Journal of Chromatography, 285 (1984) 423–441
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,430

IDENTIFICATION OF ORGANIC COMPOUNDS OBTAINED FROM INCINERATION OF MUNICIPAL WASTE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC FRACTIONATION AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

H. Y. TONG, D. L. SHORE and F. W. KARASEK*
Chemistry Department, University of Waterloo, Waterloo, Ontario, N2L 3G1 (Canada)
and
P. HELLAND and E. JELLUM
Institute of Clinical Biochemistry, University of Oslo, Oslo (Norway)
(Received November 7th, 1983)

SUMMARY

A gradient, normal-phase high-performance liquid chromatographic (HPLC) separation procedure that effectively provides a compound class separation was applied to the analysis of extracts of flyash from municipal incinerators in Ontario, Canada and Oslo, Norway. Each fraction collected from the HPLC procedure was analysed by capillary gas chromatography and capillary gas chromatography–mass spectrometry. More than 200 organic components were identified in the Canadian extract. The polychlorinated dibenzodioxins (PCDDs) were exclusively eluted in one fraction, which facilitated their quantitation. The retention indices of some environmentally important polychlorinated organic compounds were determined with an average standard deviation of 0.023. The average recovery of the PCDD compounds in the HPLC separation step was 105% with a relative standard deviation of 6.8%.

INTRODUCTION

For the economical disposal of urban refuse and to produce energy, many large cities dispose of their municipal waste by incineration. However, municipal incinerators burning garbage generate thousands of tons of flyash throughout the world each year[1]. Flyash is composed of 70–95% inorganic matter, on which a number of organic compounds are adsorbed at concentrations in the ppm to ppb range. Flyash can enter the environment as particulate matter which escapes to the atmosphere with the stack gases[2]. About 100 organic components have been identified in flyash from a municipal incinerator. These compounds include hydrocarbons, phthalates, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated organic compounds[2–4].

Some of the compounds identified in identified in flyash are known to be very toxic. Most of the polychlorodibenzodioxins (PCDDs) are known to be present.

0021-9673/84/$03.00

Some isomers of the PCDD are toxic at the ppm to ppb level and carcinogenic, teratogenic, and mutagenic at the ppb to ppt level[1].

The identification and quantification of the organic compounds in flyash from municipal incinerators have received increasing attention. However, the analysis is difficult because the incineration of garbage at high temperatures creates extremely complex mixtures of compounds. In addition, the determination of some highly toxic compounds, such as PCDDs, must be performed at an extremely low concentration level. To bring the concentration of PCDD in the sample up to the detection limit of the methods employed, extraction and concentration by factors of several thousand are commonly required. Other organic compounds that are present at much higher concentrations interfere in the determination of PCDDs by gas chromatographic–mass spectrometric (GC–MS) techniques. A pre-separation procedure is required for the effective analysis of the components in such complex mixtures.

The most common and conventional pre-separation procedure for complex environmental samples is column chromatography. However, the use of high-performance liquid chromatography (HPLC) for pre-separation has definite advantages. HPLC with a Porasil column and hexane–chloroform gradient elution has been used for the clean-up of drinking water extracts and atmospheric particulate samples[5,6]. Schuetzle *et al.*[7] successfully applied normal-phase HPLC with gradient elution to pre-separate the extract of diesel particulate exhaust. Lamparski and Nestrick[8] reported a sophisticated and complex clean-up procedure to determine specific PCDD isomers in environmental samples, including flyash. This method consists of multiple column chromatography, isocratic reversed-phase HPLC and isocratic normal-phase HPLC to achieve the isomer-specific determination of PCDDs at the ppt level. However, this clean-up procedure with its multiple steps seems too complicated and inconvenient in instances where the survey of many samples is desired. A simpler but effective procedure would be desirable.

In this study, a one-step pre-separation procedure using normal-phase HPLC with gradient elution is described for the detailed analysis of the components and the quantitative determination of the PCDD content of organic extracts from flyash samples. Two flyash samples, one from a municipal incinerator in Ontario, Canada and one from Oslo, Norway are used to demonstrate this method. Several hundred organic compounds were separated into five HPLC eluate fractions according to their relative polarities. More than 200 organic components were identified by analysing each fraction by capillary GC and GC–MS. The PCDDs were exclusively located in HPLC fraction 2 and quantified for the demonstrated samples. The modified retention indices of some environmentally important polychlorinated compounds were determined with an average standard deviation of ± 0.023. This one-step HPLC pre-separation shows a high recovery for the PCDD standards.

EXPERIMENTAL

Solvent and standards

All solvents used were distilled in glass chemicals of UV grade from Caledon Laboratories. The standards of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1234-TCDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (123478-H_6CDD), 1,2,3,4,6,7,8-heptachlo-

rodibenzo-*p*-dioxin (1234678-H_7CDD) and octachlorodibenzo-*p*-dioxin (OCDD) were purchased from Ultra Scientific (Hope, RI, U.S.A.) all glassware was cleaned by ultrasonic agitation in SUD'N detergent (BDH Chemicals, Toronto, Canada) for 30 min. Copious amounts of tap water and deionized water were used for rinsing prior to drying at 250°C for 3 h. The glassware was rinsed three times with benzene immediately before its use.

Sample collection and extraction

The Ontario flyash sample was collected in kilogram amounts by grab sampling from the electrostatic precipitator of a municipal incinerator in Toronto, Canada. The incinerator was operated at a temperature near 900°C. The sample was stored away from sources of light.

Four 50-g Ontario flyash samples were separately extracted with 500 ml of benzene in four large Soxhlet extractors for 48 hs. After the first extraction step, each thimble was replaced with a second thimble containing another 50-g sample and extraction continued for another 48 h. Suitable amounts of fresh benzene were added to keep 500 ml of solvent in the extractor during this transfer step.

Eventually, all extracts were combined to form a combined extract from 383 g of flyash. This combined extract was first concentrated to about 10 ml by rotary evaporation under aspirator vacuum, and then transferred into a 25-ml pear-shaped flask and reduced to 1 ml by further rotary evaporation under aspirator vacuum, and then transferred into a 1.0-ml reacti-vial. Final concentration to 600 μl was achieved by blowing a gentle stream of high-purity nitrogen across the top of the vial. The concentrated extract was stored in a reacti-vial with a PTFE-lined screw-cap in a freezer at -15°C.

A 43-g sample of Oslo flyash sample collected from a municipal incinerator in Oslo, Norway was extracted with 360 ml of benzene in a large Soxhlet extractor for 36 h. The concentration procedure for this sample was similar to that described above, except that this extract was brought to dryness under a gentle stream of high-purity nitrogen. The residue was stored in a 1.0-ml reacti-vial in a freezer at -15°C.

A 500-ml volume of benzene solvent that underwent Soxhlet extraction and the concentration procedure was used as the extraction blank. No impurities were found by GC analysis of this extraction blank.

High-performance liquid chromatographic pre-separation

The concentration of the Ontario flyash extract was adjusted with benzene to be equivalent to *ca.* 0.3 g of flyash per microlitre for the HPLC separation. The residue of the Oslo flyash extract was dissolved in benzene to give a concentration equivalent to *ca.* 0.1 g of flash per microlitre for HPLC separation. The instrument used for the HPLC separation was a Spectra-Physics SP-8000 equipped with an SP-8400 UV–visible detector and an SP-4100 integrator. The monitoring wavelength was 254 nm. A 10-μm semi-preparative Spherisorb silica column (250 × 9.4 mm I.D.; Terochem, Toronto, Canada) was employed with a 140-μl sample loop. The gradient elution programme developed was based on previous work[9]. It consisted of 100% *n*-hexane for 20 min, programmed to 100% dichloromethane over 30 min, held at 100% dichloromethane for 20 min, programmed to 100% acetonitrile over

10 min, held at 100% acetonitrile for 1 min, programmed back to 100% dichloromethane in 5 min and finally to 100% *n*-hexane in another 5 min. During this gradient programme, five separate fractions were collected at elution times of 0 to start of first peak, 20, 50, 70 and 91 min. The flow-rate was 5 ml/min.

A blank was obtained in the same manner as above but with benzene injected. The HPLC system was cleaned by running three mobile phases prior to injecting the sample or benzene blank.

Following the procedure described under *Solvent collection and extraction,* each collected fraction was separately concentrated to 50 μl for the Oslo sample and to 70 μl for Ontario sample for GC and GC–MS analysis. Evaporation to dryness was avoided in this concentration step.

Gas chromatographic analysis

GC analyses were carried out on a Hewlett-Packard HP-5880A gas chromatograph equipped with a flame-ionization detector and cool on-column injection for the capillary column. A 30 m × 0.32 mm I.D. Durabond DB-5 fused-silica capillary column (J&W Scientific, Rancho Cordova, CA, U.S.A.) was used. The GC conditions were as follows: injection port temperature < 50°C; column temperature, programmed from 80 to 300°C at 3°C/min with initial 1-min and final 10-min isothermal periods; detector temperature, 320°C; and helium carrier gas flow-rate, 3 ml/min measured at room temperature.

A BASIC program retention index for PAHs was written to calculate the retention indices of aromatics for the HP5880A GC based on the modified equation of Van den Dool and Kratz[10–12]. Once a sample GC run was finished and the report printed, the sample volume, retention time and numbers of aromatic rings in the reference compounds were input. A report containing the retention time (to three decimal places), retention index (to three decimal places) and peak area per microlitre of each GC component was generated from the raw data stored in the HP5880A terminal. The retention index and peak area per microlitre were used for the qualitative and quantitative analyses, respectively.

Gas chromatographic–mass spectrometric analysis

Two GC–MS systems were used to provide compound identification. At the Institute of Clinical Biochemistry, University of Oslo, a Finnigan Model 4021 equipped with an INCOS 2000 data system capable of executing a modified Probability Based Matching (PBM) search with a library of 32,000 reference compounds was used. GC separation was achieved with a 30 m × 0.25 mm I.D. DB-5 fused-silica capillary column (J&W Scientific), splitless injection and temperature programmed from 110 to 280°C at 4°C/min.

At the University of Waterloo, a Hewlett-Packard HP5992 GC–MS system equipped with a calculator-based data system employing two floppy disc units and limited special libraries of reference compounds was used. An HP59916A glass capillary effluent splitter interface and on-column injection for capillary column were also used for HP5992 GC–MS. The same capillary column as used in GC analysis was transferred to HP5992 GC–MS and similar chromatographic conditions to those described for GC analysis, with the exception of a temperature programming rate of 6°C/min, were established for GC/MS analysis. The helium carrier gas flow-rate was

3 ml/min at room temperature and the capillary effluent splitter allowed about 0.5 ml/min to enter the mass spectrometer. Electron-impact ionization at 70 eV was used. The quadrupole mass spectrometer was scanned from 500 to 50 a.m.u. Mass spectra were taken at the top of eluting GC peak and mass spectra taken prior to the GC peak were used for later background subtraction. User-developed software called DUAL-MODE allowed total ion current and mass chromatograms to be obtained in addition to individual mass spectra.

Compound identification in this study is primarily based on matching the measured mass spectra with those obtained from injected standards, from reference libraries of two GC–MS systems and from reference spectra published in the EPA/NIH Mass Spectral Data Base (U.S. Government Printing Office, 1978) and the Eight Peak Index of Mass Spectra (Mass Spectrometry Data Centre, 2nd edition, 1974). In some instances, the mass spectra search system, Probability Based Matching (PBM) and Self-Training Interpretive Retrieval System (STIRS), which is accessed by telephone link to Cornell University, was used for compound identification. The retention indices of components in a sample were also used to facilitate compound identification. These identification procedures provide identification with varying degrees of certainty. The only way to provide a more certain identification is to obtain the mass spectra and retention behaviour of pure standards directly for each compound, which was not possible for the many compounds treated here. The mass spectra of compounds identified by name were well matched with their reference spectra. When only the type of compound was indicated, mass spectra characteristic of that type were clearly obtained. Not having standards of the many isomers of the PCDD and PCDF compounds, it was not possible to indicate these isomers individually. If no identification resulted from this procedure, the compound was labelled as unidentified.

Quantification of PCDDs was performed by HP5992 GC–MS with selected ion monitoring (GC–MS–SIM). Six characteristic ions were monitored in the GC–MS–SIM analysis, *viz.*, *m/z* 319.9 and 321.9 for TCDD, 355.9 for pentachlorodibenzo-*p*-dioxin (P_5CDD), 389.8 for H_6CDD, 425.8 for H_7CDD and 459.7 for OCDD. The column temperature programme used in GC–MS–SIM analysis was 150°C for 1 min, then programmed to 300°C at 6°C/min. SIM areas of the sample and external references were used for quantitative analysis.

RESULTS AND DISCUSSION

Fig. 1 is a combined diagram showing the gradient programme for the HPLC separation used in this study (upper trace), fraction collection interval and HPLC trace of the Ontario flyash extract (lower trace). This modified HPLC separation with gradient elution separates organic compounds into non-polar, medium polar and polar compounds[7,9]. A benzene blank was run through the HPLC system using the same gradient programme. Fig. 2 indicates the presence of system impurities found in fraction 5. These impurities were identified by GC and GC–MS data and taken into account in the sample components identified from fraction 5 of the sample.

Fig. 3 is a gas chromatogram obtained from HPLC fraction 1 of the Ontario flyash extract showing a typical pattern of aliphatic compounds. More than 200 compounds are present in this fraction and most of them are well separated on the

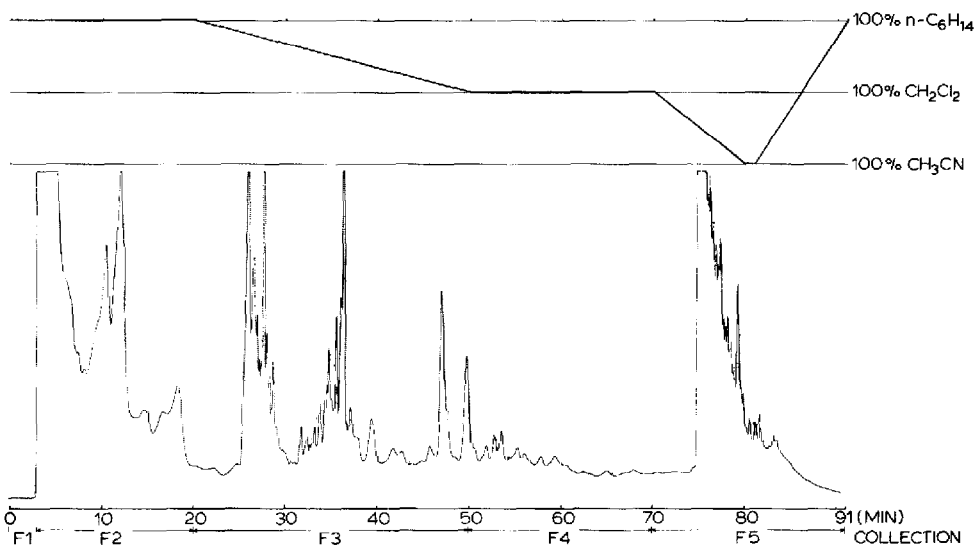

Fig. 1. Combined diagram of gradient programme for HPLC pre-separation (upper trace), liquid chromatogram of Ontario Flyash extract (lower trace) and fraction collection interval. HPLC conditions as described under Experimental.

gas chromatogram. A total ion current trace with a similar pattern and 115 clear mass spectra were also obtained from the GC–MS analysis. Most of those compounds in HPLC fraction 1 can be identified using their mass spectra and retention indices. The compound identification confirms that all compounds in HPLC fraction 1 are aliphatic, except a trace amount of hexachlorobenzene. Large amounts of hexachlorobenzene were found in HPLC fraction 2. Two homologous series, *n*-alkanes and alkenes, are illustrated in Fig. 3. From Fig. 3, it can be seen that a large amount of aliphatic compounds covering a wide range of boiling points was present in this

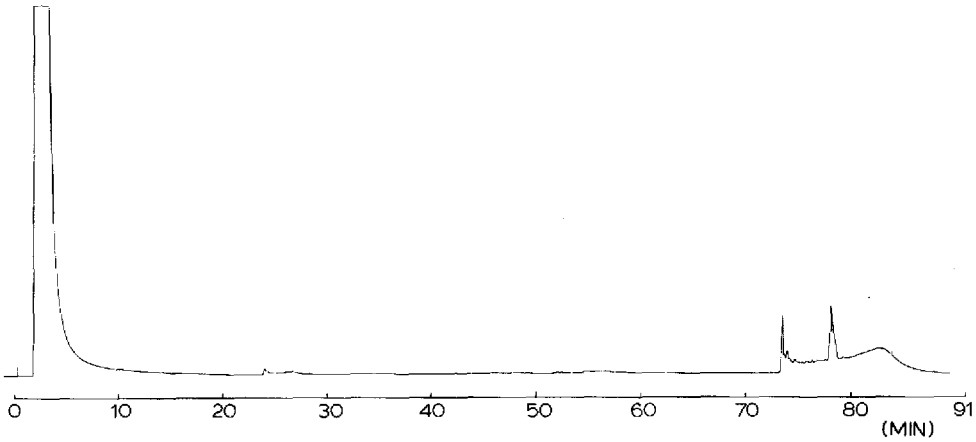

Fig. 2. Liquid chromatogram of benzene blank. HPLC conditions and gradient programme as in Fig. 1.

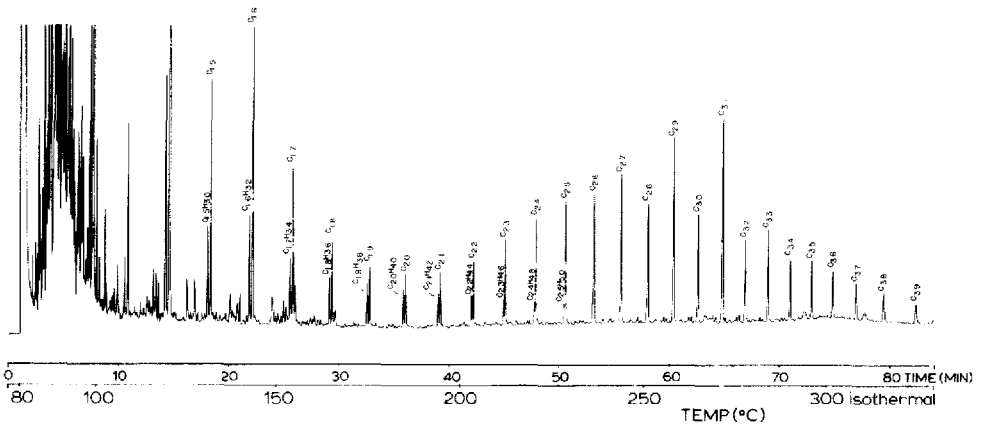

Fig. 3. Gas chromatogram of HPLC fraction 1 of Ontario flyash extract. Chromatographic conditions: 30 m × 0.32 mm I.D. DB-5 fused-silica column; temperature, 80°C for 1 min, programmed to 300°C at 3°C/min; flame-ionization detector. C_n are *n*-alkanes and C_nH_{2n} are alkenes; subscript *n* indicates the number of carbon atoms.

flyash sample. No significant amount of aliphatic compounds was found in other HPLC fractions.

Figs. 4 and 5 show the analytical results for HPLC fraction 2 of the Ontario flyash extract; the former is the gas chromatogram and the latter is the total ion current (TIC) trace from GC–MS analysis. The two traces shown in Figs 4 and 5 are very similar, except that more peaks appear in the GC analysis. In the analysis for this study, the qualitative similarity between the gas chromatogram and the TIC trace from GC–MS applied to all five HPLC fractions. It is therefore easy to locate the corresponding peaks in these two traces.

Table I lists the compounds identified, or tentatively identified, in HPLC fraction 2 of the Ontario flyash extract. The corresponding numbers of the compounds

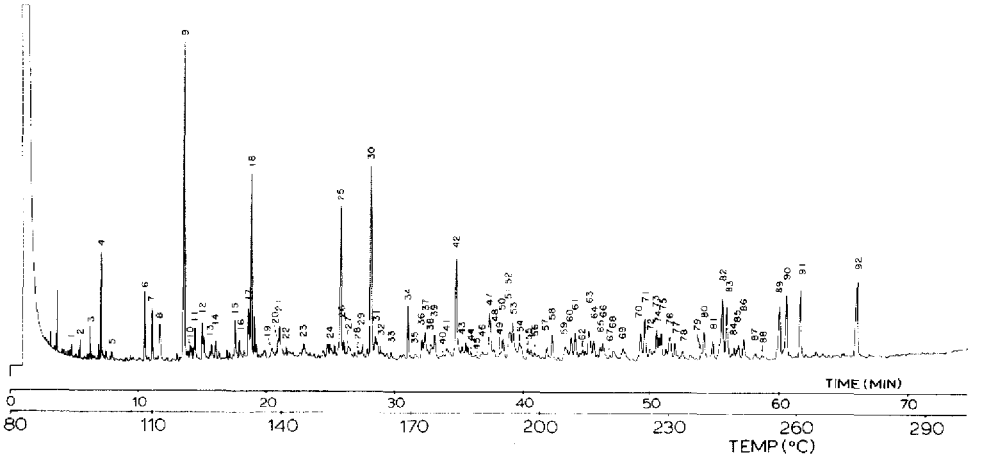

Fig. 4. Gas chromatogram of HPLC fraction 2 of Ontario flyash extract. Chromatographic conditions as in Fig. 3.

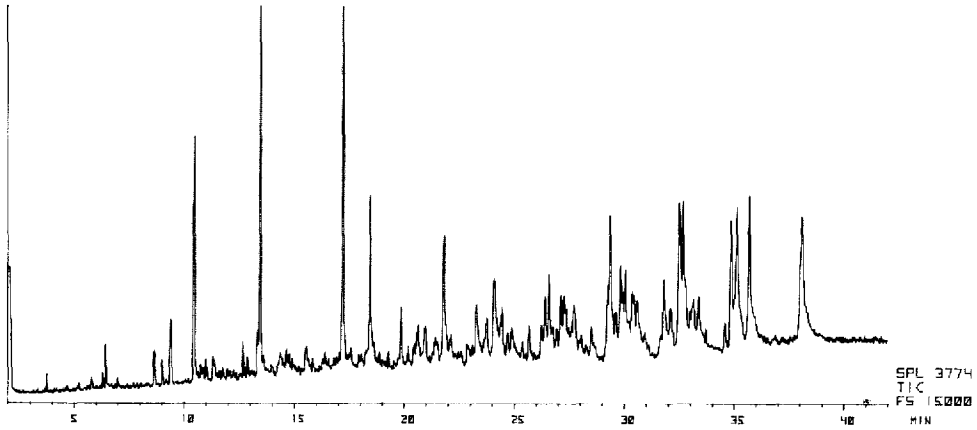

Fig. 5. Total ion current (TIC) trace of HPLC fraction 2 of Ontario flyash extract in GC–MS analysis. Chromatographic conditions: 30 m × 0.32 mm I.D. DB-5 fused-silica column; temperature, 80°C for 1 min, programmed to 300°C at 6°C/min. FS = full-scale value.

in Table I refer to the GC trace in Fig. 4. The identification of the compounds in Table I is primarily based on matching their mass spectra obtained from the GC–MS run with those obtained from references, as mentioned under Experimental.

The retention index system modified by Lee and co-workers[11,12] was also used to facilitate compound identification. The retention indices of more than 200 aromatic standards are available[11]. The retention indices of compounds can be used to aid identification for specific compounds where the mass spectra of isomers are too similar to differentiate them. As suggested by Lee and co-workers, four polycyclic aromatic compounds, naphthalene, phenanthrene, chrysene and picene, are used as reference compounds to form the retention index system for aromatics. Using our programme, the retention index of compounds can be calculated automatically and printed out by the HP5880 instrument at the end of the GC analyses. Triplicate GC injections of HPLC fraction 2 were made and the retention indices were calculated with an average standard deviation of 0.023. The retention index of the compounds determined in our sample was not always identical with that published for the standards[11,12]. Small differences in GC conditions, such as an initial isothermal period, is one of the reasons discussed by Vassilaros *et al.*[12]. The earlier eluates seem to be more sensitive to the initial isothermal period in the GC conditions. Another factor may be the difference in sample matrices. A complex sample matrix may affect the properties of the thin stationary phase film, especially for capillary columns. This slight change in the properties of the stationary phase can affect the retention behaviour of compounds in a mixture to varying extents. This may contribute to the small deviation of the retention index of a compound determined in different matrices even when internal references were used. The published retention indices, however, can still be used for isomer identification according to the relative elution order of isomers. Thus, 1- and 2-methylnaphthalene have identical mass spectra but 2-methylnaphthalene has a lower published retention index. On a comparative basis, the earlier eluate of two methylnaphthalene isomers is identified as 2- and the latter as 1-methylnaphthalene. This application of published retention indices was also used

TABLE I

COMPOUNDS IDENTIFIED IN HPLC FRACTION 2 OF ONTARIO FLYASH

Individual isomers of some compounds are not identifiable by techniques used in this procedure.

No.	*Compound*	*Mol. wt.*	*Identified by MS*	*Identified by retention index*	*Average retention index**	*Standard deviation**	*Retention index in ref. 11*	*Retention index in ref. 12*
1	Tetramethylbenzene isomer	134	×		191.711	0.013		
2	Tetramethylbenzene isomer	134	×		192.199	0.011		
3	Trichlorobenzene isomer	180	×		195.903	0.008		
4	Naphthalene	128	×	×	200.000	0.000	200.00	200.00
5	Trichlorobenzene isomer	180	×		203.904	0.003		
6	2-Methylnaphthalene	142	×		216.185	0.005	218.14	220.22
7	1-Methylnaphthalene	142	×		218.900	0.012	221.04	223.01
8	Tetrachlorobenzene isomer	214	×		221.637	0.015		
9	Biphenyl	154	×		230.795	0.026	233.96	236.44
10	1-Ethylnaphthalene	156	×		233.527	0.012	238.55	238.55
11	2,6- or 2,7-dimethylnaphthalene	156	×		234.920	0.061	237.58	240.28
12	1,3- or 1,7- or 1,6-dimethylnaphthalene	156	×		237.437	0.111	240.25	243.30
13	2,3- or 1,4- or 1,5-dimethylnaphthalene	156	×		240.900	0.003	243.55	246.03
14	Acenaphthylene	152	×		242.459	0.017	244.63	246.92
15	Methylbiphenyl	168	×		249.673	0.012		255.41
16	3- or 4-methylbiphenyl	168	×		251.189	0.007	254.71	256.69
17	Dibenzofuran	168	×		254.578	0.024	257.17	258.77
18	Pentachlorobenzene	248	×		255.626	0.035		
19	2,3,6-Trimethylnaphthalene	170	×	×	263.252	0.006	263.31	265.09
20	Nonylbenzene	204	×		265.511	0.005		
21	2,4,6-Trichlorophenol	196	×		266.230	0.011		
22	Unidentified	182	×		269.354	0.014		
23	Bromotetrachlorobenzene	292	×		275.065	0.012		
24	Decylbenzene	218	×		284.697	0.006		
25	Hexachlorobenzene	282	×		288.808	0.015		
26	1- or 2-methylfluorene	180		×	289.807	0.007	289.03	295.20
27	Bromodichloromethylphenol	254	×		291.684	0.031		
28	Dibenzothiophene	184	×	×	295.312	0.007	295.81	295.39

(*Continued on p. 432*)

TABLE I (*continued*)

No.	*Compound*	*Mol. wt.*	*Identified by MS*	*Identified by retention index*	*Average retention index**	*Standard deviation**	*Retention index in ref. 11*	*Retention index in ref. 12*
29	2,3,4,6-Tetrachlorophenol	230	×		296.864	0.006		
30	Phenanthrene	178	×	×	300.000	0.000	300.00	300.00
31	Tetrachloroacenaphthylene isomer	288	×		301.660	0.025		
32	Unidentified compound with 6 chlorines	306	×		303.494	0.023		
33	Tetrachloroacenaphthylene isomer	288	×		307.521	0.007		
34	Dihydropyrene or dihydrofluoranthene	204	×		313.830	0.002		
35	Dichlorodibenzofuran	236	×		316.456	0.000		
36	3-Methylphenanthrene	192	×	×	319.061	0.011	319.46	319.19
37	Tetrachlorobenzene dicarbonitrile isomer	264	×		320.218	0.008		
38	2-Methylanthracene	192		×	321.410**	0.026	321.57	
39	1-Methylanthracene (or 1-, 4- or 9-methylphenanthrene)	192	×		323.766	0.008	323	323.64
40	Tetrachlorobenzene dicarbonitrile isomer	264	×					
41	2,3-dichlorodibenzodioxin	252	×		328.387	0.038		
42	Phenylnaphthalene	204	×		331.911	0.018	332.59	332.64
43	Chlorophenylethynylbenzene	212	×		334.164	0.018		
44	2-Ethylphenanthrene	206		×	337.144	0.017	337.50	
45	Trichlorodibenzofuran isomer	270	×		341.831	0.024		
46	Trichlorodibenzofuran isomer	270	×		343.080	0.013		
47	Fluoranthene	202	×	×	344.560	0.054	344.01	344.51
48	Trichlorodibenzofuran isomer	270	×		346.077	0.023		
49	Trichlorodibenzofuran isomer	270	×		348.545	0.064		
50	Pentachloronaphthalene isomer	298	×		349.626	0.002		
51	Trichlorodibenzodioxin isomer	286	×		352.163	0.099	351.22	351.51
52	Pyrene	202	×	×	352.163	0.099		
53	Trichlorodibenzofuran isomer	286	×		353.355	0.006		
54	Pentachloronaphthalene isomer	298	×		356.373**	0.025		
55	Trichlorodibenzodioxin isomer	286	×		360.136	0.018		
56	Pentachloronaphthalene isomer	298	×		361.353	0.025		
57	Tetrachlorobiphenyl isomer	290	×		366.177	0.012		
58	Tetrachlorodibenzofuran isomer	304	×		368.051	0.019		
59	Tetrachlorodibenzofuran isomer	304	×		372.849	0.020		
60	Tetrachlorodibenzodioxin isomer	320	×		375.210	0.023		
61	Tetrachlorodibenzodioxin isomer	320	×		376.735	0.013		
62	Tetrachlorodibenzofuran isomer	304	×		379.565	0.018		

63	Tetrachlorodibenzodioxin isomer	320	×		381.895	0.024		
64	Hexachloronaphthalene isomer	332	×		383.695	0.054		
65	1,2,3,4-Tetrachlorodibenzodioxin	320	×	×	386.063	0.033		
66	Tetrachlorodibenzodioxin isomer	320	×		389.670	0.029		
67	Tetrachlorodibenzodioxin isomer	320	×		389.670	0.028		
68	Benzo[*ghi*]fluoranthene	226	×		391.059	0.021	389.60	389.92
69	Pentachlorodibenzofuran isomer	338	×		395.390**	0.009		
70	Pentachlorodibenzofuran isomer	338	×		401.425	0.035		
71	Pentachlorodibenzodioxin isomer	354	×		403.240	0.009		
72	Pentachlorodibenzofuran isomer	338	×		405.420	0.039		
73	Pentachlorodibenzodioxin isomer	354	×		408.477	0.037		
74	Pentachlorodibenzodioxin isomer	354	×		409.582	0.034		
75	Pentachlorodibenzodioxin isomer	354	×		410.516	0.041		
76	Pentachlorodibenzodioxin isomer	354	×		414.382***			
77	Pentachlorodibenzodioxin isomer	354	×		416.286	0.023		
78	Pentachlorodibenzodioxin isomer	354	×		418.641	0.018		
79	Hexachlorodibenzofuran isomer	372	×		427.697**	0.071		
80	Hexachlorodibenzofuran isomer	372	×		428.958	0.039		
81	Hexachlorodibenzodioxin isomer	388	×		432.550	0.027		
82	Hexachlorodibenzodioxin isomer	388	×		432.734	0.017		
83	Hexachlorodibenzodioxin isomer	388	×		438.646	0.026		
84	Hexachlorodibenzofuran isomer	372	×		441.693	0.004		
85	1,2,3,4,7,8-Hexachlorodibenzodioxin	388	×	×	443.667	0.021		
86	Hexachlorodibenzodioxin isomer	388	×		445.887	0.009		
87	Nonachlorobiphenyl isomer	460	×		450.866	0.036		
88	Decachlorobiphenyl	494	×		460.183	0.047		
89	Heptachlorodibenzofuran isomer	406	×		461.396	0.048		
90	1,2,3,4,6,7,9-Heptachlorodibenzodioxin	422	×	×	464.455	0.002		
91	1,2,3,4,6,7,8-Heptachlorodibenzodioxin	422	×	×	470.457	0.014		
92	Octachlorodibenzodioxin	456	×	×	494.399	0.024		

* Based on three determinations.
** Data obtained from two GC runs.
*** Data obtained from one GC run.

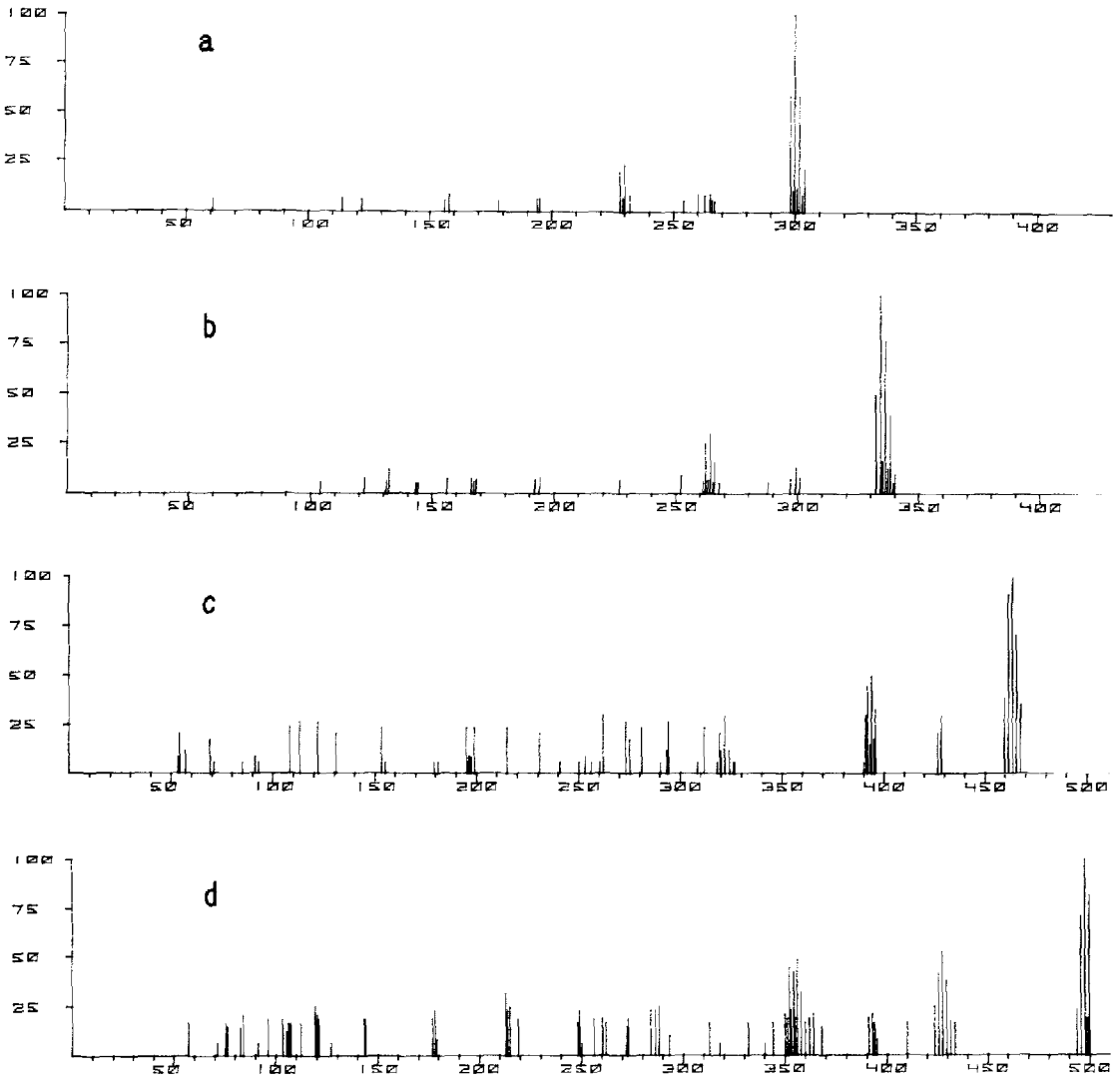

Fig. 6. Mass spectra of (a) pentachloronaphthalene, (b) hexachloronaphthalene, (c) nonachlorobiphenyl and (d) decachlorobiphenyl identified in HPLC fraction 2 of Ontario flyash extract.

in the differentiation of dimethylnaphthalene and methylanthracene isomers, and to distinguish phenanthrene from anthracene.

The retention indices of the later eluates are consistent with those published to the last integral place. Consequently, they can be used to confirm the identification by GC–MS. A few compounds in HPLC fraction 2 are tentatively identified using retention index data. In this instance, auxiliary information was used to confirm identity. Table I lists the retention indices of the compounds identified in HPLC fraction 2 of the Ontario flyash extract, the standard deviation obtained from triplicate injections and some retention index data published in the literature. Some of

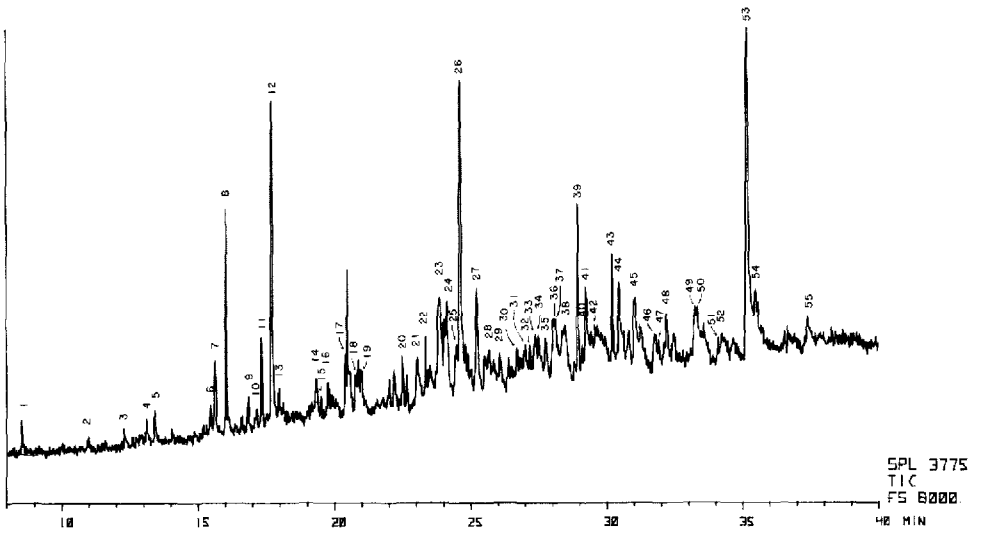

Fig. 7. Total ion current (TIC) trace of HPLC fraction 3 of Ontario flyash extract. Chromatographic conditions as in Fig. 5. FS = full-scale value.

the PCDD isomers are specified in Table I as a result of matching with retention indices of injected isomeric PCDD standards. For some compounds which can not be isomer specified, the compound class is given.

The data in Table I show that in this separation procedure the major compounds in fraction 2 are PAHs with 2–3 rings, sulphur-containing PAHs, polychlorinated PAHs with 1–3 rings, PCDDs and polychlorinated dibenzofurans (PCDFs). A few mass spectra of highly chlorinated PAH found in fraction 2 are shown in Fig. 6. In terms of environmental concern, HPLC fraction 2 is most important.

Analysis of a mixture of PCDD standards by this HPLC separation procedure has shown that PCDDs elute exclusively in fraction 2. To confirm the reliability of this result, GC–MS with selected ion monitoring was used to scan the other four HPLC fractions (fractions 1,3,4 and 5) of Ontario and Oslo flyash extracts. GC–MS–SIM was set to monitor the characteristic ions for TCDD, P_5CDD, H_6CDD, H_7CDD and OCDD. No significant amount of PCDDs was found in these four fractions. We conclude that all of the PCDDs elute exclusively in fraction 2. Generally, this exclusive elution applies to most compounds determined in HPLC fraction 2, which makes the quantitation of PCDDs easier.

The analytical results for HPLC fraction 3 of the Ontario flyash extract are illustrated in Fig. 7 and Table II. The compounds identified in this fraction are correspondingly labelled by number on the total ion current trace and in Table II. As there is a significant deviation in the retention index data between our determined and the published data for some compounds in this fraction, retention index data are used only to aid identification. On a comparative basis, the published retention indices help to identify the isomers of terphenyl and quaterphenyl and differentiated benzo[*e*]pyrene from benzo[*a*]pyrene. The compound identification in HPLC fraction 3 is based primarily on the GC–MS data.

The data in Table II show that HPLC fraction 3 contains major compounds

such as nitrogen-containing PAHs, oxygenated PAHs (oxy-PAHs), chlorinated oxy-PAHs and PAHs with three or more rings. Apparently the compounds in fraction 3 have a greater polarity than those in fraction 2. Compounds with lower symmetry of the oxygen such as benzofuran are more polar than those with higher symmetry such as the dibenzofurans. The former is found in fraction 3 and the latter are eluted in fraction 2.

Table III lists the compounds identified in HPLC fractions 4 and 5. Compound identification in these two fractions is based on mass spectra from GC–MS data. More than 30 distinctive mass spectra were obtained from fractions 4 and 5. However, few of them have been identified positively owing to a lack of standard compounds and auxiliary information. According to the data in the blank run in Fig. 2, the compounds in HPLC fraction 5 from the sample are mixed with polar impurities from the dichloromethane and *n*-hexane mobile phases. Compounds present that originate from impurities in the system are butylbenzl phthalate, dioctyl phthalate and an unidentified compound with a molecular weight of 171. A large amount of the unidentified compound of molecular weight 171 is also present in the flyash extract.

The usefulness of this gradient HPLC separation as applied to the analysis of

TABLE II

COMPOUNDS IDENTIFIED IN HPLC FRACTION 3 OF ONTARIO FLYASH EXTRACT

No.	*Compound*	*Mol.wt.*
1	4,7-Dimethylbenzofuran	146
2	Benzoic acid	122
3	Nitrogen-containing PAH	177
4	Naphthaldehyde	156
5	1,2-Diphenylethane	182
6	Biphenylamine isomer	169
7	Diphenylmethanone	182
8	1,3-Diphenylpropane	196
9	Nitrogen-containing PAH	181
10	Biphenylamine isomer	169
11	Unidentified	208
12	9-Fluorenone	180
13	Bis(methylphenyl)diazene	210
14	Phenyl benzoate	198
15	1,3-Diphenyl-2-propen-1-one	208
16	Unidentified	210
17	*o*-Terphenyl	230
18	Chloro-9-fluorenone isomer	214
19	Chloro-9-fluorenone isomer	214
20	Chloro-9-fluorenone isomer	214
21	4H-Cyclopenta[*def*]phenanthren-4-one	204
22	Nitrogen-containing PAH	239
23	Dichloro-9-fluorenone isomer	248
24	Dichloro-9-fluorenone isomer	248
25	1-Chloro-9,10-anthraquinone	242
26	*m*-Terphenyl	230
27	*p*-Terphenyl	230
28	Dichloro-9-fluorenone isomer	248

TABLE II (*continued*)

No.	*Compound*	*Mol.wt.*
29	Unidentified*	244
30	Trichloro-9-fluorenone isomer	282
31	Trichloro-9-fluorenone isomer	282
32	Trichloro-9-fluorenone isomer	282
33	Unidentified	244
34	Unidentified**	314
35	7H-Benz[*de*]anthracen-7-one (or 11-benzo[*a*]fluorenone)	230
36	Methylphenylindole	207
37	Trichloro-9-fluorenone isomer	204
38	Trichloro-9-fluorenone isomer	284
39	Methylphenylindole	207
40	Triphenylene	228
41	Chrysene	228
42	Tetrachloro-9-fluorenone isomer	316
43	Diisooctyl phthalate	390
44	Methylphenyl-1H-indole	207
45	Tetrachloro-9-fluorenone isomer	316
46	Benzo[*cd*]pyrenone	254
47	Pentachloro-9-fluorenone isomer	350
48	*o*-Quaterphenyl	306
49	Benzo[*e*]pyrene	252
50	Pentachloro-9-fluorenone isomer	350
51	1,7-Diphenylnaphthalene	280
52	Benzo[*a*]pyrene	252
53	*m*-Quaterphenyl	306
54	Unidentified	354
55	Unidentified	320

* Tentatively identified as 6-methylene-3,3-diphenyl-1,4-cyclohexadienene.
** Tentatively identified as methyl octahydrodimethyl-(1-methylethyl)-1-phenanthrenecarboxylate.

PCDDs in a complex sample is illustrated in Figs. 8 and 9. Fig. 8 shows the SIM trace of the non-fractionated extracts (traces A and B) compared with the SIM trace of HPLC fraction 2 (traces C and D) of the Oslo flyash sample. The instrument was set to monitor two characteristic ions, *m/z* 319.9 and 321.9, for TCDD. Traces A and B show the apparent presence of TCDD in the Oslo sample due to the alignment of peaks in those two traces. Even when monitoring four characteristic ions for TCDD (*m/z* 319, 322, 259 and 257), this alignment appeared to be valid. However, after the original extract was fractionated by HPLC and the collected fraction 2 was analysed, the peaks originally shown on trace B had disappeared. The compounds responsible for the *m/z* 321.9 ions are found in the HPLC fraction 1. From these results, it can be concluded that there is no TCDD present in the Oslo flyash sample at the detection limit used in this study. However, the other PCDD compounds listed in Table IV were present.

Fig. 9 shows the contrasting SIM traces for monitoring TCDD in the Ontario flyash extract with and without the HPLC separation. The upper trace was obtained from the GC–MS–SIM analysis of HPLC fraction 2 and the lower trace from the GC–MS–SIM analysis of the original extract without the HPLC fractionation. Some

TABLE III

COMPOUNDS IDENTIFIED IN HPLC GRACTIONS 4 AND 5 OF ONTARIO FLYASH EXTRACT

No.	*Fraction*	*Compound*	*Mol.wt.*
1	4	1-(Methylphenyl)ethanone	134
2		Biphenylamine isomer	169
3		Unidentified	186
4		Unidentified	196
5		Xanthen-9-one	196
6		Ketone derivative of PAH	196
7		Dibutyl phthalate	278
8		9,10-Anthraquinone	208
9		Unidentified	258
10		Quinone derivative of PAH	220
11		Ketone derivative of PAH	230
12		Diisooctyl phthalate	390
13		Nitrogen-containing PAH	209
14		Unidentified phthalate	
1	5	Unidentified	198
2		Diethyl phthalate	222
3		Biphenylamine isomer	169
4		Caffeine	194
5		Dibutyl phthalate	278
6		Benzo[*c*]cinnoline	134
7		Butylbenzyl phthalate (impurity)	312
8		Unidentified	171
9		Dioctyl phthalate (impurity)	390
10		Unidentified	330

TABLE IV

CONCENTRATION OF PCDDs DETERMINED IN FLYASH

Flyash	*Compound*	*Concentration (ng/g flyash)*	*Relative standard deviation (%)**
Ontario	TCDD	541	19
	P_5CDD	467	16
	H_6CDD	591	22
	H_7CDD	434	23
	OCDD	467	19
Oslo	TCDD	Not detected	
	P_5CDD	11	15
	H_6CDD	51	5
	H_7CDD	119	15
	OCDD	186	9

* Two determinations.

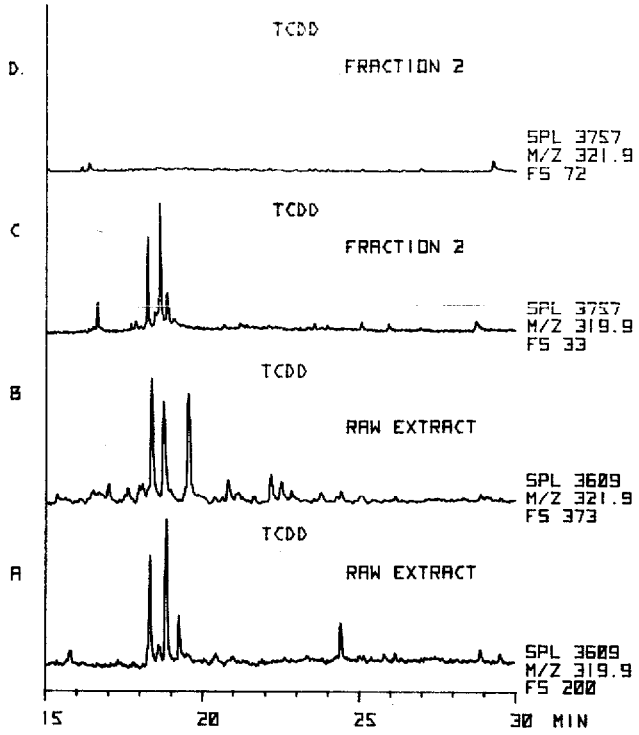

Fig. 8. SIM data for monitoring TCDD in non-fractionated extract (traces A and B) and HPLC fraction 2 (traces C and D) of Oslo flyash sample. TCDD elutes between 15 and 20 min under these chromatographic conditions. FS = full-scale value.

improvement in the resolution and reduction in background can be seen from the upper trace, although it is apparent that there were no major interferents with the TCDD in this sample.

Quantitation of PCDDs in the Ontario and Oslo flyash extracts was performed by GC–MS–SIM with external PCDD standards. Owing to the absence of various specified isomers of PCDD, the summarized integration area of all isomers having the same number of chlorine atoms in the sample was used for the comparison with the integration area obtained from each PCDD standard injected under the same conditions. Thus, the quantitative result is non-isomer specific. For P_5CDD, the quantitation was carried out using a response factor intermediate between 1234-TCDD and 123478-H_6CDD.

A standard mixture containing 1234-TCDD, 123478-H_6CDD, 1234678-H_7CDD and OCDD was used to test the recovery of the HPLC fractionation step. The average recovery for these four standards was determined to be 105% with a relative standard deviation of 6.8%. Kooke *et al.*[14] reported a recovery of 90% for the Soxhlet extraction of PCDD. O'Keefe *et al.*[15] found no significant loss of TCDDs in the drying procedure of vacuum evaporation–nitrogen evaporation. The recovery of each step was considered in the quantification of PCDDs in Ontario and Oslo flyash. Quantitative results for PCDDs in these flyash samples are listed in Table IV.

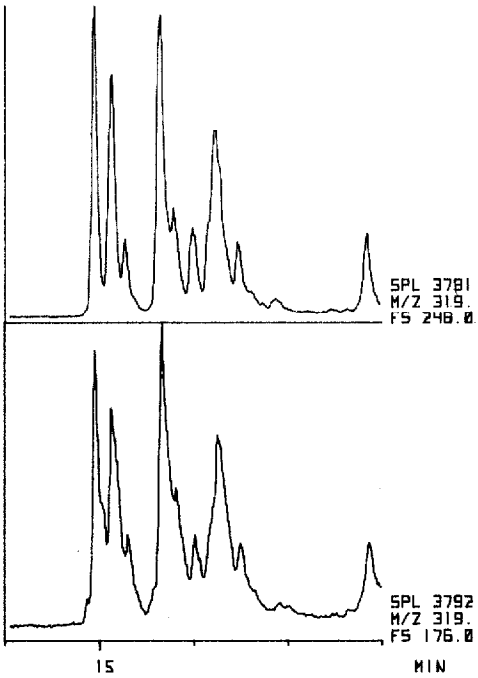

Fig. 9. SIM data for monitoring TCDD in non-fractionated extract (lower trace) and in HPLC fraction 2 (upper trace) of Ontario flyash sample. FS = full-scale value.

CONCLUSIONS

The one-step HPLC separation for the analysis of flyash from municipal incinerators is effective. Several hundred organic compounds are separated into five fractions. The complexity of each category and interferences among the compounds are greatly reduced. More compounds can be identified. Only five fractions and exclusive elution of PCDDs in one fraction make a set of survey analyses and quantification of PCDD in samples much easier. This separation procedure is simple and time saving. Further fractionation of each fraction by reversed-phase HPLC can be carried out to give even further separation. Preliminary experiments indicate this to be a very effective step.

ACKNOWLEDGEMENT

Financial support from the Natural Sciences and Engineering Research Council of Canada, is gratefully acknowledged.

REFERENCES

1 F. W. Karasek and F. I. Onuska, *Anal. Chem.*, 54 (1982) 309A.
2 G. A. Eiceman, R. E. Clement and F. W. Karasek, *Anal. Chem.*, 51 (1979) 2344.
3 K. Olie, P. L. Vermeulen and O. Hutzinger, *Chemosphere*, 6 (1977) 455.
4 H. R. Buser, A. Bosshardt, C. Rappe and R. Lindehl, *Chemosphere*, 7 (1978) 417.

5 A. D. Thruston, Jr., *J. Chromatogr. Sci.*, 16 (1978) 254.
6 W. C. Eisenberg, *J. Chromatogr. Sci.*, 16 (1978) 145.
7 D. Schuetzle, F. S. C. Lee and T. J. Prater, *Int. J. Environ. Anal. Chem.*, 9 (1981) 93.
8 L. L. Lamparski and T. J. Nestrick, *Anal. Chem.*, 52 (1980) 2045.
9 F. W. Karasek, J. A. Sweetman, R. E. Clement, F. P. Vance and H. Y. Tong, *Chemical Characterization of Organic Compounds Associated with Diesel Engine Particulate Emissions, Contract Report to Ontario Ministry of Environment April 1982.*
10 H. van den Dool and P. Dec. Kratz, *J. Chromatogr.*, 11 (1963) 463.
11 M. L. Lee and D. L. Vassilaros, *Anal. Chem.*, 51 (1979) 768.
12 D. L. Vassilaros, R. C. Kong, D. W. Later and M. L. Lee, *J. Chromatogr.*, 252 (1982) 1.
13 A. C. Viau and F. W. Karasek, *J. Chromatogr.*, 270 (1983) 235.
14 R. M. M. Kooke, J. W. A. Lustenhouwer, K. Olie and O. Hutzinger, *Anal. Chem.*, 53 (1981) 461.
15 P. O'Keefe, C. Meyer and K. Dillon, *Anal. Chem.*, 54 (1982) 2623.