,2-benzanthracene				t	t	t				+	+
28		0.906	0.37[h]	0.26[h]	–	–	–	–	–			
	Dimethyltriphenylene		M	M							+	+
	Dimethylchrysene		M	M							+	+

Table 1 (cont'd.).

Peak No.	Compound[a]	RRT[b]	Gel fraction							Criteria of identification		
			45	46	47	48	49	50+51	52-55			
			Percent composition[c]							GC-RT[d]	UV[e]	MS[f]
29		0.946	5.27[h]	4.54[h]	2.83[h]	1.70	0.63	0.53	0.32			
	Benzo(b)fluoranthene		M	M	M	M	M	M	M	+	+	+
	Benzo(j)fluoranthene		m	m	m	m	m	m	m		+ (19)	+
	Benzo(k)fluoranthene		t	t	t	t	t	t	t		+ (19)	+
30		0.966	2.63	2.86	1.87	1.19	0.58	0.51	0.32			
	Benzo(a)fluoranthene		M	M	M	M	M	M	M		+ (19)	+
	Dimethyl-1,2-benzanthracene		t	t	t	t	–	–	–		+	
31		0.978	0.10	<0.05	–	–	–	–	–			
	Tetramethylpyrene											+
32		1.000	8.25	17.35	25.98	30.93	26.16	11.79	6.02			
	Benzo(e)pyrene									+	+	+
	Benzo(a)pyrene									+	+	+
33		1.018	1.06	1.45	3.07	4.32	4.52	2.84	1.11			
	Perylene		M	M	M	M	M	M	M	+	+	+
	Methylbenzo(j)fluoranthene										+	+
	Methylbenzo(b)fluoranthene										+	+
34		1.039	0.96	0.33	0.24	0.08	0.09	–	–			
	Methylbenzo(j)fluoranthene										+	+
	Methylbenzo(b)fluoranthene										+	+
	Methylbenzo(a)pyrene		t	t	t	t	t				+	+
35		1.062	3.51	5.34	4.29	3.13	1.78	0.71	0.60			
	Methylbenzo(e)pyrenes		M	M	M	M	M	M	M		+	+
	Methylbenzo(a)pyrene		m	m	m	m	m	m	m		+	+
36		1.087	4.97	7.69	7.71	5.91	4.04	1.75	1.11			
	Methylbenzo(e)pyrenes										+	+
	Methylbenzo(a)pyrenes										+	+
	Methylperylenes										+	+
37		1.110	0.20[h]	0.97[h]	0.91[h]	1.40[h]	1.08[h]	0.28[h]	<0.05			
	Methylbenzo(a)pyrene				t	t	–	–	–		+	+
	Methylperylene		M	M	M	M	M	M	M		+	+
	Dimethylbenzo(e)pyrene		t	t	–	–	–	–	–		+	+
38		1.121	0.16[h]	0.57[h]	0.26[h]	0.17[h]	<0.05	–	–			
	Methylperylene										+	+
	Methylbenzo(a)pyrenes										+	+
	Methylbenzo(e)pyrenes										+	+
	Dimethylbenzo(e)pyrene		t	t	–	–	–				+	+
39		1.143	0.13	0.18	0.19	–	–	–	–			
	Indenofluoranthene		M	M	M						+ (19)	+
	Dimethylbenzo(e)pyrenes		m	m	m						+	+
	Dimethylperylene		t	t	t						+	+

Table 1. Composition of gel filtration fractions 45 to 55 (cont'd.).

Peak No.	Compound[a]	RRT[b]	Gel fraction 45	46	47	48	49	50+51	52-55	Criteria of identification GC-RT[d]	UV[e]	MS[f]
			Percent composition[c]									
40		1.158	0.98	0.53	0.53	<0.05	–	–	–			
	Dibenz(a,j)anthracene		t	t	t	t				+	+	+
	Dimethylbenzo(e)pyrenes		M	M	M	M					+	+
	Dimethylperylene										+	+
	Dimethylbenzo(a)pyrenes										+	+
41		1.183	1.00[h]	1.22[h]	1.50[h]	1.01[h]	0.94[g]	0.85[g]	0.51[g]			
	Dibenz(a,c)anthracene		t	t	t	t	–	–	–	+	+	+
	Dibenz(a,h)anthracene		t	t	t	t	–	–	–	+	+	+
	Dimethylbenzo(e)pyrenes										+	+
	Dimethylperylene										+	+
	Dimethylbenzo(a)pyrenes										+	+
42		1.201	2.73	4.53	6.11	5.17	4.27	2.44	1.16			
	Picene		t	t	t	t	t	–	–	+	+	+
	Indenopyrene		M	M	M	M	M	M	M	+	+	+
	Dimethylbenzo(e)pyrenes		m	t	t	–	–	–	–		+	+
	Dimethylperylene		m	t	t	–	–	–	–		+	+
	Dimethylbenzo(a)pyrenes		m	t	t	–	–	–	–		+	+
	Trimethylbenzo(e)pyrenes		t	t	–	–	–	–	–		+	+
43		1.255	0.07[g]	0.52[h]	1.41[h]	6.36	17.05	33.73	43.10			
	Benzo(ghi)perylene		M	M	M	M	M	M	M	+	+	+
	Methyldibenz(a,c)anthracene		t	t	–	–	–	–	–		+	+
	Dimethylbenzo(a)pyrene		m	t	–	–	–	–	–		+	+
	Trimethylbenzo(e)pyrene		t	t	–	–	–	–	–		+	+
44		1.272	<0.05	1.52	0.61	2.18	7.35	9.35	6.49			
	Anthanthrene		M	M	M	M	M	M	M	+	+	+
	Methylindenopyrene		m	m	t	–	–	–	–		+	+
45		1.303	0.64[h]	0.77[h]	0.22[h]	<0.05	–	–	–			
	Methyldibenz(a,c)anthracene		t	t	t	–					+	+
	Methylindenopyrene		M	M	M	M					+	+
	Trimethylbenzo(e)pyrene		t	t	–						+	+
46		1.332	0.99[h]	1.11[h]	0.64[h]	0.55	0.27	0.05	–			
	Trimethylbenzo(e)pyrene		t	t	–	–	–	–			+	+
	Methylindenopyrene		M	M	M	M	M	M			+	+
47		1.360	0.14[h]	0.52[h]	0.76	1.27	1.98	2.03	1.85			
	Methylindenopyrenes		M	M	m	m	m	m	m		+	+
	Methylbenzo(ghi)perylene		m	M	M	M	M	M	M		+	+
48		1.413	0.30[h]	0.98[h]	3.22[h]	6.50[h]	11.77[h]	13.41[h]	9.27[h]			
	Dimethylindenopyrene		m	–	–	–	–	–	–		+	+
	Methylbenzo(ghi)perylenes		M	M	M	M	M	M	M		+	+
	Methylanthanthrenes		m	M	M	M	M	m	t		+	+
49		1.432	0.14	0.24	0.36	0.81	0.28	0.63	<0.05			
	Methylbenzo(ghi)perylene		M	m	m	t	–	–	–		+	+
	Methylanthanthrenes		m	M	M	M	M	M	M		+	+
	Dimethylindenopyrene		M	m	t	t	–	–	–		+	+
50		1.492	0.12[g]	0.12[g]	<0.05[g]	–	–	–	–			
	Methylbenzo(ghi)perylene		M	M	M	M					+	+
	Methylanthanthrene		m	m	M	M					+	+
	Dimethylindenopyrenes		M	M	m	t					+	+

Table 1 (cont'd.).

Peak No.	Compound[a]	RRT[b]	Gel fraction 45	46	47	48	49	50+51	52-55	Criteria of identification GC-RT[d]	UV[e]	MS[f]
			Percent composition[c]									
51		1.523	0.25	0.22	0.47	0.05	–	–	–			
	Dibenzo(b,j)fluoranthene		m	m	m	m	m	t			+ (20)	+
	Methylbenzo(ghi)perylene		m	m	m	m	–	–			+	+
	Dimethylindenopyrene		M	m	t	–	–	–			+	+
	Dimethylbenzo(ghi)perylene		M	M	M	M	M	M			+	+
52		1.545	0.53[h]	0.51[h]	1.17[h]	1.35[h]	1.11[h]	1.22[h]	0.23[h]			
	Dibenzo(a,e)fluoranthene		M	M	M	M	M	m	–		+ (20)	+
	Dibenzo(a,l)pyrene		t	m	m	m	m	m	–	+	+	+
	Dimethylindenopyrene		m	t	–	–	–	–	–		+	+
	Dimethylbenzo(ghi)perylenes		M	M	M	M	M	M	M		+	+
	Dimethylanthanthrenes		m	M	M	M	m	–	–		+	+
53		1.616	0.15	0.44	0.91	1.41	1.79	4.31	1.71			
	Dibenzofluoranthenes		t	m	M	M	M	M	M		+	+
	Dimethylbenzo(ghi)perylene		M	M	M	M	m	t	t		+	+
54		1.638	0.11	0.36	0.70	–	–	–	–			
	Dibenzofluoranthene		M	M	M	M					+	+
	Dimethylbenzo(ghi)perylene		M	M	M	M					+	+
	Dimethylanthanthrene		m	m	m	m					+	+
55		1.678	0.17[h]	0.36[h]	0.76[h]	1.41[h]	1.72[h]	4.06	2.36			
	Dibenzo(a,e)pyrene		t	M	M	M	M	M	M	+	+	+
	Dimethylbenzo(ghi)perylenes		M	m	m	t	t	t	t		+	+
	Trimethylindenopyrene		t	t	t	t	t	–	–		+	+
	Trimethylbenzo(ghi)perylene		t	t	t	t	t	–	–		+	+
56		1.711	–	<0.05	0.19	2.49	0.98	3.62	16.68			
	Benzo(b)perylene			m	M	M	M	M	m		+ (19)	+
	Dibenzo(a,i)pyrene		M	M	M	M	M	M	m	+	+	+
	Coronene			t	t	m	M	M	M	+	+	+
	Dimethylbenzo(ghi)perylene		M	M	m	m	m	–	–		+	+
	Dimethylanthanthrene		m	m	m	m	–	–	–		+	+
	Trimethylindenopyrenes		t	t	–	–	–	–	–		+	+
	Trimethylbenzo(ghi)perylenes		t	t	–	–	–	–	–		+	+
57		1.877	<0.05	0.05[h]	0.20[h]	0.20[h]	0.05[h]	–	–			
	Dibenzo(a,h)pyrene			M	M	M	M			+	+	+
	Dibenzo(e,l)pyrene			m	m	m	m				+ (19)	+
	Trimethylbenzo(ghi)perylene			t	t	–	–				+	+
58		2.015		<0.05	0.24[h]	0.83[h]	0.36[h]	2.19[h]	5.42[h]			
	Methylcoronene										+	+
59		2.300		–	–	–	–	0.50[h]	0.83[h]			
	Dimethylcoronene										+	+

a: Whenever possible with multiple component GC peaks, the following designations are used under gel fraction number: M — major component, greater than 30% of composition; m — minor component, less than 30% of composition; t — trace amount, less than 10% of composition.
b: Relative to benzo(a)pyrene; a factor of 70.8 converts RRT to minutes from point of injection.
c: Based on total GC volatiles in gel fraction assuming unitary detector response.
d: GC retention time identical to standard.
e: UV spectra identical to standard, identical to literature (reference given), or analogous to parent compound.
f: Molecular ion and fragmentation pattern correlation.
g: Contains other unidentified material.
h: Major component(s) unidentified.
M: Major component.

Table 2. Identification data[a].

GC peak No.	HPLC RRT[b]	Compound	λ_{max}[c]	Mass (m/e)[d]
2	1.10	Unidentified	232, 238, 278, 320, 335, 355, 373, 400, 425	192, 190
3	1.39	Dimethylphenanthrene	252, 278, 287, 300	206, 191
		Unidentified (possibly dimethylenephenanthrene)	250-4B	204
5	1.15	Acephenanthrylene	232, 252, 260, 286, 298, 317, 328, 345, 363	202[e]
	1.22	Unidentified (possibly aceanthrylene)	228, 244, 271, 323	202
		Unidentified		208
7	1.38	Unidentified	217, 228, 240-8B, 272, 278, 287, 350, 367	218
9	1.17	3,4-Benzofluorene		216
	1.23	2,3-Benzofluorene		216
	1.39	1-Methylfluoranthene		216
		2-Methylfluoranthene		216
12		Methyl-2,3-benzofluorene	262	230, 215
		Dimethylpyrene	334	230, 215
		Dimethylfluoranthene	239, 288, 362	230, 215
13	1.28	Methyl-2,3-benzofluorene (probably the 9-methyl isomer)	252, 265, 306-10B, 328, 333, 342	230, 215
	1.36	Methyl-1,2 and/or 2,3-benzofluorene	265	230, 215
	1.45	Unidentified	233, 240, 264, 274, 335	216[e]
	1.59	Methyl-1,2 and/or 2,3-benzofluorene	254, 263	230, 215
		Dimethylpyrene	240, 323, 337	230, 215
		Dimethylfluoranthene	239, 288	230, 215
	1.69	Dimethylpyrene	235, 245, 263, 276, 308, 321, 337	230, 215
14	1.75	Dimethylpyrene	234, 243, 265, 276, 308, 321, 337	230, 215
15	1.51	Dimethylpyrene	234, 244, 265, 276, 308, 322, 337	230, 215
16	1.65	Dimethylpyrene	242, 265, 276, 321, 343	230, 215
	1.68	Dimethylpyrene	242, 265, 276.5, 322, 337	230, 215

Table 2. (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max} [c]	Mass (m/e)[d]
17	1.42	Dimethylpyrene	242, 265, 276, 328, 347	230, 215
19	1.43	Methyl-2,3-benzofluorene	256, 266, 275, 285, 306, 319	230, 215[e]
	1.46	Unidentified	253, 267, 285, 299, 309, 336, 361	242, 240[e]
	1.49	Unidentified (isomer of HPLC 1.46)	253, 267, 286, 300, 311, 336, 360	242, 240[e]
	1.55	3,4-Dimethylenepyrene		228
	1.65	Dimethylpyrene	233, 242, 265, 276, 312, 327, 343	230, 215
	1.76	Dimethylpyrene	242, 254, 266, 277, 327, 342	230, 215
	1.86	Dimethylpyrene	247, 266, 278, 323, 330	230, 215
20	1.65	Unidentified	226, 235, 242, 253, 270, 276, 281, 295, 310, 327, 343, 357, 374, 394	242
	1.87	Trimethylpyrene (2 isomers)	236, 245, 269, 279, 325, 340, 345	244, 229
	1.92	Trimethylpyrene	236, 245, 267, 278, 324, 339	244, 229
21	1.47	Unidentified (pyrene-type — possibly methyl-3,4-dimethylenepyrene)	246, 267, 275, 281, 326, 340	242
	1.55	Dimethyl-1,2 and/or 2,3-benzofluorene	255, 265	244, 229
		Methyl-3,4-dimethylenepyrene	234	242
	1.89	Trimethylpyrene	245, 266, 278, 326, 339	244, 229
22	1.58	Methyl-1,2-benzanthracene	275, 287	242, 227
		Dimethyl-1,2 and/or 2,3-benzofluorene	253, 262	244, 229
		Unidentified (pyrene-type — possibly methyl-3,4-dimethylenepyrene)	275, 338, 344, 365, 386	242
	1.63	Unidentified	242	
	1.74	Methylbenzo(ghi)fluoranthene	232, 245, 279, 290, 333, 348	240
	1.95	Trimethylpyrene	234, 243, 266, 278, 328, 344	244, 229
	2.01	Trimethylpyrene	236, 245, 267, 279	244, 229
23	1.66	3-Methylchrysene		242
	1.71	Methyltriphenylene	248, 258	242
	1.73	2-Methylchrysene		242
	1.88	Trimethylpyrene	235, 245, 279, 327, 344	244
	1.92	Trimethylpyrene	235, 244, 268, 279	244
24		Methyl-1,2-benzanthracene	280B, 288	242
		Methyl-1,2-benzanthracene	280B, 292	242

Table 2. Identification data[a] (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max}[c]	Mass (m/e)[d]
24 (cont'd.)		Trimethylpyrene	240, 246, 280B, 322, 345	244
		Unidentified		240
25	1.61	4,5-Methylenetriphenylene	250, 257	240[e]
	1.66	1-Methylchrysene		242
		Unidentified	223, 243, 248	256, 242[e]
	1.68	6-Methylchrysene		242
	1.79	Methyl-1,2-benzanthracene	288	242
	1.89	Trimethylpyrene	235, 245, 268, 279, 330, 345	244
26	1.60	Unidentified	247, 256	
	1.64	Unidentified	258, 264, 267	
	1.72	Dimethylchrysene	268	256, 241
		Unidentified (possibly methylene-1,2-benzanthracene)	286	240
	1.86	3,4-Trimethylenepyrene		242, 240
27	1.48	Unidentified	221, 232, 242, 260, 274, 284, 293	
	1.51	Unidentified	258, 282, 292, 295, 367, 380, 402, 425	254, 252[e]
	1.62	Unidentified	250, 257, 266, 280, 315, 390, 410	
	1.64	4,5-Methylenechrysene	259, 268, 311	240[e]
	1.75	Unidentified (possibly dimethyldimethylenepyrene)	247, 263, 280, 329, 344, 378, 388	256[e]
	1.84	Dimethyltriphenylene	251, 260	256
		Unidentified (possibly cyclopentenopyrene)	236, 244, 268, 278, 328, 345	242, 240
	1.94	Dimethylbenzo(ghi)fluoranthene	233, 246, 278, 290	254[e]
		Dimethylchrysene	260, 270	256[e]
	1.96	Dimethylchrysene	260, 270	256
		Dimethyl-1,2-benzanthracene	280, 290	256
28		Unidentified (pyrene-type)	245, 271, 282, 349, 355, 368+252	
		Dimethyltriphenylene	250, 259	256, 241
		Dimethylchrysene	259, 270	256, 241
29	1.80	Benzo(j)fluoranthene		252
	1.84	Benzo(b)fluoranthene + benzo(k)fluoranthene		252
		Unidentified	258, 269, 288, 289	

Table 2 (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max}[c]	Mass (m/e)[d]
30	1.74	Benzo(a)fluoranthene		252
	1.90	Dimethyl-1,2-benzanthracene	280, 290	256
	2.00	Unidentified	256, 263, 300, 312, 360	
31		Tetramethylpyrene	spectra too weak	258
32	1.66	Benzo(e)pyrene		252
	1.73	Benzo(a)pyrene		252
33	1.66	Perylene		252
	1.86	Methylbenzo(j)fluoranthene	241, 318, 332, 363, 383	266
	1.88	Methylbenzo(b)fluoranthene	256, 276, 292, 301	266
34	1.78	Methylbenzo(a)pyrene	253, 263, 282, 295, 363, 377, 383	266[e]
	1.85	Methylbenzo(j)fluoranthene	241, 316, 331, 365, 384	266
	1.88	Methylbenzo(j)fluoranthene	256, 289, 299	266
35	1.65	Methylbenzo(e)pyrene	236, 256, 267, 278, 289, 319, 333	266
	1.71	Methylbenzo(e)pyrene	237, 257, 266, 277, 288, 316, 331	266
	1.80	Methylbenzo(a)pyrene	255, 265, 285, 296, 365, 378, 384, 405	266
36	1.73	Methylbenzo(e)pyrene	255, 266, 278, 290, 307, 320, 335	266
		Methylperylene	253, 409, 429, 434	266
	1.77	Methylbenzo(e)pyrene	221, 236, 265, 278, 288, 307, 319, 332	266
		Methylbenzo(a)pyrene	360, 366, 380, 386	266
	1.82	Methylbenzo(a)pyrene	255, 265, 286, 296, 350, 366, 386	266
		Methylperylene	254, 406, 436	266
37	1.76	Unidentified (trace)	250, 300, 310, 357, 386, 408	280, 266, 264
	1.83	Unidentified (trace)	257, 266, 277, 289	
	1.90	Methylbenzo(a)pyrene	263, 286, 297, 370, 390	266
		Methylperylene	253, 370, 390, 412, 438	266
	2.11	Dimethylbenzo(e)pyrene	277, 289, 319, 332	280, 265
38	1.75	Unidentified	275, 285, 309, 408	264

Table 2. Identification data[a] (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max}[c]	Mass (m/e)[d]
38 (cont'd.)	1.86	Methylperylene (carryover)	246, 252, 388—92, 410, 438	266
		Methylbenzo(a)pyrene	296, 370, 388—91	266
		Methylbenzo(e)pyrene	279, 287, 320, 335	266
	1.91	Methylbenzo(a)pyrene	254, 265, 285, 297, 368, 382, 388	266
		Methylbenzo(e)pyrene	278, 286, 317, 332	266
	2.06	Dimethylbenzo(e)pyrene	278, 290, 318, 333	280, 265
39	1.90	Indenofluoranthene		276
	2.09	Dimethylbenzo(e)pyrene	268, 279, 291, 319, 334	280, 265
		Dimethylperylene	254, 410, 436	280, 265
	2.22	Dimethylbenzo(e)pyrene	267, 286, 299, 365, 383—87	280, 265
	2.26	Dimethylbenzo(e)pyrene	268, 286, 298, 365, 383—87	280, 265
40	1.91	Dibenz(a,j)anthracene		278
	2.04	Dimethylbenzo(e)pyrene	225, 237, 269, 280, 291, 322, 333—35	280, 265
	2.12	Dimethylbenzo(e)pyrene	238, 258, 267, 280, 291, 321, 333	280, 265
		Dimethylperylene	258, 423, 438	280, 265
		Dimethylbenzo(a)pyrene	267, 368, 380, 388	280, 265
	2.21	Dimethylbenzo(a)pyrene	258, 266, 286, 298, 367, 387	280, 265
	2.25	Dimethylbenzo(a)pyrene	267, 286, 299, 365, 388	280, 265
41	1.83	Dibenz(a,c)anthracene		278
	1.88	Dibenz(a,h)anthracene		278
	1.92	Unidentified	258, 284, 297, 336, 386, 409	276[e]
	1.95	Unidentified + trace of indenopyrene	242, 269	276[e]
	2.10	Dimethylbenzo(e)pyrene	238, 259, 268, 280, 291, 322, 337	280, 265
		Dimethylbenzo(e)pyrene	238, 254, 266, 280, 292, 322, 336	280, 265
		Dimethylperylene	254, 414, 440	280, 265
	2.16	Dimethylbenzo(a)pyrene	255, 266, 287, 298, 342, 359, 379	280, 265
	2.20	Dimethylbenzo(a)pyrene	255, 266, 288, 299, 352, 369, 389	280, 265
42	1.90	Picene		278
	1.94	Indenopyrene		276
	2.10	Dimethylbenzo(e)pyrene	238, 281, 292, 314, 322, 337	280, 265
	2.14	Dimethylbenzo(e)pyrene	222, 237, 283, 294, 314, 328, 342	280, 265
		Dimethylperylene	250, 392, 412, 436, 440	280, 265
	2.19	Dimethylbenzo(a)pyrene	256, 266, 287, 297, 371, 390	280, 265

Table 2 (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max}[c]	Mass (m/e)[d]
42 (cont'd.)	2.23	Dimethylbenzo(a)pyrene	256, 266, 287, 299, 353, 370, 389	280, 265
		Dimethylbenzo(a)pyrene	300, 375, 393	280, 265
	2.37	Trimethylbenzo(e)pyrene	282, 292, 334	294, 279
	2.43	Trimethylbenzo(e)pyrene	281, 293, 321, 335	294, 279
43	1.89	Benzo(ghi)perylene		276
	1.97	Methyldibenz(a,c)anthracene	276, 286	292, 277
	2.02	Methyldibenz(a,c)anthracene	275, 286	292, 277
	2.13	Unidentified	260, 286, 297, 357	292, 290
	2.17	Dimethylbenzo(a)pyrene	291, 300, 360B, 388	280, 265
	2.39	Trimethylbenzo(e)pyrene	281, 292, 338	294, 279
44	2.00	Anthanthrene		276
	2.16	Methylindenopyrene	250, 290, 303, 316, 360	290, 275
45	2.04	Methyldibenz(a,c)anthracene	275, 286	292, 277
	2.18	Unidentified	285	292[e]
	2.26	Methylindenopyrene	250, 290, 302, 315, 361, 375, 385	290, 275
	2.45	Trimethylbenzo(e)pyrene	280, 292, 340	294, 279
46	2.10	Unidentified	242, 276, 284, 326, 343, 358, 362	292, 290[e]
	2.35	Trimethylbenzo(e)pyrene	292, 328, 343	294, 279
	2.40	Methylindenopyrene	249, 301, 314, 344, 361, 377, 385	290, 275
47	2.11	Unknown (possibly methyl isomer of peak 46, HPLC RRT 2.10)	242—250, 275, 285, 301, 315, 356, 363, 374, 388	306
	2.15	Methylindenopyrene	250, 300, 314, 358	290, 275
	2.22	Methylindenopyrene	250, 275, 290, 300, 314, 344, 361, 380, 384	290, 275
		Methylbenzo(ghi)perylene	275, 288, 299, 329, 346, 362, 381, 384	290, 275
48	2.06	Unidentified (possibly methyl isomer of peak 46, HPLC RRT 2.10)	275, 285, 355+374, 388	306, 391
	2.19	Dimethylindenopyrene	250, 299, 313, 356	304
		Methylbenzo(ghi)perylene	277, 289, 300, 320, 380—85	290, 275
	2.21	Methylbenzo(ghi)perylene	276, 288, 300, 329, 350, 364, 382—85	290, 275
	2.25	Methylanthanthrene	290, 305, 406, 420, 430	290, 275
	2.39	Methylanthanthrene	260, 295, 308, 405, 424, 428, 456	290

Table 2. Identification data[a] (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max} [c]	Mass (m/e)[d]
49	2.29	Methylbenzo(ghi)perylene	288, 300, 347, 363, 380—85B	290
		Methylanthanthrene	292, 306.5, 404, 412, 421, 429, 436	290
	2.38	Methylanthanthrene	294, 306.5, 399, 405, 412, 420, 428, 436	290
	2.49	Methylanthanthrene	293, 304, 405, 420, 428, 436	290
	2.73	Dimethylindenopyrene	253, 292, 303, 315, 363, 380—90B	304
50	2.48	Unidentified	295, 305, 323, 358	286[e]
	2.64	Methylbenzo(ghi)perylene	289, 300.5, 363, 384	290[e]
		Methylanthanthrene	292, 305, 422, 429, 434	290[e]
	2.79	Dimethylindenopyrene	253, 288, 300, 315, 363	304
	2.81	Dimethylindenopyrene	251.5, 291, 302, 315, 363, 375—85B	304
	2.86	Dimethylindenopyrene	251, 291—92, 302, 315, 363, 385	304
51	2.13	Dibenzo(b,j)fluoranthene		302
	2.51	Methylbenzo(ghi)perylene	288, 300, 363, 380—86B	290
	2.65	Dimethylindenopyrene	251, 303, 315, 363, 387	304
	2.74	Dimethylbenzo(ghi)perylene	291, 302, 364, 382, 387	304
52	2.13	Unidentified (probably dibenzo(a,e)fluoranthene)	240, 254, 264, 285, 300, 316, 332 (20)	302
	2.16	Unidentified (possibly dibenzo(j,l)fluoranthene)	248, 284, 303, 319, 330, 343 (21)	302
		Dibenzo(a,l)pyrene		302
	2.22	Unidentified (probably a dibenzofluoranthene)	249, 269, 280, 305, 362, 382	302
	2.47	Dimethylindenopyrene	251, 316, 358	304
		Dimethylbenzo(ghi)perylene	278, 290, 301, 360, 386	304
	2.53	Dimethylbenzo(ghi)perylene	278, 290, 302, 330, 350, 365, 384, 387	304
	2.70	Dimethylanthanthrene	233, 261, 297, 309, 406, 439	304
	2.75	Dimethylanthanthrene	263, 297, 309, 438	304
	2.84	Dimethylanthanthrene	234, 262, 297, 310, 439	304
53	2.29	Unidentified (probably a dibenzofluoranthene)	224, 227, 248, 291, 362, 377, 382	302[e]
	2.68	Dimethylbenzo(ghi)perylene	275B, 291, 302, 331, 367, 390	304
54	2.15	Unidentified (probably a dibenzofluoranthene)	243, 260, 270, 303, 315, 399	302[e]

Table 2 (cont'd.).

GC peak No.	HPLC RRT[b]	Compound	λ_{max}[c]	Mass (m/e)[d]
54 (cont'd.)	2.70	Dimethylbenzo(ghi)perylene	275B, 291, 302, 331, 369, 390	304
		Dimethylanthanthrene	309, 433	304
55	2.14	Dibenzo(a,e)pyrene		302[e]
	2.18	Unidentified (probably a dibenzofluoranthene)	282, 294, 308, 344, 361, 381	302[e]
	2.55	Dimethylbenzo(ghi)perylene	291, 302, 367, 389	304
		Dimethylbenzo(ghi)perylene	291, 302, 366, 384, 389	304
	2.67	Dimethylbenzo(ghi)perylene	292, 302, 365B, 389B,	304
	3.04	Trimethylindenopyrene	253, 317	318[e]
56	2.08	Benzo(b)perylene		302
	2.35	Dibenzo(a,i)pyrene		302
	2.59	Coronene		300
		Dimethylbenzo(ghi)perylene	292, 302, 365, 383, 387	304
		Dimethylanthanthrene	308, 436	304
	3.18	Dimethylbenzo(ghi)perylene	291, 303, 368, 385	304
		Dimethylanthanthrene	310, 435	304
	3.34	Trimethylindenopyrene	253	318
		Trimethylbenzo(ghi)perylene	293, 303, 366B, 386B	318
	3.50	Trimethylindenopyrene	253	318
		Trimethylbenzo(ghi)perylene	279, 292, 304, 367B, 389B	318
57	2.35	Dibenzo(a,h)pyrene		302
	2.45	Unidentified (possibly dibenzo(e,l)pyrene)	223, 274, 286, 329 (19)	302
	3.00	Unidentified (possibly methyldibenzo(a,e)pyrene)	274, 286, 302	316[e]
		Unidentified (probably a methyl isomer of peak 55, HPLC RRT 2.28)	272, 284, 295, 309, 363, 383	316[e]
		Trimethylbenzo(ghi)perylene	289, 302	318
58		Methylcoronene	291, 303, 325, 335, 340	314
		Unidentified (probably a methyldibenzopyrene)		316
59		Dimethylcoronene	291, 303, 340	328
		Unidentified		330, 326

a: Except to indicate HPLC RRT for selected PAH, this table presents identification data for compounds whose GC retention time and/or literature UV data are lacking.
b: Relative to anthracene; a factor of 48 converts HPLC RRT to time in minutes from point of injection (see text for limitations of HPLC RRT).
c: 85 % CH_3OH/H_2O; B — broad.
d: Unless otherwise noted, mass obtained by GC-MS techniques.
e: Mass obtained by submitting trapped HPLC peak to probe MS analysis.

Figure 6. HPLC chromatogram of GC peak number 9. (Anthracene added as internal standard.)

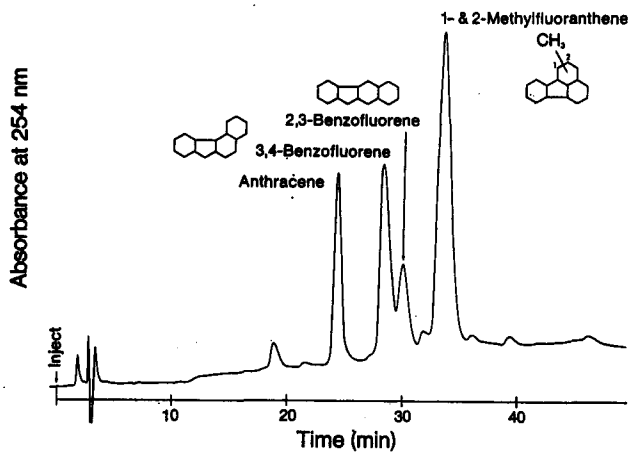
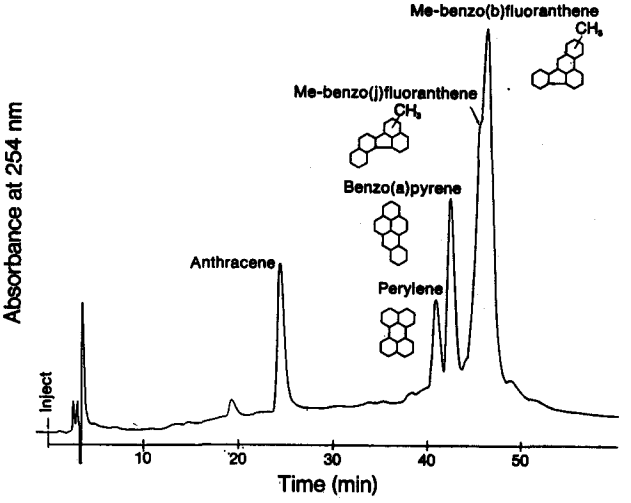


Figure 7. HPLC chromatogram of GC peak number 33. (Anthracene added as internal standard.)



Several examples of the remarkable separations achieved by HPLC are shown in Figures 6–10. The HPLC separations of GC peak number 9 of GF fraction 49 is shown in Figure 6. Resolved compounds were 3,4-benzofluorene, 2,3-benzofluorene, and two methyl fluoranthenes. UV data confirmed that the broadness and asymmetry of the methyl fluoranthene peak was due to the presence of the 1- and 2-methyl isomers. The HPLC separation of GC peak number 33 of GF fraction 45 is given in Figure 7. Perylene, benzo(a)pyrene, and two methylbenzofluoranthenes were separated. The benzo(a)-pyrene was a carryover from peak number 32. Such carryover was found in many cases with the high molecular weight, low volatility PAH. The complexity of some GC peaks is illustrated in Figure 8. HPLC of peak number 41 of GF fraction 45 separated dibenz(a,c)-anthracene, dibenz(a,h)anthracene, a trace of indeno-pyrene, a dimethylperylene, two dimethylbenzo(e)-pyrenes, and two dimethylbenzo(a)pyrenes. HPLC RRT and MS analyses of the two large unknown peaks indicated that they were unsubstituted PAH with m/e of 276. Lack of literature UV data on several possible candidates

Figure 8. HPLC chromatogram of GC peak number 41. (Anthracene added as internal standard.)

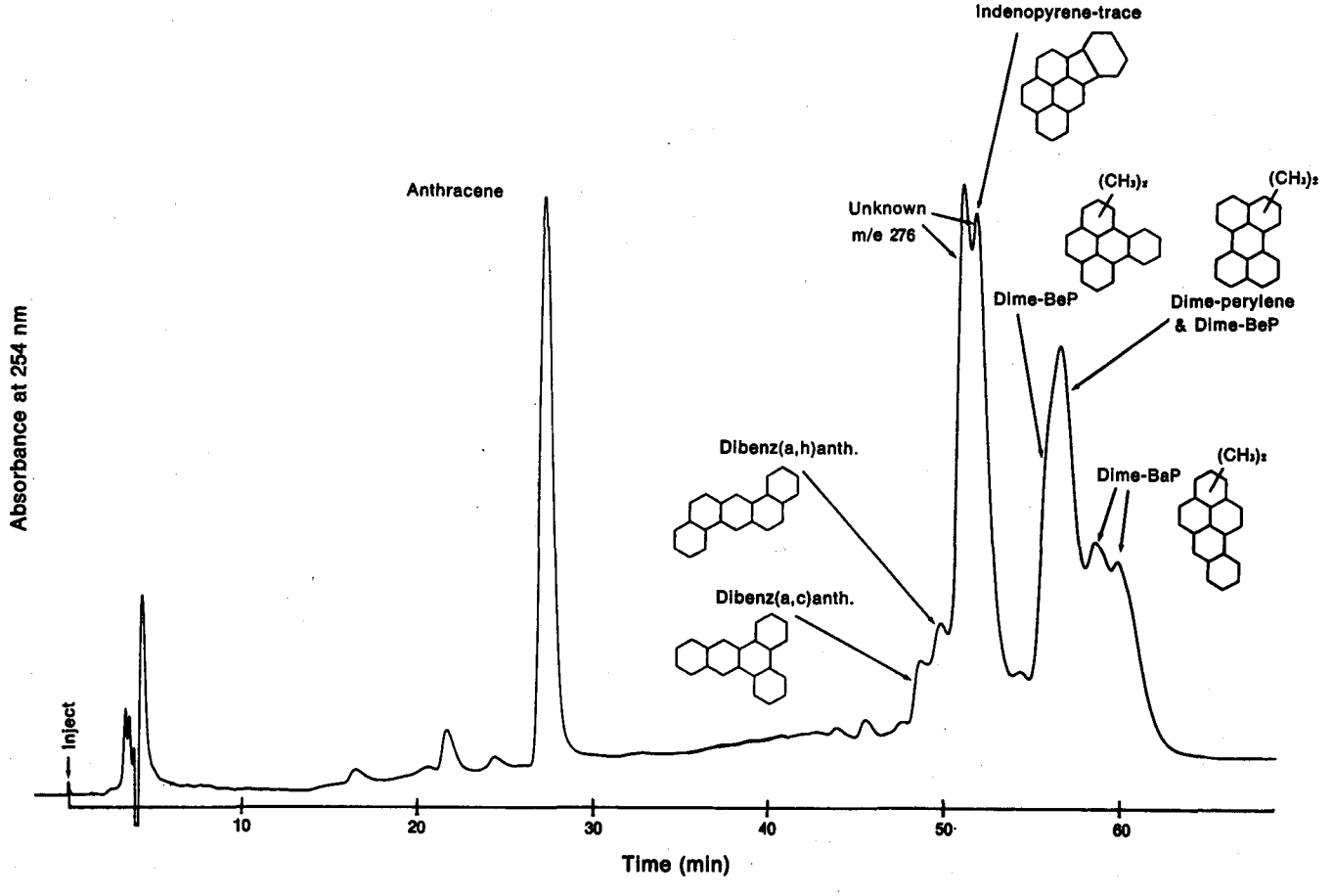
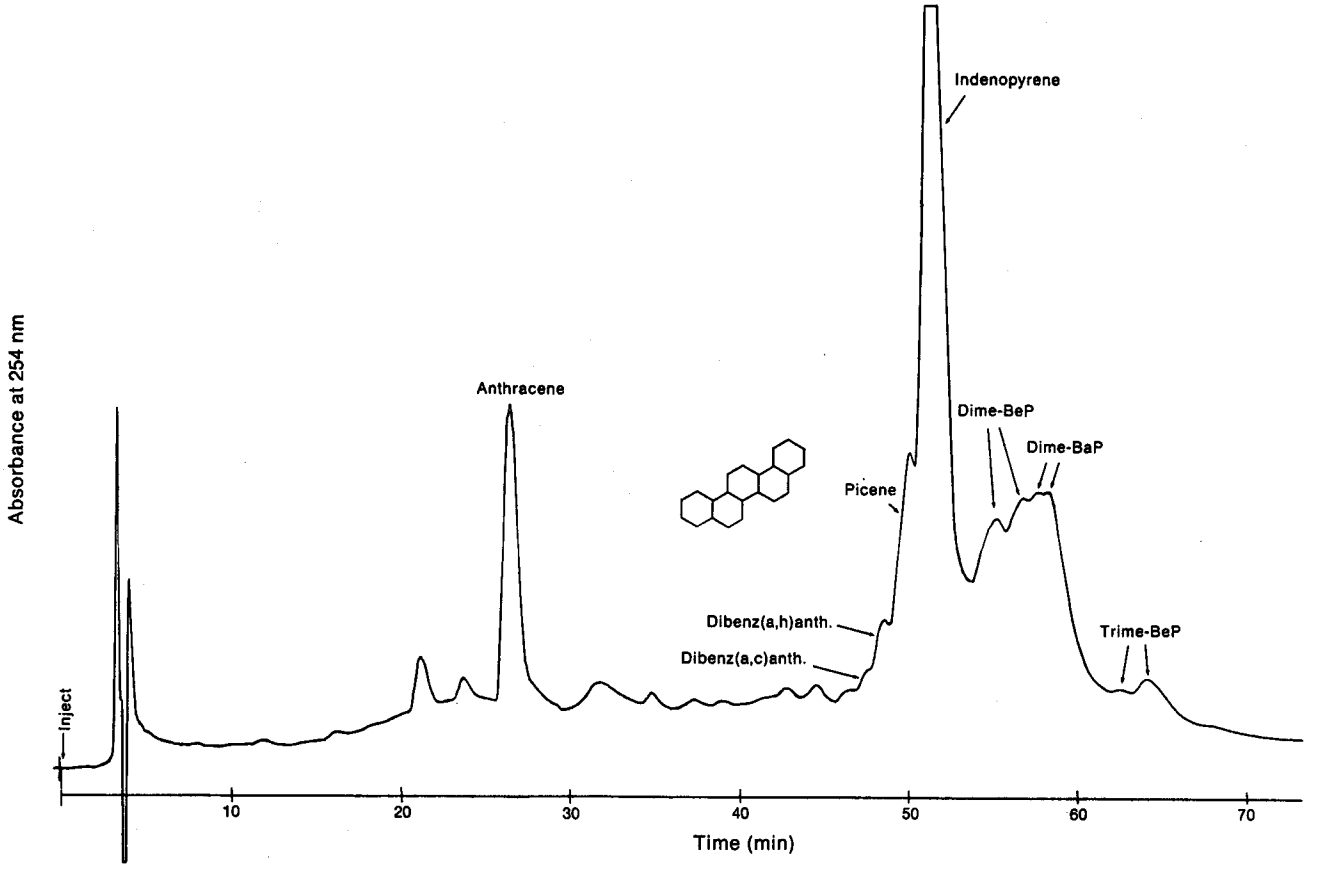


Figure 9. HPLC chromatogram of latter half of GC peak number 42. (Anthracene added as internal standard.)



for these peaks prevented conclusive identification. Possible assignments are aceperylene and dibenzo(b,ghi)-fluoranthene or dibenzo(b,mno)fluoranthene. The HPLC resolution of the backslope of GC peak number 42 of GF fraction 47 is shown in Figure 9. Identified compounds were: traces of dibenz(a,c)anthracene and dibenz-(a,h)anthracene, picene, indenopyrene, two dimethylbenzo(a)pyrenes, two dimethylbenzo(e)pyrenes, and a trimethylbenzo(e)pyrene. The decreasing quantities of dimethyl and trimethyl derivatives in GF fraction 47 are quite apparent. The final example of the HPLC separations (Figure 10) shows components from GC peak

Figure 10. HPLC chromatogram of GC peak number 44; a — unidentified. (Anthracene added as internal standard.)

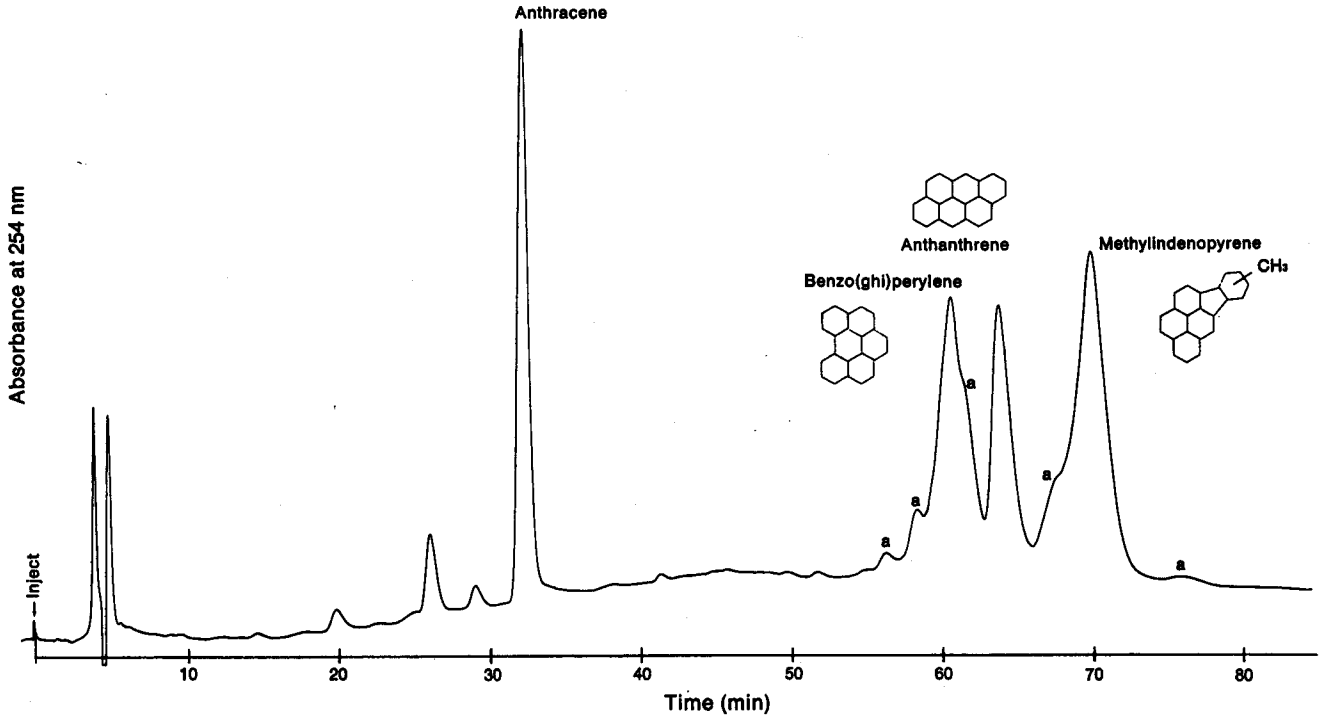
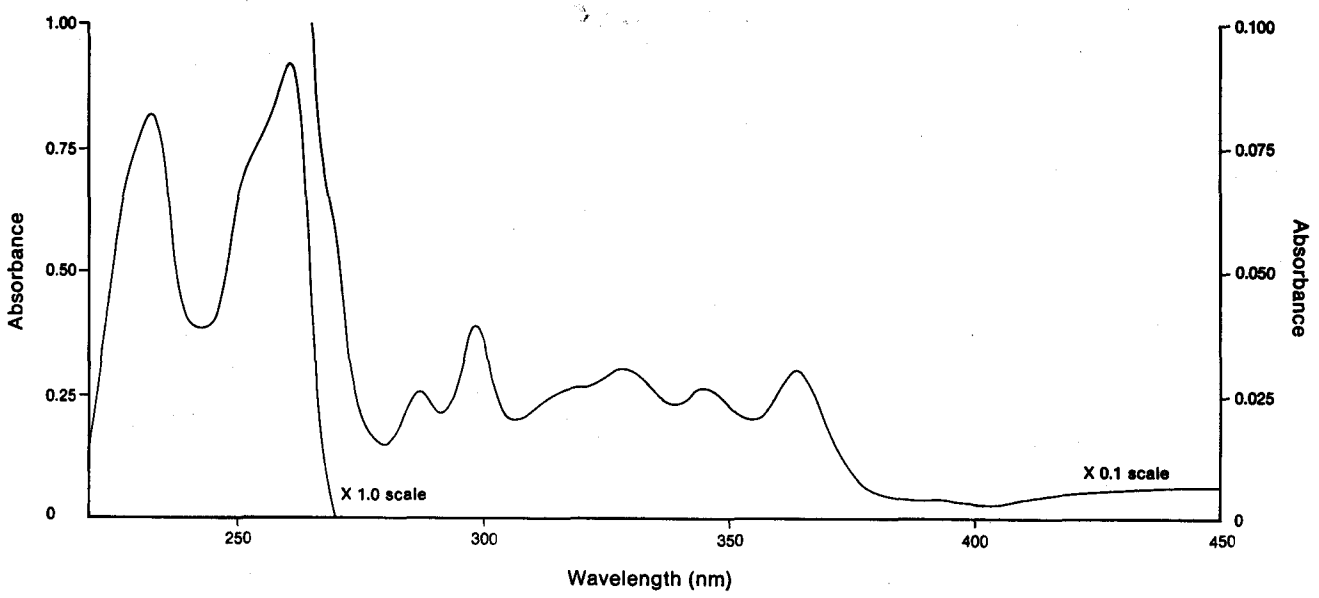


Figure 11. UV spectrum of collected GC peak number 5.



number 44 of GF fraction 47. Benzo(ghi)perylene (carry-over from peak number 46), anthanthrene, and methyl-indenopyrene were identified. Additional data on the above separations can be found in Table 2, under the corresponding GC peak number.

In this study, several new components of CSC were identified. These include 3,4-dimethylenepyrene, 3,4-trimethylenepyrene, cyclopenta(c,d)pyrene, 4,5-methylenetriphenylene, benzo(b)perylene, and several dibenzofluoranthenes. The lack of available standards and literature UV spectra for many PAH has hindered further identifications. This was particularly true with the dibenzofluoranthene series. For the ten possible dibenzofluoranthenes, only four literature UV spectra could be found.

Some of the difficulties in assigning conclusive identifications are illustrated by the following case. The UV spectrum of the major component in GC peak number 5 is shown in Figure 11. This compound had a molecular weight of 202 and it was tentatively identified as acephenanthrylene. To our knowledge, the UV spectrum of acephenanthrylene has not been reported; however, acephenanthrylene has been reported to elute from a SE-30 GC column between fluoranthene and pyrene (14). This seems reasonable as acephenanthrylene is an isomer of fluoranthene.

Table 3. Levels of selected large ring PAH in cigarette smoke condensate from 1R1 cigarettes.

PAH	Amount[a] (μg/100 cigarettes)
Benzo(a)pyrene/benzo(e)pyrene	2.4
Perylene	0.3
Indenopyrene	0.6
Benzo(ghi)perylene	0.5
Anthanthrene	0.3
Methylbenzo(ghi)perylenes	0.5
Coronene	0.1

a: Values obtained using flame ionization detector and employing internal standard methods. Methyl derivatives were assumed to yield detector response identical to parent compounds.

The levels of several high molecular weight PAH in CSC were determined. For this purpose, 810 research cigarettes were smoked, and the PAH were isolated and quantitated by our accelerated technique (11, 12). The results (Table 3) show that the level of each of the larger PAH was smaller than that of the benzopyrenes. The data in Table 3 compare favorably with previous results (15).

On the basis of the data in Table 1, we evaluated the relative concentrations of the large PAH in cigarette smoke. Compounds that occurred in major amounts are: benzofluoranthenes, benzo(a)pyrene, benzo(e)pyrene, perylene, indenopyrene, benzo(ghi)perylene, anthanthrene, coronene, and their methyl and dimethyl derivatives. PAH that occur in minor to trace quantities are dibenzanthracenes, dibenzophenanthrenes, dibenzofluoranthenes, and the dibenzopyrenes. Several dibenzanthracenes, dibenzophenanthrenes, and dibenzopyrenes have been identified in CSC (15, 16). Surprisingly, however, the more abundant methyl and dimethyl derivatives of indenopyrene and benzo(ghi)perylene have not been isolated and characterized previously.

This report concludes our identification studies on the high MW PAH of cigarette smoke and complements the results on the middle region (9). Current work on the low MW, highly-alkylated PAH will be described in this journal in the near future.

SUMMARY

A gel filtration chromatography method was developed for the isolation and concentration of the high molecular weight polynuclear aromatic hydrocarbons (PAH) contained in the most biologically active fraction of cigarette smoke condensate (CSC). The unusually complex

mixture of large PAH found in CSC necessitated the use of preparative gas chromatography followed by high-pressure liquid chromatography to achieve separation and identification. Mass spectral, ultraviolet absorption, and chromatographic retention data were needed for the comprehensive identification of the large molecular weight PAH components of CSC. The majority of the more than 200 isolated compounds were identified. Compounds newly identified in CSC included 3,4-dimethylenepyrene, 3,4-trimethylenepyrene, cyclopenta(c,d)pyrene, 4,5-methylenetriphenylene, benzo(b)perylene, and several dibenzofluoranthenes.

ZUSAMMENFASSUNG

Es wurde ein gel-chromatographisches Verfahren entwickelt zur Isolierung und Konzentrierung der polycyclischen aromatischen Kohlenwasserstoffe (PAH) hohen Molekulargewichts, die in der biologisch am stärksten aktiven Fraktion des Cigarettenrauchkondensates (CSC) enthalten sind. Die ungewöhnlich komplexe Mischung dieser im Rauchkondensat gefundenen Kohlenwasserstoffe erforderte zur Erzielung von Trennung und Identifizierung präparative Gaschromatographie mit nachfolgender Hochdruck-Flüssig-Chromatographie (HPLC). Zur umfassenden Identifizierung der im Kondensat befindlichen polycyclischen aromatischen Kohlenwasserstoffe mit hohem Molekulargewicht waren massenspektrometrische Daten, UV-Absorptionswerte und chromatographische Retentionszeiten notwendig. Von den mehr als 200 isolierten Verbindungen wurde der größte Teil identifiziert. Im Cigarettenrauchkondensat erstmalig identifizierte Verbindungen waren neben anderen 3,4-Dimethylenpyren, 3,4-Trimethylenpyren, Cyclopenta(c,d)pyren, 4,5-Methylentriphenylen, Benz(b)perylen und mehrere Dibenzfluoranthene.

RÉSUMÉ

On a développé une méthode de chromatographie à perméation de gel pour l'isolation et la concentration des hydrocarbures polynucléaires aromatiques (PAH) de haut poids moléculaire, présents dans la fraction biologiquement la plus active du condensat de fumée de cigarette (CSC). Le mélange particulièrement complexe de ces PAH dans le CSC a exigé l'utilisation des techniques de chromatographie préparative en phase gazeuse suivie de chromatographie liquide à haute pression, afin de pouvoir isoler et identifier les composés. Les données de spectrographie de masse, d'absorption U.V. et de rétention chromatographique ont été requises pour l'identification complète des PAH de haut poids moléculaire du CSC. On a identifié la majorité des 200 composés isolés. Parmi les nouveaux composés identifiés dans le CSC, l'on peut citer le 3,4-diméthylène-pyrène, le 3,4-triméthylène-pyrène, le cyclopenta(c,d)pyrène, le 4,5-méthylènetriphénylène, le benzo(b)pérylène et plusieurs dibenzofluoranthènes.

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