Journal of Chromatography, 398 (1987) 227–237
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19553

SEPARATION AND IDENTIFICATION OF ORGANIC COMPOUNDS IN AIR PARTICULATE EXTRACTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

K. P. NAIKWADI*, G. M. CHARBONNEAU and F. W. KARASEK
Department of Chemistry, University of Waterloo, Waterloo, Ontario N2L 3G1 (Canada)
and
R. E. CLEMENT
Ontario Ministry of the Environment, 125 Laboratory Services Branch Rexdale, Rexdale, Ontario M9W 5L4 (Canada)
(Received March 10th, 1987)

SUMMARY

High-volume sampling was used to collect particulate emissions from a residental wood burning furnace. Organic compounds were extracted by Soxlet extraction and separated into acid, base and neutral fractions by acid–base extraction. The neutral fraction consisted of a large number of aliphatic and aromatic compounds. Semi-preparative high-performance liquid chromatography (HPLC) was required to further separate the extremely complex neutral fraction in to several subfractions. Retention behavior of the aromatic compounds on a normal phase column in HPLC was typical, in that capacity factors increased with the number of rings, and was effected little by the alkyl substitution. Compound identification was done by gas chromatography–mass spectrometry (GC–MS) technique. The acid–base extraction procedure followed by HPLC fractionation prior to GC–MS analysis greatly facilitated the analysis.

INTRODUCTION

Numerous organic compounds have been identified as products of wood combustion, and have been detected on particulate emissions from wood stoves. Prominent among those are many polynuclear aromatics, some of which are considered by the U.S. Environmental Protection Agency to be priority pollutants, because they are mutagenic and carcinogenic[1]. Particulate emissions from wood stoves are often sampled by high-volume filtration using glass fiber filters[2,3]. The techniques for the extraction of the organic compounds from the collected particulate matter have been described previously[4,5].

A complete identification of the polynuclear aromatics (PAHs) extracted from the particulate emitted from combustion sources is generally complicated by a large

0021-9673/87/$03.50

background of aliphatic compounds. Various methods for the separaton and identification of aliphatic and PAH compounds in such complex samples have been reviewed[6–11]. Previously, methods using dimethyl sulfoxide and pentane[12] or acid–base extraction to separate compounds in to acidic, basic and neutral fractions have been reported[13–15]. Recently, polymeric liquid crystal capillary columns have been developed and used for isomer specific separation and identification of PAH isomers[16–19].

A great deal of interest has been shown in the use of high-performance liquid chromatography (HPLC) for the separation of compounds based on their polarities. Semi-preparative HPLC has been used for the separation and isolation of various compounds into definite compound classes. An optimized HPLC program developed using standards can be used for the analysis of real samples because of the high reproducibility of HPLC method. Unfortunately, because of the high capacity and low efficiency of semi-preparative HPLC the identification of specific compounds of interest in complicated mixtures is difficult using conventional HPLC detectors. However, the HPLC fractions after concentration can be analysed very easily by capillary gas chromatography–mass spectrometry GC–MS. Thus combination of more than one technique for the analysis of the complex samples results in the separation and the ability to identify a large number of compounds. We will report in this paper the use of acid–base extraction, semi-preparative HPLC and GC–MS for the complete analysis of the organic compounds in air particulate extract. Isomer specific identification using retention indices method will be demonstrated.

EXPERIMENTAL

Chemicals and methods

The solvents used in this method were distilled in glass, UV grade from Caledon Lab., (Georgetown, Canada). The standard PAHs were purchased from Ultra Scientific, (Hope, RI, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.). All the standards were prepared by dissolving 4–10 mg of each PAH in an appropriate amount of benzene or mixture of benzene and dichloromethane and stored below 0°C when not in use.

The general scheme for the separation of organic compounds in air particulate extracts is shown in Fig. 1. The air particulate extracts dissolved in toluene contained suspended particulate matter which was removed using a centrifuge. The compounds were then separated into three major groups, acidic, basic and neutral using acid–base extraction. The neutral organic compounds were further separated according to their polarities using HPLC.

Semi-preparative HPLC

HPLC separation and fraction isolation was achieved using a Varian 5000 liquid chromatograph with a Vista 401 data system which has the capability to recalculate and replot the stored data of an analysis. A semi-preparative μBondapack (10 μm) amine column (250 mm × 9.4 mm I.D.; Waters Scientific, Mississauga, Canada) was used. The samples were injected by an automated Rheodyne injector with 100 μl sample loop. A gradient solvent program was developed for the separation of a standard mixture containing two–five ring PAHs. The program consisted of 98% hexane and 2% dichloromethane for 12 min, programmed to 100% dichloro-

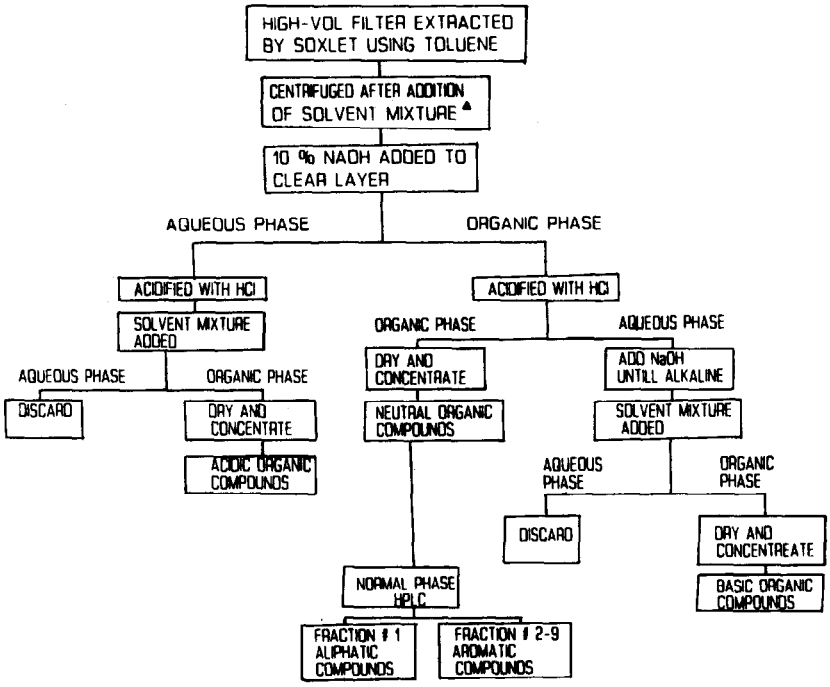

Fig. 1. General scheme for the separation of organic compounds in air particulate extract. Solvent mixture is 20% dichloromethane in benzene.

methane in 45 min, held for 10 min and then linearly programmed to 100% acetonitrile, held for 10 min and then programmed back to 100% dichloromethane and finally to hexane using a flow-rate of 1 ml/min. The fractions were collected between 6–14, 20, 24, 28, 33, 37, 43, 55 and 80 min. All fractions were concentrated to 200 μl each for GC and GC–MS analysis.

GC analysis

The HPLC fractions were analyzed on a Hewlett-Packard 5880A GC equipped with flame ionization and electron-capture detectors using a 30 m × 0.32 mm I.D. DB-5 fused-silica capillary column (J & W Scientific, Rancho Cordova, CA, U.S.A.). The same column was used for the separation and characterization of individual compounds by GC–MS.

GC–MS analysis

GC–MS analysis was performed on a Hewlett Packard 5987A instrument with an HP 1000 data system which stores the raw data and permits drawing the mass spectra of any peak on the total ion current (TIC) trace. Computer identifications were carried out by the Probability Based Matching (PBM) system based on a library containing 78 000 reference spectra. The ion source temperature was 200°C, and GC conditions were similar to those described previously. A user-developed, BASIC programme stored in the GC–MS data system allowed the calculation of the retention indices of PAHs in the sample injected.

RESULTS AND DISCUSSION

It is very difficult to perform complete analysis of air particulate extracts for organic compounds by GC alone because of sample complexity and peak overlap. Complex samples must be simplified into less complex sub-samples by separating compounds into different classes based on their physical and chemical properties such as acidity, polarity, volatility and structural characteristics prior to GC–MS analysis. The general scheme for the separation of compounds into different classes is shown in Fig. 1. The acid–base extraction is a very important step for removing water soluble inorganic impurities, and separating highly polar acidic and basic organic compounds from the neutral fraction. From our experiments, we observed that the composition of the neutrals obtained after acid base extraction did not change for a period of several months. The probable reason for this stability might have been due to the removal of the catalytic inorganic impurities by the acid–base extraction. Such impurities can catalytically effect the changes in composition of untreated extracts[20]. Similarly, we have observed that the GC column deteriorates almost immediately and peaks lose their symmetry if a sample without acid–base extraction was injected.

The neutral fraction after acid–base extraction consists mainly of aliphatics and aromatics. The aliphatic compounds were highly concentrated and co-eluted with environmentally important PAHs in the GC–MS analysis, and prevented comprehensive compound identification. However, the aliphatic compounds were effectively separated from the aromatics using normal-phase HPLC. Reproducible separation of aliphatic compounds from aromatics was obtained on both silica and amine HPLC columns. The gradient solvent program was optimized for HPLC using a mixture of standard PAHs. It was observed that the amine column is more selective than the silica column for PAHs separation according to the number of rings. It was also observed that the best separation of aromatics having two and three rings can be obtained using hexane for a longer period of time. Prolonged elution using hexane, however, results in peak broadening for the larger PAHs and they elute in more than one HPLC fraction. To overcome this difficulty, the initial gradient program was modified from 100% hexane to a composition of hexane–dichloromethane (98:2). Using this solvent mixture it was observed that the PAHs eluted faster, whereas the retention order remained the same. This program was used to separate neutral organics in various fractions.

In Fig. 2, the UV trace of HPLC analysis of organic neutrals is shown. The HPLC fraction 1, exclusively consisted of aliphatic compounds. This HPLC fraction was analysed by GC–MS and the compounds were identified by their mass spectra, PBM search and an injection of standard mixture of alkanes (C_{12}–C_{40}) under identical conditions. The TIC trace and the compounds identified are shown in Fig. 3. The mass spectra and PBM search of peaks eluted after 45 min indicate the presence of alkenes. The identification of individual peaks was not possible, because of non-availability of standards. Aliphatic compounds have retention times from 20 to 50 min in GC analysis and co-elute with the PAHs of environmental importance. Thus HPLC separation prior to GC–MS analysis of PAHs proved to be advantageous.

The HPLC fraction 2 consisted of the two-ring aromatic compounds such as naphthalene, biphenylene and their alkyl substituents. In HPLC the amine column

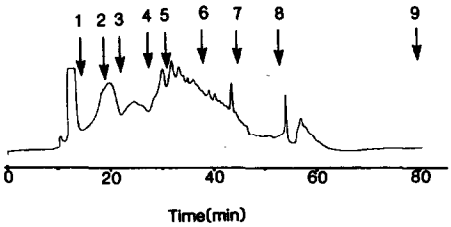

Fig. 2. UV trace of HPLC separation of neutral fraction of organics. HPLC conditions are described in the text.

is more selective[9] than the silica column, but it is extremely difficult to separate definite aromatic ring containing PAHs in a specific fraction. There is always some overlapping of compounds in conjugative fractions. The physical properties such as vapour pressure and polarity of PAHs are not consistent with the number of aromatic rings, hence in GC analysis several PAHs have similar retention times (Table I, HPLC fractions 2–4). Due to their different polarities and hence interaction with the amine column, their separation is achieved by HPLC.

HPLC fraction 5 consisted of a large number of 4-ring PAHs (Fig. 7). In the HPLC fractions 2–6 numerous peaks can be seen in corresponding TIC traces (Figs. 4–8). For most of the peaks in the TIC traces, mass spectra, molecular weight and best match by PBM search were obtained. However, based on these methods alone the identification of isomeric PAHs is not possible. The isomer-specific identification of PAHs can be carried out by injecting the mixture of standard isomeric PAHs under identical conditions and comparing the retention times and retention indices of standards and the compounds in the sample.

The air particulate extracts contained a large number of PAHs. It is beyond the reach of a laboratory to keep all the PAHs standards, moreover several PAHs

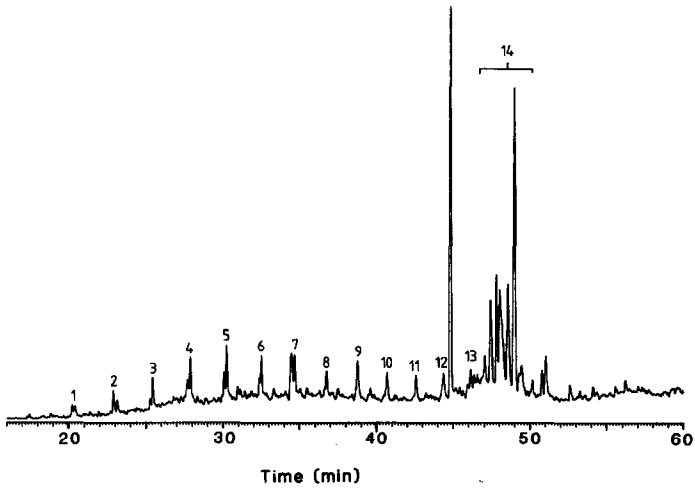

Fig. 3. Total ion current trace of HPLC fraction 1 of air particulate extract. Chromatographic conditions: 30 m × 0.32 mm I.D., DB-5 fused-silica capillary column; temperature, 80°C for 1 min, programmed to 300°C at 4°C/min, 20 min at 300°C. Peaks: 1–13 correspond to C_{18}–C_{30} aliphatic hydrocarbons.

TABLE I

ORGANIC COMPOUNDS IDENTIFIED IN HPLC FRACTIONS 2–6 OF AIR PARTICULATE EXTRACT

Peak No.	*Compound*	*Mol. wt.*	*Retention time (min)*	*Retention index*	*Reported retention index*	*Method of identification**
Fraction 2 (Fig. 4)						
1	Naphthalene, 2-methyl	146	8.53	218.21	218.14	a, b, c, d
2	1,1′-Biphenyl	154	10.89	232.86	233.96	a, b, d
3	Naphthalene, 2,6-dimethyl	156	11.53	236.80	237.58	a, b, c, d
4	Naphthalene, 2,3-dimethyl	156	12.48	242.64	243.55	a, b, c, d
5	Dibenzofuran	168	14.63	256.05	257.17	a, b, d
6	Naphthalene, 2,3,4-trimethyl	170	16.01	264.56	263.31	a, b, d
7	9H-Fluorene	166	16.49	267.53	268.17	a, b, c, d
8	9H-Fluorene, 9-methyl	180	17.17	271.72	272.38	a, b, d
9	Biphenyl, 4,4′-dimethyl	182	17.61	274.44	274.59	a, b, d
10	Dibenzofuran, methyl	182	17.97	276.66	–	a, b
11	9H-Fluorene, 2-methyl	180	19.69	287.28	288.21	a, b, d
12	9H-Fluorene, 1-methyl	180	20.22	290.55	289.03	a, b, d
13	9H-Fluorene, 2,3-dimethyl	194	22.41	300.04	–	a, b
14	9H-Fluorene, dimethyl	194	23.00	308.01	–	a, b
15	Naphthalene, 1-phenyl	204	23.97	314.23	315.19	a, b, d
16	9H-Fluorene, dimethyl	194	24.22	315.83	–	a, b
17	Naphthalene, 1-(phenylmethyl)	218	26.88	332.88	–	a, b
18	1,1′-Biphenyl, 2,3,3′,4,4′-pentachloro	324	32.02	365.83	366.12	a, b
Fraction 3 (Fig. 5)						
1	Phenanthrene	178	21.80	300.10	300.00	a, b, c, d
2	Anthracene	178	22.00	301.92	301.92	a, b, c, d
3	Naphthalene, 1-phenyl	204	23.99	314.35	315.19	a, b, d
4	Phenanthrene/anthracene-methyl	192	24.16	315.44	–	a, b
5	Phenanthrene/anthracene-methyl	192	24.79	319.48	–	a, b
6	4H-Cyclopenta[*def*]phenanthrene	190	25.31	322.82	322.08	a, b, d
7	Phenanthrene, 9-methyl	192	25.46	323.78	323.06	a, b, c, d
8	Naphthylene, 2-phenyl	204	26.77	332.17	332.59	a, b, d
9	Phenanthrene, 9-ethyl	206	27.52	336.98	337.05	a, b, d
10	Indenoindene	204	28.24	341.60	–	a, b
11	Naphthalene, 2-phenylmethyl	218	29.44	349.29	–	a, b
12	Phenanthrene, trimethyl	220	31.02	359.42	–	a, b
13	Azulene, 2,6-dimethyl-4-phenyl	232	33.03	372.30	–	a, b
Fraction 4 (Fig. 6)						
1	Phenanthrene	178	21.85	300.64	300.00	a, b, c, d
2	Anthracene	178	22.02	301.73	301.69	a, b, c, d
3	Phenanthrene, 3-methyl	192	24.78	319.55	319.46	a, b, d
4	Phenanthrene, 2-methyl	192	24.91	320.25	320.17	a, b, d
5	Phenanthrene, 1-methyl	192	25.48	323.91	323.90	a, b, d
6	Naphthalene, 2-phenyl	204	26.74	331.98	332.59	a, b, d
7	Phenanthrene, 3,6-dimethyl	206	27.78	338.65	337.83	a, b, d
8	Naphthalene, 2-phenylmethyl	218	29.63	350.51	–	a, b
9	Pyrene	202	29.86	351.98	351.22	a, b, c, d
10	Benzophenanthrene	228	31.69	363.71	–	a, b
11	Pyrene, 2-methyl	216	32.70	370.19	370.15	a, b, d

TABLE I (*continued*)

Peak No.	*Compound*	*Mol. wt.*	*Retention time (min)*	*Retention index*	*Reported retention index*	*Method of identification**
Fraction 5 (Fig. 7)						
1	Fluoranthene	202	28.82	345.32	244.01	a, b, c, d
2	Aceaphenanthrene	202	29.34	348.65	–	a, b, d
3	Pyrene	202	30.00	352.88	351.22	a, b, c, d
4	11H-Benzo[*a*]fluorene	216	32.19	366.92	366.74	a, b, d
5	11H-Benzo[*b*]fluorene	216	32.61	369.61	369.39	a, b, c, d
6	Pyrene, 1-methyl	216	33.13	372.97	373.55	a, b, c, d
7	Pyrene, 1-ethyl	230	35.26	386.60	385.35	a, b, d
8	Benzo[*ghi*]fluoranthene	226	35.98	391.21	389.60	a, b, d
9	Benz[*a*]anthracene	228	37.21	399.10	398.50	a, b, c, d
10	Chrysene/triphenylene	228	37.21	400.19	400.00	a, b, c, d
11	Benz[*a*]anthracene,11-methyl	242	38.91	412.05	412.72	a, b, d
12	Triphenylene, 1-methyl	242	39.75	415.45	416.32	a, b, d
13	Chrysene, 5-methyl	242	39.94	420.01	419.68	a, b, d
Fraction 6 (Fig. 8)						
1	Furan, 2-butyltetrahydro	128	17.30	272.53	–	a, b
2	9H-Fluoren-9-one	180	20.80	294.13	294.79	a, b, c
3	1-Phenanthrenol	194	23.04	308.26	–	a, b
4	Pheniondione	222	29.03	346.66	347.47	a, b, c
5	Benzo[*gh*]fluoranthene	226	36.93	391.41	389.60	a, b, c, d
6	Chrysene/triphenylene	228	37.31	399.74	400.00	a, b, c, d
7	Benzofluoranthene	252	43.33	446.21	–	a, b
8	Benzo[*e*]pyrene	252	43.79	449.76	450.73	a, b, c, d
9	Perylene	252	44.63	456.25	456.22	a, b, c, d
10	Benz[*e*]acephenanthrylene	252	45.16	460.35	–	a, b
11	Benz[*j*]aceanthrylene, 3-methyl	252	46.16	468.08	–	a, b
12	Dibenzo[*a,h*]anthracene	276	49.71	495.51	495.45	a, b, c
13	Picene/benzo[*ghi*]perylene	276	50.25	499.69	500.00	a, b, c, d
14	Dibenzo[*def,mno*]chrysene	276	50.74	–	–	a, b
15	Dibenzo[*a,k*]pyrene	302	55.84	–	–	a, b
16	Coronene	300	56.93	–	–	a, b

* (a) Identified by molecular weight from MS; (b) identified by PBM library search; (c) identified by comparison of GC retention time and retention index data with authentic reference standard; (d) identified by retention index reported for authentic standard (ref. 21).

are not commercially available. However, it is possible to apply a common method of identification and use the data available for several standards in different laboratories. In particular, the retention index method developed by Lee *et al.*[21] was applied and retention indices (RI) were obtained for a mixture of 20 PAHs using a RI programme stored in a HP 1000 data system, which was accessible to the HP 5987A GC–MS system. The RI obtained for a standard PAHs mixture in our experiments were identical to that reported[21]. This shows the applicability of RI data in different laboratories and hence access to positive identification of several isomeric PAHs in environmental samples by comparing RI values of standard PAHs reported in the literature. It is very important to note that the environmental samples such as air particulate extracts have hundreds of peaks and a particular peak showing a RI value

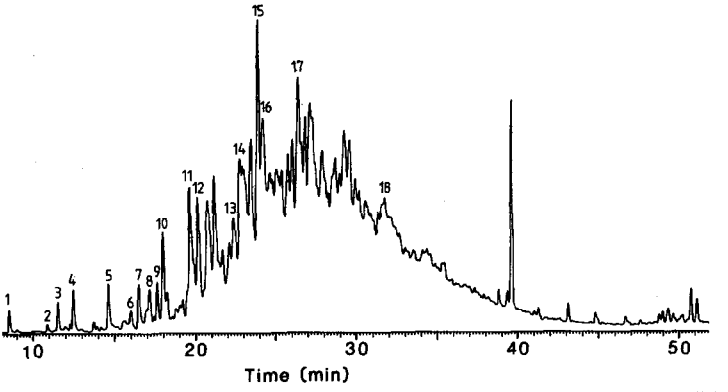

Fig. 4. Total ion current trace of HPLC fraction 2. Chromatographic conditions as in Fig. 3. Peaks as numbered in Table I.

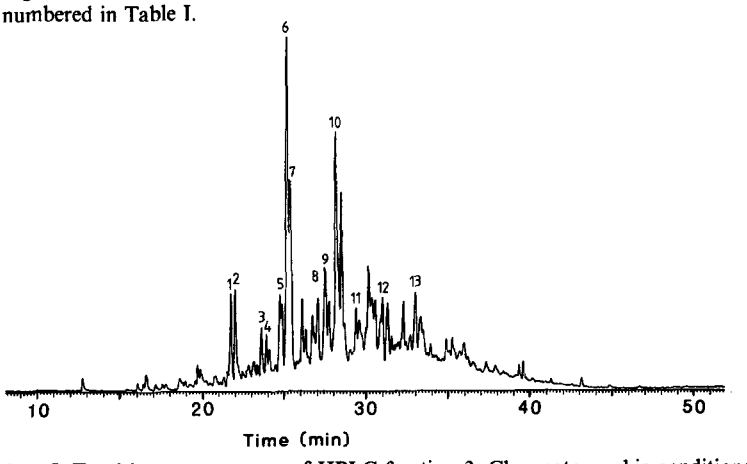

Fig. 5. Total ion current trace of HPLC fraction 3. Chromatographic conditions as in Fig. 3. Peaks as numbered in Table I.

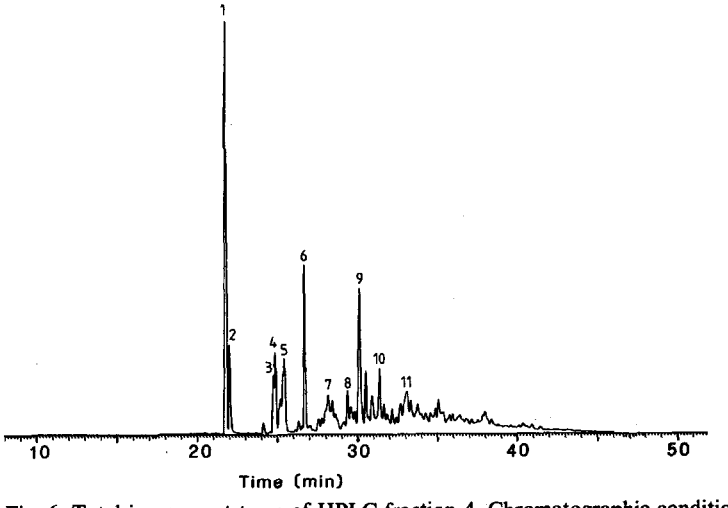

Fig. 6. Total ion current trace of HPLC fraction 4. Chromatographic conditions as in Fig. 3. Peaks as numbered in Table I.

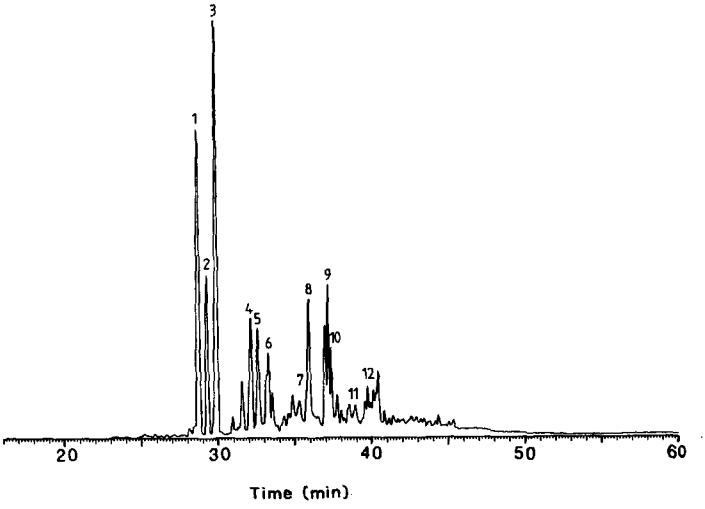

Fig. 7. Total ion current trace of HPLC fraction 5. Chromatographic conditions as in Fig. 3. Peaks as numbered in Table I.

similar to that of standard may not be the expected compound. Hence, RI values should be used as complementary to the other data such as mass spectra, molecular weight, and PBM search. Thus using several methods, the peaks in the HPLC fractions 2–6 were identified and are reported in Table I.

The HPLC retention behaviour of polar compounds is different from the PAHs when an amine column is used. Even though the three-ring PAHs eluted in fraction 3, the three-ring aromatics containing a carbonyl group were eluted in fraction 6 (Fig. 8). This shows that the amine column has a high selectivity for the separation

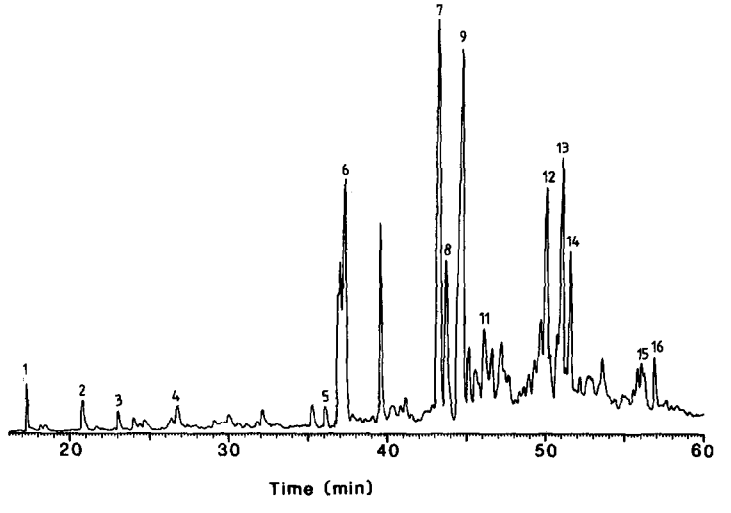

Fig. 8. Total ion current trace of HPLC fraction 6. Chromatographic conditions as in Fig. 3. Peaks as numbered in Table I.

TABLE II

ACIDIC COMPOUNDS IDENTIFIED IN AIR PARTICULATE EXTRACT (FIG. 9)

Peak No.	*Compound*	*Retention time (min)*	*Mol wt.*
1	Phenol, 4-ethyl-2-methoxy	6.78	152
2	Phenol, 2,6-dimethoxy	8.88	154
3	Benzoic acid, 4-hydroxy-3-methoxy	11.36	168
4	2-Naphthalenol	12.98	144
5	Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)	13.76	182
6	1-Naphthalenol, 4-methyl	16.06	158
7	(1,1'-Biphenyl)-3-ol	18.66	170
8	Unknown	22.68	182
9	Methanone, (2-hydroxyphenyl)-phenyl	25.50	198
10	Phenanthrenol	28.96	194
11	Phenol, 2,6-dichloro-4-(1-methylpropyl)	34.42	218

of aromatic hydrocarbons according to the number of rings, whereas carbonyl-substituted aromatics are retained very strongly. HPLC fractions 7–9 do not show any peak in GC–MS analysis.

The highly polar and reactive (acidic) compounds isolated in the acid–base extraction and identified by GC–MS using computer matching are reported in Table II (fig. 9). The acid–base extraction has the advantages of removing inorganic impurities and the water soluble compounds that may be permanently retained on the HPLC column, and to separate the polar reactive organic compounds which otherwise require the application of very strong solvents and high flow-rate for their elution from the HPLC amine column. The recoveries for selected PAH, after acid base

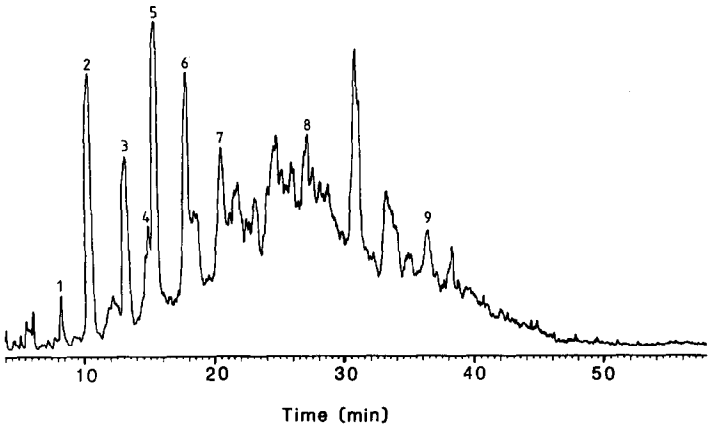

Fig. 9. Total ion current trace of acidic compounds separated by acid–base extraction. Gas chromatographic conditions as in Fig. 3. Peaks as numbered in Table II.

extraction were above 85%. This indicates the simplicity and usefulness of an acid–base extraction prior to HPLC and GC–MS analysis.

The recoveries of a standard mixture of 20 PAHs in acid–base extraction and HPLC separations were determined. By correcting the amounts of PAHs detected in different fractions to recoveries of standard PAHs under identical conditions, it was observed that PAHs detected in the particulate matter varied from 0.1 to 1.1 μg/g.

REFERENCES

1 M. Moller and J. Alfhein, *Atmos. Environ.*, 14 (1980) 83.
2 H. Y. Tong, J. D. Centen, F. W. Karasek, E. Jellum and P. Helland, *J. Chromatogr.*, 324 (1985) 373.
3 F. W. Karasek, D. W. Denney, K. W. Chan and R. E. Clement, *Anal. Chem.*, 50 (1978) 82.
4 D. F. S. Natusch and B. A. Tomkins, *Anal. Chem.*, 50 (1978) 1429.
5 M. L. Lee, D. L. Vassilaros and D. W. Later, *Int. J. Environ Anal. Chem.*, 11 (1982) 251.
6 K. D. Bartle, M. L. Lee and S. A. Wise, *Chem. Soc. Rev.*, 10 (1983) 113.
7 P. W. Jones and P. Leber (Editors), *Polynuclear Aromatic Hydrocarbons*, Ann Arbor Science Publishers, Ann Arbor, MI, 1979.
8 A. Bjorseth and A. J. Dennis (Editors), *Polynuclear Hydrocarbons: Chemistry and Biological Effect*, Battelle, Columbus, OH, 1980.
9 L. H. Keith (Editor), *Identification and Analysis of Organic Pollutants in Air*, Butterworth, Boston, London, Sydney and Toronto, 1984.
10 M. L. Lee, M. Novotny and K. D. Bartle (Editors), *Analytical Chemistry of Polycyclic Aromatic Hydrocarbons*, Academic Press, New York, 1981.
11 D. J. Futoma, S. R. Smith, J. Tanaka and T. E. Smith, *CRC. Crit. Rev. Anal. Chem.*, 12 (1981) 69.
12 R. W. Coutant, *Environ. Sci. Technol.*, 11 (1977) 871.
13 G. Grimmer and H. Bohnke, *Z. Anal. Chem.*, 261 (1972) 310.
14 G. Grimmer, K. W. Naujack and D. Schneider, *Z. Anal. Chem.*, 311 (1982) 475.
15 W. Cautreels and K. Van Cauwenberghe, *Water Air Soil Poll.*, 6 (1976) 103.
16 K. E. Markides, M. Nishioka, B. J. Tarbet, J. S. Bradshaw and M. L. Lee, *Anal. Chem.*, 57 (1985) 1296.
17 M. A. Apfel, H. Finkelmann, G. M. Janini, R. J. Laub and B. A. Smith, *Anal. Chem.*, 57 (1985) 651.
18 K. P. Naikwadi, A. L. Jadhav, S. Rokushika, H. Hatano and M. Oshima, *Macromol. Chem.*, 187 (1986) 1407.
19 K. P. Naikwadi, A. M. McGovern and F. W. Karasek, *Can. J. Chem.*, in press.
20 R. E. Clement and F. W. Karasek, *Intern. J. Environ. Anal. Chem.*, 7 (1979) 109.
21 M. L. Lee, D. L. Vassilaros, C. M. White and M. Novotny. *Anal. Chem.*, 51 (1979) 768.