Clean-up by high-performance liquid chromatography of polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans on a pyrenylethylsilica gel column

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ABSTRACT

A clean-up step prior to the determination of polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) based on normal-phase HPLC on a 2-(1-pyrenyl)ethyldimethyl silylated silica column is presented. With hexane as mobile phase, polychlorobiphenyls (including highly chlorinated non-*ortho* isomers) and organochlorine pesticides are eluted in the first fraction. The total fraction of PCDDs and PCDFs can be eluted by backflushing. By application of a gradient, all congeners are eluted within 75 min. The retention order of the PCDDs and PCDFs is predominantly governed by the degree of chlorination, but the substitution pattern also has a strong influence. Subfractions of the PCDD/PCDF fraction can be taken. The fraction containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was isolated. The three tetrachlorodibenzo-*p*-dioxin isomers of this fraction were baseline separated on a non-polar capillary GC column.

INTRODUCTION

Clean-up by HPLC prior to the determination of polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) has been already successfully utilized by several groups [1–17] and proved to be a valuable tool for isolating the PCDDs and PCDFs from very complex natural matrices. Most of the workers employed reversed-phase HPLC with alkylsilylsilica phases either for the improvement of isomer specifity [2,6,9] or for the removal of interfering matrix components [3,5,11,14] after matrix decomposition by chemical digestion with concentrated

sulphuric acid or a silica column loaded with sulphuric acid.

Some groups utilized a two-step LC fractionation employing normal- and reversed-phase HPLC with silica and alkylsilica columns, respectively [4,7,14,15]. Swerev and Ballschmiter [10] reported the fractionation of PCDDs on cyanopropyl-, diphenyl- and phenylsilica gel. For clean-up HPLC with microparticulate alumina [1,16], aminopropylsilica [17] and nitro-bonded [8] stationary phases has also been reported.

Creaser and Al-Haddad [12] described the separation of polychlorobiphenyls (PCBs) and organochlorine pesticides from the PCDD/ PCDF fraction on a porous graphitic carbon HPLC column. Owing to strong peak tailing and a high affinity of PCDDs and PCDFs for this

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phase, the method is solvent consuming and backflushing, which does not permit heart-cut techniques, is necessary.

A strong driving force behind the development of sophisticated clean-up steps by HPLC with various stationary phases was also the possibility of avoiding a final determination step for PCDDs and PCDFs by high-resolution GC–MS [7,13]. Expensive instrumentation impedes the general investigation of human food for PCDD/PCDF content. With the less selective but more sensitive electron-capture detection (ECD), the determination limit for PCDDs and PCDFs can be lowered with respect to the determination limit of a quadrupole MS detector.

In a previous paper [18], we reported the separation of thirteen tetrachlorodibenzo-*p*-dioxin (TCDD) isomers on a 2-(1-pyrenyl)ethyl-dimethylsilylated silica gel (PE-SG) column. PE-SG was designed as an electron-donor (ED) phase for donor–acceptor complex (DAC) LC. We were able to show that the formation of DACs can be assumed to be the predominant retention mechanism of PCDDs on PE-SG if the chromatographic separation is performed in the normal-phase mode. In a previous study by Barnhart *et al.* [19], the selectivity of PE-SG for TCDDs was only studied in the reversed-phase mode with methanol as mobile phase.

In this paper, we report PE-SG as a stationary phase in normal-phase HPLC as a clean-up step in PCDD/PCDF analysis. We studied not only the separation of PCDDs and PCDFs from interferring PCBs and organochlorine pesticides, but also the evaluation of PE-SG for the isolation of 2378-TCDD from its isomers and from TCDFs by fractionation of the sample extract.

EXPERIMENTAL

Chemicals

All solvents were of HPLC grade (Rathburn, Walkerburn, UK). Eleven TCDD isomers were purchased from Cambridge Isotope Labs. (Woburn, MA, USA) as solutions in nonane (50 ± 5 μg/ml); 1,2,3,6-, 1,2,3,9-, 1,2,6,7-, 1,2,8,9-, 1,3,7,8-, 1,4,7,8- and 2,3,7,8-TCDD were received as individual isomers and 1,2,3,7/1,2,3,8- and 1,3,6,8/1,3,7,9-TCDD as mixtures

of two isomers. Peak assignment of the mixtures was described in previous papers [18,20]. The isomers 1,2,3,4- and 1,2,7,8-TCDD were received as crystals from Cambridge Isotope Labs. The pentachlorobiphenyls and organochlorine pesticides were provided by Promochem (Wesel, Germany).

A synthetic mixture of all PCDD congeners from mono- to octachlorodibenzo-*p*-dioxin (total concentration 10 μg/ml) and a purified fly-ash extract were provided by Dr. F.W. Karasek (Department of Chemistry, University of Waterloo, Ontario, Canada). A synthetic standard mixture of PCBs from mono- to decachlorobiphenyl was available as standard reference material (SRM 2262; NIST, Gaithersburg, MD, USA).

Apparatus

Fractionation was performed on an HP 1050 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a quaternary gradient pump and autoinjector in conjunction with an LDC Spectromonitor III UV detector. The PE-SG column (Cosmosil Pye, $d_p = 5$ μm, 250 mm × 4.6 mm I.D.) was supplied by Nacalai Tesque (Kyoto, Japan).

Fractions of the synthetic PCDD mixture were investigated using an HP 5890A gas chromatograph with ECD. Separations were performed on an apolar capillary column (50 m × 0.2 mm I.D. × 5.5 μm, PONA, Hewlett-Packard). Fractions of the purified fly-ash extract were further investigated using an HP 5890A gas chromatograph coupled with an HP 5971 mass-selective detector. The capillary column employed [CP-SIL 8 CB (0.2 μm) for pesticides, 50 m × 0.25 mm I.D.] was manufactured by Chrompack (Middelburg, Netherlands).

Separation studies

All retention data were obtained under isocratic and isothermal (25°C) conditions. The flow-rate was 1 ml/min in all instances. The UV detector was operated at 235 nm. Retention times were measured with solutions of the standards in 2-propanol or hexane, respectively. The solutions in nonane were diluted with appropriate solvents. In the case of mixtures, the

nonane was evaporated before dissolving the standard in 2-propanol in order to avoid peak broadening or distortion due to solvent effects of the sample solution.

The PCB-containing standard mixture (SRM 2262, NIST, Gaitherburg, MD, USA) and fractions were investigated using an HP 5890A gas chromatograph with ECD under the following conditions: injection mode, splitless; temperature programme, initially 50°C (0.3 min), increased at 1°C/min to 290°C; column, DB-5 (0.1 μm), 60 m × 0.20 mm I.D. (J & W, Folsom, CA, USA).

Fractionations

The PCDD mixture was fractionated either under isocratic conditions with hexane as mobile phase or by employing the following gradient: 0-24 min, 100% hexane, 0.8 ml/min; 24-60 min, linear gradient from 100% hexane to dichloromethane-hexane (90:10, v/v), linear gradient of flow-rate from 0.8 to 1.5 ml/min. The temperature of the column was not controlled. The UV detector was operated at 235 nm. The volume injected was 20-30 μl.

The fractions were collected in 10-ml glass vials, 20 μl of nonane were added and the solvent was concentrated in a gentle stream of nitrogen to the volume of the added nonane.

Gas chromatographic conditions and acquisition parameters

The GC–ECD operating parameters for the analysis of the concentrated fractions were the following: injection mode, splitless; injection port temperature, 300°C; injection volume, 1 μl; column, PONA (5.5 μm), 50 m × 0.20 mm I.D.; column head pressure, 280 kPa of helium; oven programme, initial temperature, 130°C; initial time, 0.5 min; ramp rate, 30°C/min to 230°C then 1°C/min to the final temperature, 290°C; detector temperature, 300°C; make-up gas (nitrogen) flow-rate, 60 ml/min.

The GC–MS operating parameters for the analysis of the concentrated fractions were as follows: injection port, on-column; inlet programme: initial temperature, 50°C; ramp rate, 20°C/min; final temperature, 300°C; injection volume, 1 μl; column, CP-SIL 8 CB (0.2 μm),

TABLE I

IONS MONITORED IN MS DETECTION OF PCDD AND PCDF ISOMERS

Time (min)	m/z
20.0-28.5	303.9, 305.9, 319.9, 321.9
28.5-33.5	339.9, 341.9, 355.9, 357.9
33.5-41.0	373.8, 375.8, 389.8, 391.8
41.0-50.0	407.8, 409.8, 423.8, 425.8
50.0-65.0	441.7, 443.7, 457.7, 459.7

50 m × 0.25 mm I.D., column head pressure, 100 kPa of helium at 50°C, constant-flow mode (0.415 ml/min); oven programme, initial temperature, 50°C; ramp rate, 10°C/min to 230°C then 1°C/min to the final temperature, 280°C; transfer line temperature, 290°C; ionization mode, electron impact (70 eV); solvent delay, 20 min; detection mode, single-ion monitoring; dwell time, 300 ms. The ions monitored are listed in Table I.

RESULTS AND DISCUSSION

Isolation of TCDDs from PCBs and organochlorine pesticides

The capacity factors, k', listed in Table II and the chromatograms presented in Fig. 1 show that the pyrene column tested is able to separate TCDD isomers from those classes of compounds which might interfere in TCDD determination, *i.e.*, organochlorine pesticides and pentachlorobiphenyls. Isolation of the TCDD fraction can be done much faster in the normal-phase mode. The best results were obtained with the solvent mixture hexane-methanol (95:5, v/v). In this instance, all possible interferents eluted within 8 min. The isolated fraction of TCDDs can be collected between 12 and 18 min. The conditions with hexane as mobile phase are similar. The retention times are longer, but working with hexane facilitates the evaporation of the solvent prior to GC analysis.

In order to study whether all PCB congeners elute before the TCDDs, we injected a synthetic standard mixture of PCBs containing 28 congeners of all degrees of chlorination, *ortho-*, 1-*ortho-*

TABLE II

CAPACITY FACTORS OF VARIOUS TCDD ISOMERS, PENTACHLOROBIPHENYLS (P5CBs) AND OR-GANOCHLORINE PESTICIDES ON THE PE-SG COL-UMN

Data for TCDD isomers were taken from ref. 18.

Solute	k' (methanol)	k' (hexane)
1,2,3,4-TCDD	17.32	3.19
1,2,3,6-TCDD	18.34	4.18
1,2,3,7-TCDD	15.01	3.90
1,2,3,8-TCDD	14.41	3.63
1,2,3,9-TCDD	15.94	4.22
1,2,6,7-TCDD	12.34	4.04
1,2,7,8-TCDD	13.28	4.01
1,2,8,9-TCDD	10.51	3.78
1,3,6,8-TCDD	19.26	3.42
1,3,7,8-TCDD	17.04	4.02
1,3,7,9-TCDD	16.47	3.63
1,4,7,8-TCDD	19.26	4.78
2,3,7,8-TCDD	15.59	4.74
2,2',4,5,5'-P5CB	1.34	0.48
3,3',4,4',5-P5CB	2.45	0.81
3,3',4,5,5'-P4CB	4.42	1.26
p,p'-DDT	1.15	0.87
o,p'-DDE	0.66	0.37
p,p'-DDE	0.94	0.45
o,p'-DDD	0.68	1.03
p,p'-DDD	0.95	2.03

and non-*ortho* substituted congeners. Haglund *et al.* [21] used fractionation on PE-SG for the isolation of the most toxic planar PCB congeners from the non-planar isomers. They observed that the non-*ortho* isomers elute in the last position within a group of PCBs of the same degree of chlorination. With hexane as mobile phase all congeners, contained in an industrial product, were eluted within 10 min at a flow-rate of 0.70 ml/min. The dimensions of the PE-SG column were 150 mm × 4.6 mm I.D.

In accordance with the results of Haglund *et al.*, we observed that all peaks of the synthetic PCB mixture eluted within 12 min with hexane as mobile phase. The eluate was collected. Comparison of the gas chromatogram of the concentrated eluate with that of the synthetic mixture proved that also highly chlorinated congeners and non-*ortho* congeners were eluted from PE-SG in the first 12 ml under the chosen chromatographic conditions.
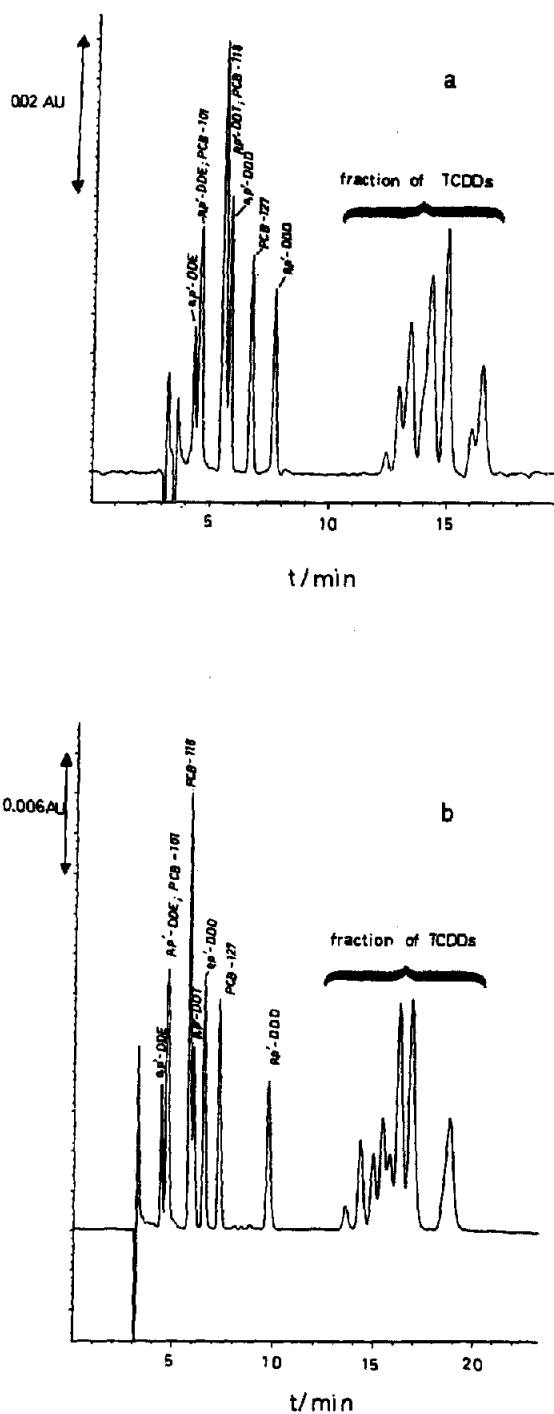
Fig. 1. Isolation of TCDD isomers from organochlorine pesticides and pentachlorobiphenyls. Column, PE-SG (250 mm × 4.6 mm I.D.); mobile phase, (a) hexane–methanol (95:5, v/v) and (b) hexane; temperature, 25°C; detection wavelength, 235 nm; flow-rate, 1 ml/min.

Fractionation of a synthetic PCDD mixture

Under isocratic conditions with hexane as mobile phase at a flow-rate of 0.8 ml/min only part of the injected congeners (mono- to penta-chlorodibenzo-*p*-dioxins) eluted within 80 min. The monitored UV absorption peaks were identified by GC–ECD from the collected fractions. With TCDDs, identification was also possible by comparison of the HPLC trace of the synthetic PCDD mixture with that of thirteen TCDD isomers run under the same conditions on the PE-SG column [18] and comparison of the gas chromatograms of the fractions with a published gas chromatogram [22] of all TCDD isomers obtained with the same type of GC column as used in our studies.

Fig. 2 shows the HPLC results for the synthetic PCDD mixture. Only mono- to penta-chlorodibenzo-*p*-dioxins were eluted within 80 min. The retention order is predominantly governed by the degree of chlorination. From the theory of DAC-LC [23], this retention order would be expected. The potential to form DACs as electron acceptors increases with increasing number of chlorine substituents. In Fig. 2 it can be seen that the substitution pattern of the PCDDs itself influences the retention to a great extent. A fraction of three TCDD isomers (t_R = 22–24 min), containing 2,3,7,8-, 1,2,6,9- and 1,4,7,8-TCDD, can easily be separated from higher chlorinated congeners and other TCDD isomers. In Fig. 3 a chromatogram of the isolated 2,3,7,8-TCDD-containing fraction is presented. On the apolar column employed, 2,3,7,8-TCDD is baseline separated from the other two isomers, which co-eluted on the PE-SG column.

In order to study the retention of the higher chlorinated congeners on PE-SG, a gradient from hexane to dichloromethane–hexane (90:10, v/v) was employed. With this gradient all congeners were eluted within 70 min. Also, the retention order of the higher chlorinated congeners is predominantly governed by the degree of chlorination. There is no overlapping of fractions containing isomers of the same degree of chlorination. The gradient causes an extreme drift of
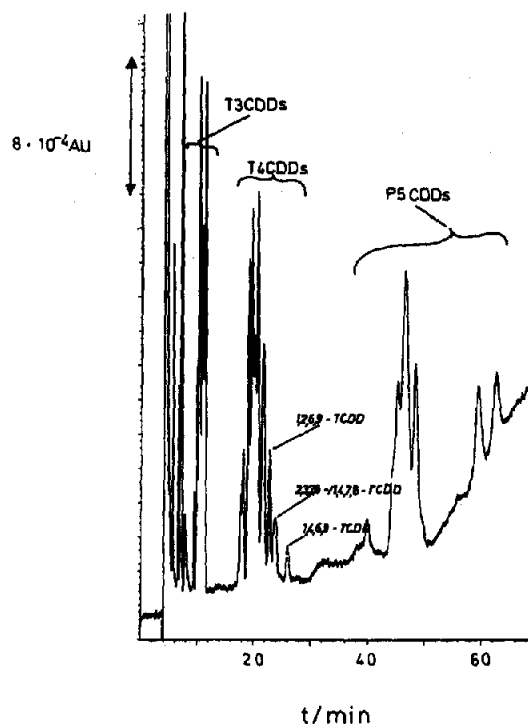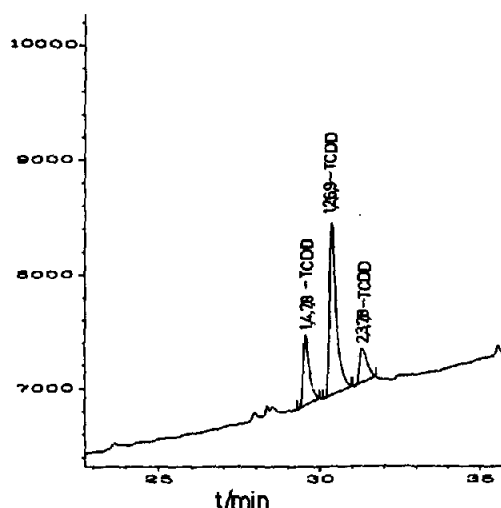
Fig. 2. Chromatogram of a synthetic PCDD mixture eluted from PE-SG. Column, 250 mm × 4.6 mm I.D.; mobile phase, hexane; temperature, ambient; detection wavelength, 235 nm; flow-rate, 0.8 ml/min. T3 = Tri; T4 = tetra; P5 = penta.

Fig. 3. Chromatogram of the isolated 2,3,7,8-TCDD-containing fraction analysed by GC–ECD. Injection mode, splitless; injection port temperature, 300°C; injection volume, 1 µl; column, PONA (5.5 µm), 50 m × 0.20 mm I.D.; column head pressure, 280 kPa of helium; initial oven temperature, 130°C; initial time, 0.5 min; ramp rate, 30°C/min to 230°C then 1°C/min to final temperature, 290°C.

the baseline at a detection wavelength of 235 nm.

Fractionation of a fly-ash extract

The same gradient as used for the fractionation of PCDDs was employed for the fractionation of a purified fly-ash extract containing PCDDs and PCDFs. A very high absorption, monitored during the elution of the first 10 ml of the mobile phase, indicates the elution of impurities. The elution of TCDDs could not be monitored directly by UV detection because of the presence of broad peaks, which are presumably partly due to impurities and partly to the large number of congeners that elute in this region.

Monitoring of the elution of higher chlorinated congeners was hampered by the baseline drift that resulted from the gradient of mobile phase, but was not impossible. Octachlorodibenzo-*p*-dioxin (OCDD) is separated from octachlorodibenzofuran (OCDF). OCDF elutes in the last position. Quantitative analysis of the octachlorinated and heptachlorinated congeners might be possible by HPLC on PE-SG with UV detection, as can be seen in Fig. 4. Fractions of the eluate were taken and analysed by GC–MS.

Generally, the retention order of the PCDFs follows the retention order of the PCDDs. It is mainly governed by the degree of chlorination. The regions where PCDDs and PCDFs of the same degree of chlorination elute overlap. Also, slight overlapping of the TCDF and pentachlorodibenzofuran (P5CDF) fractions occurred.

The fraction containing 2,3,7,8-TCDD was isolated. Analysis by GC–MS proved that a TCDF isomer co-elutes from both the PE-SG and the non-polar GC columns employed, so that the separation sequence presented does not permit the determination of 2,3,7,8-TCDD by GC–ECD.
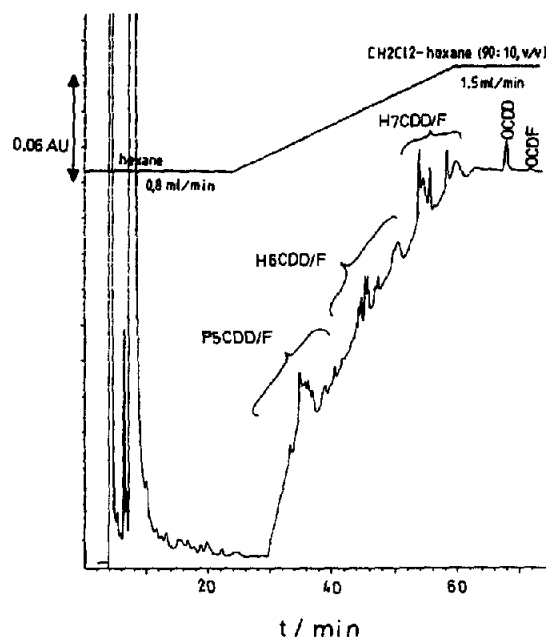
CONCLUSIONS

Normal-phase HPLC on PE-SG is a useful clean-up step in the analysis of PCDDs and PCDFs. It can be utilized as a rapid clean-up step separating possibly interferring PCBs and organochlorine pesticides from PCDDs and PCDFs. PCBs and organochlorine pesticides are eluted in the first 12 ml with hexane as mobile phase. The total fraction of PCDDs and PCDFs can be eluted by backflushing.

By application of a mobile phase with a stronger elution capacity, fractionation of the PCDDs and PCDFs is possible in the same chromatographic run in which clean-up is performed. This fractionation can be considered as a final "residue polishing" step. As could be demonstrated for 2,3,7,8-TCDD, the problem of peak overlap with isomers can be lowered to a large extent if small fractions are taken that contain only one of the seventeen congeners of the 2,3,7,8-TCDD class.

Fig. 4. Chromatogram of a fly-ash extract eluted from PE-SG. Column, 250 mm × 4.6 mm I.D.; gradient elution, 0–24 min, 100% hexane, 0.8 ml/min; 24–60 min, linear gradient of mobile phase composition from 100% hexane to dichloromethane–hexane (90:10, v/v); 24–60 min, linear gradient of flow-rate from 0.8 to 1.5 ml/min; temperature, ambient; detection wavelength, 235 nm. P5 = Penta; H6 = hexa; H7 = hepta.

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