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SEMI-PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF COMPLEX ORGANIC MIXTURES

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SUMMARY

Better understanding of the trace organic pollutants in the environment challenge us to carry on the extensive multi-component identification and quantification in an extremely complex environmental mixture. The analysis of such complex mixtures requires an effective separation method which has a high efficiency, speed, reproducibility, and recovery. This requirement is beyond the ability of any single conventional separation method, and has led to the use of high-performance liquid chromatography separation techniques in the analysis of organic mixtures.

The effectiveness of a semi-preparative high-performance liquid chromatographic separation together with gas chromatography–mass spectrometry for the analysis of organic compounds and for target analysis of dioxins in organic extracts of fly ash particulate samples from municipal incenerators has been demonstrated. The extract is a typically complex environmental mixture containing as many as 600 organic components.

INTRODUCTION

In environmental samples, traces of organic compounds are commonly present in extremely complex matrices and many of these are toxic[1,2]. Hundreds of components are known to be present in the extracts of particulates from diesel exhaust and fly ash from municipal incinerators[3,4]. Utilizing detection techniques with high sensitivity, direct analysis has been limited to a few compounds in such complex samples. More often the interference among components in the mixtures hinders direct multicomponent analysis. Therefore, a preseparation is necessary for the effective analysis of such mixtures.

A number of separation procedures have been reported for subsequent class or individual compound analysis[5–8]. For complex samples and where there is a requirement for extensive identification, the use of high-performance liquid chromatography (HPLC) has definite advantages. HPLC is gaining popularity in the fractionation and clean-up of complex environmental samples[3,9,10].

The organic extract of fly ash from municipal incinerators is extremely complex. Two classes of toxic compounds found are polychlorinated dibenzodioxins

(PCDDs) and polychlorinated dibenzofurans (PCDFs). Based on our previous work[11,12], a complete procedure is given here to show the effectiveness of HPLC separation for both multicomponent identification and target compound analysis in an extremely complex mixture. Data for extracts of fly ash samples from Canada and Japan are presented.

EXPERIMENTAL

Chemical reagents

Most polycyclic aromatic compound (PAC) standards and all polychlorinated benzene and phthalate ester standards used for compound identification and retention index calculation were of 95–99% purity and obtained from either Aldrich Chem. Co. (Montreal, P.Q., Canada) or Chem. Service Inc. (West Chester, PA, U.S.A.). 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-heptachlorodibenzo-*p*-dioxin (1234678-H_7CDD), octachlorodibenzo-*p*-dioxin (OCDD) and octachlorodibenzofuran (OCDF) were obtained from Ultra Scientific Inc. (Hope, RI, U.S.A.). The standards of 1,2,3,4,7-pentachlorodibenzo-*p*-dioxin (12347-P_5CDD) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) were obtained from Cambridge Isotope Laboratories Inc. (Woburn, MA, U.S.A.) and Foxboro/Analabs (North Haven, CT, U.S.A.), respectively.

The solvents used in this study were "distilled in glass", UV grade from Caledon Laboratories (Georgetown, Ontario, Canada).

Fly ash extracts

The Ontario fly ash sample was collected from the electrostatic precipitator of a municipal incinerator in Toronto, Canada. The 435-g sample was Soxhlet-extracted with benzene for 48 h and finally concentrated to 1.8 ml for normal-phase HPLC separation.

A total of 135 g of a Japanese fly ash sample taken from two municipal incinerators in the city of Kyoto was also Soxhlet-extracted with benzene and reduced to 0.2 ml for the HPLC separation.

The extraction and following concentration procedure have been described previously[11].

Normal-phase semi-preparative HPLC separation

The normal-phase HPLC separation was achieved on a Spectra-Physics SP8000 equipped with a SP8400 UV/vis variable wavelength detector and SP4100 integrator. A 10-μm Spherisorb silica column (250 × 9.4 mm; Terochem, Toronto, Canada) was used with a 140-μl sample loop. A 91-min gradient elution program consisting of *n*-hexane, dichloromethane and acetonitrile was employed to separate the raw extract into five fractions. The flow-rate was 5 ml/min. The details of this HPLC separation were described previously[11]. Five fractions were collected and concentrated, and then subjected to extensive detailed analysis by gas chromatography–mass spectrometry (GC–MS) and high-resolution gas chromatography (HRGC).

Reversed-phase semi-preparative HPLC separation

The instrument used for the reversed-phase HPLC separation was a Varian 5000 equipped with a Vista 402 data system, built-in UV detector and Fluorichrom detector. A MicroPak MCH-10 (C_{18}) column (300 × 8 mm; Varian Associates Inc., Walnut Creek, CA, U.S.A.) was used with a 100-μl sample loop. A gradient elution program using acetonitrile and dichloromethane was employed with a flow-rate of 2 ml/min.

Fraction 2, collected from the normal-phase HPLC separation and containing exclusively PCDDs and PCDFs, was subjected to the reversed-phase separation. Six subfractions (SFs) were separately collected during 27 min and concentrated for the target analysis of PCDDs and PCDFs. The detailed description of this reversed-phase HPLC separation has been reported previously[12].

Qualitative analysis of HPLC fractions

Five fractions obtained from the normal-phase HPLC separation were subjected to analysis by GC–MS and HRGC for compound identification. The GC–MS analyses were performed mainly on a Hewlett-Packard 5987 GC–MS–data system equipped with a 30 m × 0.32 mm I.D. DB-5 FSCC column (J & W Scientific, Rancho Cordova, CA, U.S.A.). The electron-impact ionization mode was operated at 70 eV. This system allows a comprehensive library search of unknown spectra against 70 000 stored reference spectra.

Retention indices based on polycyclic aromatic hydrocarbon reference compounds have also been used to facilitate compound identification[13]. The retention indices of unknown compounds in samples were obtained on a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector and the same FSCC column used for GC–MS analysis.

Quantitative analysis of PCDDs and PCDFs in HPLC subfractions

The subfractions 2–6 were subjected to quantitative analysis for PCDDs and PCDFs by HRGC and GC–MS with selected ion monitoring (SIM). These analyses were performed on the HP5880 GC and HP5987 GC–MS instruments, respectively. PCDDs and PCDFs in each subfraction were first confirmed by the mass chromatograms of their M^+, $[M+2]^+$ and $[M+4]^+$ ions, and then quantified on the basis of the peak area of the $[M+2]^+$ ion mass chromatogram relative to that for the standards injected[12].

The identification of PCDDs and PCDFs in gas chromatograms of subfractions was achieved by comparison of those flame ionization detector traces to their corresponding total ion-current traces obtained from GC–MS analysis. PCDD and PCDF standards were injected in order to obtain response factors for quantitative analysis. The analysis of PCDDs and PCDFs in this study was not completely isomer-specific due to the unavailability of standards for all the individual isomers.

RESULTS AND DISCUSSION

A scheme of the complete procedure for the analysis of organic compounds in a fly ash sample is given in Fig. 1. This procedure can be divided into two major parts: first, normal-phase HPLC separation for an extensive identification of com-
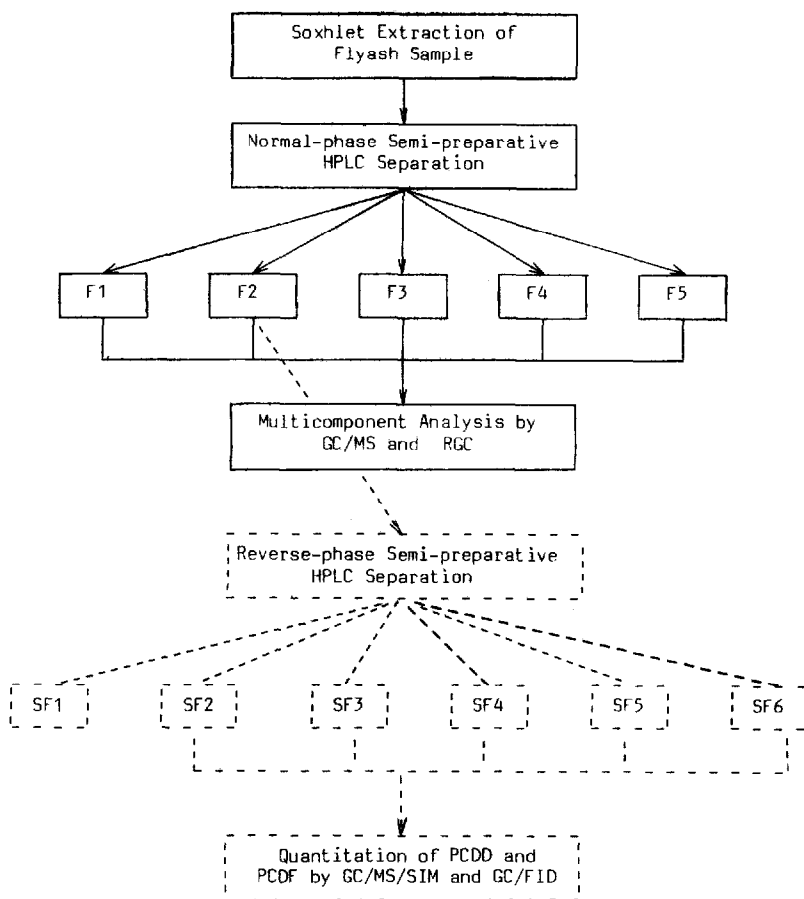
Fig. 1. Scheme of the complete procedure for the multicomponent analysis (solid line), and PCDD–PCDF target analysis of a fly ash sample.

pounds, and secondly, reversed-phase HPLC separation for a target analysis of PCDDs and PCDFs in a fly ash sample.

Fig. 2 illustrates the normal-phase HPLC separation of the Kyoto fly ash sample. This separation sorted organic compounds into non-polar, medium polar and polar categories. Five fractions (F1 to 5) were collected. Numerous components in the raw extract were grouped into different organic compound classes. The interferences among the co-eluting components had been greatly reduced in each fraction. More components could be seen and a better identification could be achieved after the components in the raw extract had been divided into several fractions.

The gas chromatogram of fraction 2 of the Kyoto sample is shown in Fig. 3. Some typical compounds identified in this fraction as well as the identification method used are listed in Table I.
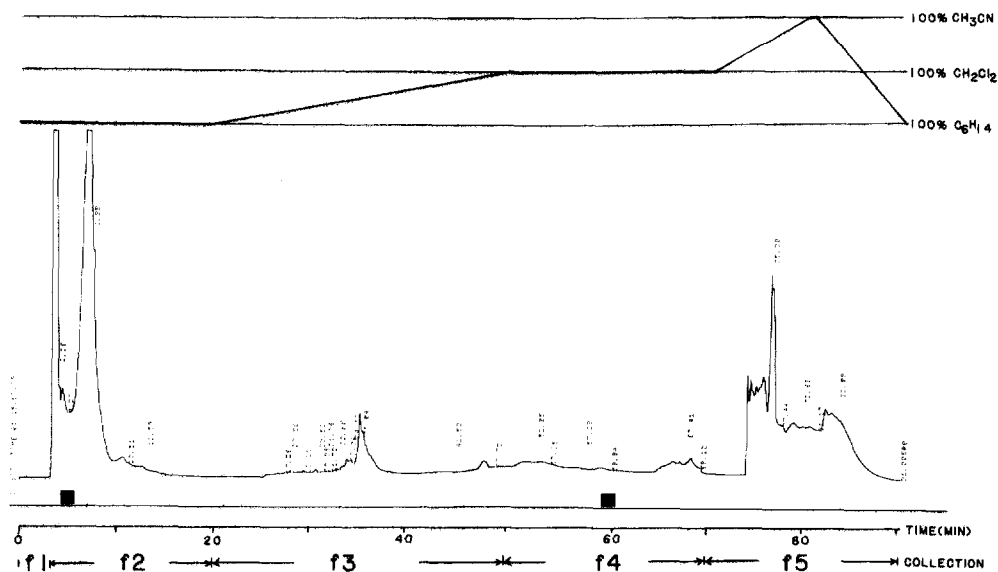
Fig. 2. Normal-phase HPLC separation of the Kyoto fly ash extract: gradient elution program (upper trace); UV chromatogram (middle trace); and fraction collection interval. For the HPLC conditions, see Experimental section.

A more complete identification of a number of organic compounds was made for five fractions of Ontario fly ash and some of the typical compounds are illustrated in Table II. More than 600 organic components were observed in the GC traces of five fractions of Ontario fly ash, more than 200 of which have been identified. Such
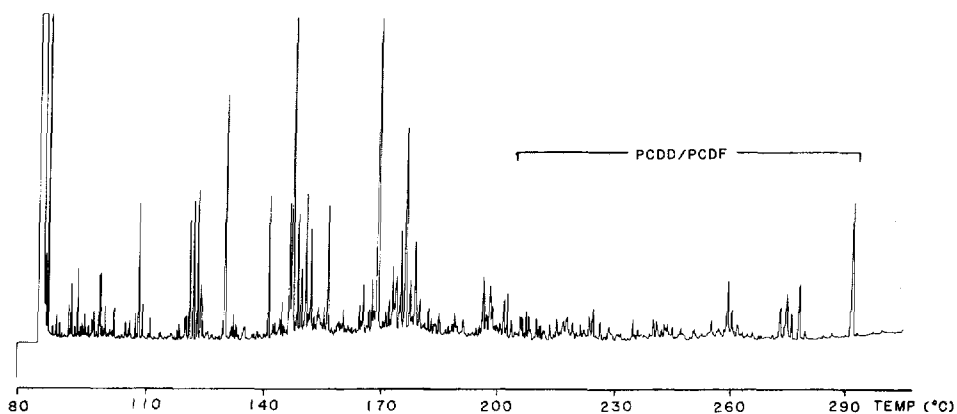

Fig. 3. Gas chromatogram of HPLC fraction 2 of the Kyoto fly ash extract. Chromatographic conditions: column 30 m × 0.32 mm I.D. DB-5 FSCC; temperature, 80°C for 1 min, programmed to 300°C at 3°C/min; flame ionization detection. The elution region for PCDDs and PCDFs (which have more than four chlorine atoms) is shown.

a comprehensive identification of organic compounds will provide a great deal of information for the better understanding of the impact of fly ash pollution on the human environment.

In the normal-phase HPLC separation, PCDDs and PCDFs are exclusively eluted in fraction 2 together with more than 100 other components. PCDDs and PCDFs are two series of compounds formed by chlorine atom substitution on the dibenzo-*p*-dioxin and dibenzofuran parent molecules. Owing to the different numbers and patterns of substitution, there are 75 PCDD and 135 PCDF isomers. When they are present together, these isomers are eluted in a narrow temperature range on the gas chromatogram. Such serious peak overlapping makes component identification very difficult and quantification by GC data impossible. In many reported clean-up procedures, even after separation, the analysis of PCDDs and PCDFs is usually achieved with a highly selective system such as GC–MS–SIM.

To facilitate an isomer-specific analysis or an analysis using non-selective detectors, a further isolation of PCDDs and PCDFs in fraction 2 of Ontario fly ash

TABLE I

COMPOUNDS IDENTIFIED IN HPLC FRACTION 2 OF THE KYOTO FLY ASH EXTRACT

Compound	MW	Retention index	Identification method[*]
Trimethylbenzene	120	177.24	a
Dichlorobenzene	146	179.21	a
Naphthalene	128	200.00	a,b,c,d
Trichlorobenzene	180	204.34	a,d
Dimethylnaphthalene	142	217.46	a,b
Tetrachlorobenzene	214	222.05	a,b,c,d
Biphenyl	154	231.38	a,b,c,d
Ethylnaphthalene	156	234.26	a,b,c
Methylbiphenyl	168	250.27	a,b,c
Pentachlorobenzene	248	256.22	a,b,c,d
Hexachlorobenzene	282	289.55	a,b,c,d
Tetrachlorophenol	230	296.69	a,b,d
Phenanthrene	178	300.00	a,b,c,d
Tetrachloroacenaphthalene	288	307.23	a,b,c
Methylanthracene	192	323.28	a,b,c,d
Phenylnaphthalene	204	331.30	a,b,d
Tetrachlorodibenzodioxin	320	375.46	a,b,d
Chrysene	228	400.01	a,b,c,d
Pentachlorodibenzofuran	338	405.74	a,b,d
Pentachlorodibenzodioxin	354	408.84	a,b,d
Hexachlorodibenzodioxin	388	427.34	a,b,d
Heptachlorodibenzofuran	406	461.57	a,b,d
Heptachlorodibenzodioxin	422	465.07	a,b,d
Octachlorodibenzodioxin	456	493.40	a,b,c,d

[*] a, Identified by sample mass spectra; b, identified by retention indices; c, identified by standard compounds injected; d, can be found in ref. 11.

TABLE II

COMPOUNDS IDENTIFIED IN FRACTIONS 1–5 OF ONTARIO FLY ASH

Compound	MW	Identification method*
Fraction 1		
C_{12}–C_{39} n-alkane	170–548	
C_{14}–C_{25} n-alkene	196–350	
Fraction 2		
Tetramethylbenzene	134	a
Trichlorobenzene	180	a
Naphthalene	128	a,b,c
Methylnaphthalene	142	a
Tetrachlorobenzene	214	a
Biphenyl	154	a
Ethylnaphthalene	156	a
Dimethylnaphthalene	156	a
Acenaphthylene	152	a
Methylbiphenyl	168	a
Dibenzofuran	168	a
Pentachlorobenzene	248	a
Trimethylnaphthalene	170	a,b
Nonylbenzene	204	a
Trichlorophenol	196	a
Bromotetrachlorobenzene	292	a
Decylbenzene	218	a
Hexachlorobenzene	282	a
Methylfluorene	180	b
Bromodichloromethylphenol	254	a
Dibenzothiophene	184	a,b,c
Tetrachlorophenol	230	a
Phenanthrene	178	a
Tetrachloroacenaphthylene	288	a
Dihydropyrene or dihydrofluoranthene	204	a
Dichlorodibenzofuran	236	a
Methylphenanthrene	192	a
Tetrachlorobenzene dicarbonitrile	264	a
Methylanthracene	192	a
Dichlorodibenzodioxin	252	a
Phenylnaphthalene	204	a
Chlorophenylethynylbenzene	212	a
Ethylphenanthrene	206	a
Trichlorodibenzofuran	270	a
Fluoranthene	202	a
Pentachloronaphthalene	298	a
Trichlorodibenzodioxin	286	a
Pyrene	202	a
Tetrachlorobiphenyl	290	a
Tetrachlorodibenzofuran	304	a
Tetrachlorodibenzodioxin	320	a
Hexachloronaphthalene	332	a
Benzo[*ghi*]fluoranthene	226	a
Pentachlorobenzofuran	338	a
Pentachlorodibenzodioxin	354	a
Hexachlorodibenzofuran	372	a

TABLE II (*continued*)

Compound	MW	Identification method[*]
Hexachlorodibenzodioxin	388	a,c
Nonachlorobiphenyl	460	a
Decachlorobiphenyl	494	a
Heptachlorodibenzofuran	406	a
Heptachlorodibenzodioxin	422	a,c
Octachlorodibenzodioxin	456	a,c
Fraction 3		
Dimethylbenzofuran	146	a
Benzoic acid	122	a
Naphthaldehyde	156	a
1,2-Diphenylethane	182	a
Biphenylamine	169	a
Diphenylmethanone	182	a
Diphenylpropane	196	a
9-Fluorenone	180	a,b,c
Bis(methylphenyl)diazene	210	a
Phenyl benzoate	198	a
1,3-Diphenyl-2-propen-1-one	208	a
Terphenyl	230	a,b
Chloro-9-fluorenone	214	a
4H-Cyclopenta[*d,e*]phenanthren-4-one	204	a
Dichloro-9-fluorenone	248	a
1-Chloro-9,10-anthraquinone	242	a
Trichloro-9-fluorenone isomer	282	a
7H-Benz[*d,e*]anthracen-7-one (or 11-benzo[*a*]fluorenone)	230	a
Methylphenylindole	207	a
Triphenylene	228	a,b
Chrysene	228	a,b,c
Tetrachloro-9-fluorenone	316	a
Diisooctyl phthalate	390	a
Methylphenyl-1H-indole	207	a
Benzo[*c,d*]pyrenone	254	a
Pentachloro-9-fluorenone isomer	350	a
Quaterphenyl	306	a,b
Benzo[*e*]pyrene	252	a,b,c
1,7-Diphenylnaphthalene	280	a
Benzo[*a*]pyrene	252	a,b,c
Fraction 4		
1-(Methylphenyl)ethanone	134	a
Biphenylamine isomer	169	a
Xanthen-9-one	196	a,c
Dibutyl phthaalate	278	a
9,10-Anthraquinone	208	a,c
Diisooctyl phthalate	390	a
Fraction 5		
Diethyl phthalate	222	a
Caffeine	194	a
Dibutyl phthalate	278	a
Benzo[*c*]cinnoline	134	a

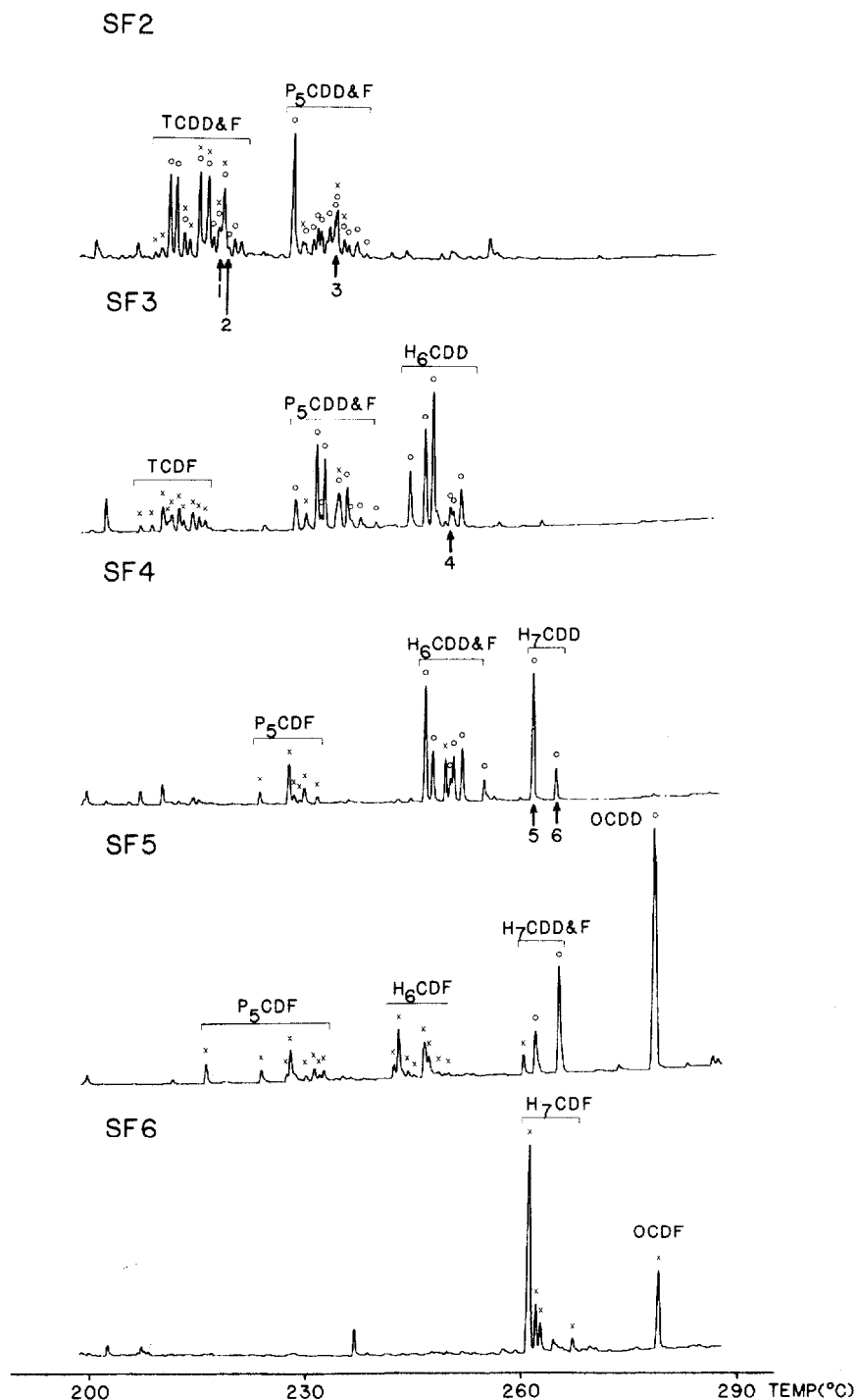[*] For a,b,c see Table I.

SF2

SF3

SF4

SF5

SF6

Fig. 4. GC traces (flame ionization detection) showing the distribution of PCDDs and PCDFs among subfractions 2–6 from the reversed-phase HPLC separation. Conditions as in Fig. 3. ○, PCDD; ×, PCDF. GC retention times: 1 = 1234-TCDD; 2 = 2378-TCDD isomer; 3 = 12347-P$_5$CDD; 4 = 123478-H$_6$CDD; 5 = 1234578-H$_7$CDD isomer; 6 = 1234678-H$_7$CDD isomer.

was carried out by reversed-phase HPLC. Six subfractions (SF1 to 6) were obtained. Fig. 4 shows the distribution pattern of PCDDs and PCDFs among SF2 to SF6. In this diagram, PCDDs and PCDFs are labelled differently. The method used for identification has been described in the Experimental section and also discussed elsewhere[12]. Most of the other components in fraction 2 were found in SF1.

Many PCDDs were separated from their corresponding PCDFs, and some positional isomers of PCDDs (or PCDFs) with the same number of chlorine atoms were distributed into different subfractions. This further spread of PCDDs and PCDFs into different subfractions and good isolation of PCDDs and PCDFs from almost all other organic components greatly facilitates their isomer-specific identification and quantification. This separation is also an important improvement in the analysis of PCDDs and PCDFs in a complex mixture using a non-selective detector. The quantitation of PCDDs and PCDFs in Ontario fly ash using GC–MS–SIM and GC analysis with flame ionization detection has been given elsewhere[12]. After this two-step HPLC separation, the results obtained by GC–MS–SIM and GC are reasonably consistent[12].

Recoveries of higher than 90% for some typical PAC, PCDD and PCDF standards have been reported for these normal- and reversed-phase HPLC separations[3,11,12]. The HPLC separations described also provide a good reproducibility.

CONCLUSIONS

The data obtained provide evidence for the effectiveness of semi-preparative HPLC when used as a separation technique for the analysis of organics in an extremely complex mixture. Using different combinations of mobile phases and columns, a large number of components can be separated into different classes or groups as needed. In this way, the complexity of each HPLC fraction or subfraction and the interferences among components are dramatically reduced. Consequently, more components can be identified and a better quantitation can be achieved by the subsequent analysis. High separation efficiency and recovery, and good reproducibility, are much more easily obtained in HPLC separations.

ACKNOWLEDGEMENT

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