ELSEVIER

Journal of Chromatography A, 786 (1997) 237–248

JOURNAL OF CHROMATOGRAPHY A

Selectivity of electron-donor- and electron-acceptor-bonded silica packing materials for hydrophobic environmental contaminants in polar and non-polar eluents

Kazuhiro Kimata[a], Ken Hosoya[b], Hiroaki Kuroki[c], Nobuo Tanaka[b,*], John R. Barr[d], P. Cheryl McClure[d], Donald G. Patterson, Jr.[d], Eva Jakobsson[e], Ake Bergman[e]

[a]*Nacalai-Tesque, 17 Ishibashi, Kaide-cho, Muko-shi, Kyoto 617, Japan*

[b]*Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan*

[c]*Daiichi College of Pharmaceutical Sciences, Fukuoka 815, Japan*

[d]*Centers for Disease Control, 4770 Buford Highway, NE, Atlanta, GA 30341-3724, USA*

[e]*Department of Environmental Chemistry, Stockholm University, S-106 91 Stockholm, Sweden*

Received 11 March 1997; received in revised form 22 May 1997; accepted 23 May 1997

Abstract

Electron-acceptor-bonded stationary phases, 2-(nitrophenyl)ethylsilyl (NPE) and 3-(*p*-nitrophenoxy)propylsilyl (NPO), and electron-donor-bonded phases, 3-(N-carbazolyl)propylsilyl (CZP), 2-(1-pyrenyl)ethylsilyl (PYE), and 5-coronenylpentylsilyl (COP), were prepared from silica particles and their selectivities were examined in both polar and non-polar solvents for specific isomers of polychlorodibenzo-*p*-dioxins (PCDDs), hexachloronaphthalenes (HxCNs) and planar and non-planar polychlorobiphenyl (PCB) congeners. Although no single stationary phase was able to separate all the isomer pairs that are coproduced during the synthesis of the PCDDs and HxCNs, pairs can be separated by selecting a suitable stationary phase and solvent. The separation of mixtures of PCDD isomers were found to be most successful with PYE and NPO phases, which yielded the opposite elution orders for each isomer pair that is produced as a mixture. Similar results were obtained for the HxCN isomers that were separated on PYE and CZP phases. The COP phase provided easier separation of non-*ortho*-substituted and mono-*ortho*-substituted PCBs from the other PCBs based on the planarity than PYE phase. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, LC; 2-(Nitrophenyl)ethylsilyl silica; 3-(*p*-Nitrophenoxy)propylsilyl silica; 3-(N-Carbazolyl)propylsilyl silica; 2-(1-Pyrenyl)ethylsilyl silica; 5-Coronenylpentylsilyl silica; Polychlorodibenzo-*p*-dioxins; Hexachloronaphthalenes; Polychlorobiphenyls; Tetrachlorodibenzofurans

1. Introduction

Chlorinated aromatic compounds of environmental concern provide unique separation problems because they include several groups of polychlorinated compounds which consist of a large number of congeners [e.g. 209 polychloro-biphenyls (PCBs), 75 polychlorodibenzo-*p*-dioxins (PCDDs), 135 polychlorodibenzofurans (PCDFs) or 75 polychloronaphthalenes (PCNs)]. Complete separation of all the congeners of any of these groups has not yet been

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00597-9

achieved even by the most efficient separation methods such as high resolution capillary gas chromatography (HRGC) [1,2] or electrokinetic chromatography. Although high-performance liquid chromatography (HPLC) is not directly applicable for the environmental analysis for these types of compounds in general due to the low efficiency, it should provide means for the preparation of reference standards (by achieving isomer separations) and for the treatment of biological and environmental samples for GC–MS analysis (by providing class separation among several groups of compounds).

In a previous report we showed the separation and peak identification of 22 PCDD isomer pairs coproduced during their synthesis. This was done by reversed-phase liquid chromatography (RPLC) using alcoholic mobile phases with an electron-donor bonded stationary phase, 2-(1-pyrenyl)ethylsilyl (PYE), and electron-acceptor bonded stationary phases, 2-(nitrophenyl)ethylsilyl (NPE) and 3-(*p*-nitrophenoxy)propylsilyl (NPO) silica [3]. In preparative HPLC, the use of a single non-polar solvent is often desirable since these solvents are more easily recycled than aqueous–organic solvent mixtures, which is important for environmental and economic purposes. Additionally, non-polar organic solvents show better solubilities for hydrophobic aromatic compounds. High structural selectivity for chlorinated aromatic compounds can be provided by stationary phases having electron-donor or acceptor groups in non-polar solvents [4–8]. Pyell et al. reported the use of several donor–acceptor bonded phases including PYE and nitroaromatic-bonded phases in methanol and in hexane for the separation of tetrachlorodibenzo-*p*-dioxin (TCDD) isomers [7], although the approach was not necessarily aimed at the separation of the isomers that are synthesized as mixtures and are difficult to separate. It has been reported that 1,2,3,4,6,7-/1,2,3,5,6,7-hexachloronaphthalenes (HxCNs) are produced as a mixture either by chlorination or by dechlorination reactions [9–11]. Six PCDD isomer pairs, 1,2,4,6-/1,2,4,9-tetrachlorodibenzo-*p*-dioxins (TCDDs), 1,2,4,7-/1,2,4,8-TCDDs, 1,2,4,6,7-/1,2,4,8,9-pentachlorodibenzo-*p*-dioxins (PnCDDs), 1,2,4,6,8-/1,2,4,7,9-PnCDDs, 1,2,3,6,7,9-/1,2,3,6,8,9-hexachlorodibenzo-*p*-dioxins (HxCDDs) and 1,2,4,6,7,9-/1,2,4,6,8,9-HxCDDs are also produced as mixtures during synthesis from chlorocatechols and chloronitrobenzenes [12,13]. These isomer pairs are particularly hard to separate [9–11,14] and present in the environment as mixtures. It would be of interest to examine how the separation of these isomer pairs are affected by changes in stationary phases and mobile phases, since variation of selectivity has been reported for the separation of TCDD isomers in non-polar solvents compared to reversed-phase mode [7].

Another area of intense interest that should be achieved by HPLC is a class separation among halogenated aromatic compounds which could be employed as pretreatment of environmental or biological samples. In such chromatographic sample preparation, the analytes could be extracted into a non-polar solvent which then can be conveniently concentrated and introduced into a GC–MS system. The PYE stationary phase shows a preference toward planar molecules [15] and has been employed for the class separation of PCBs from other halogenated compounds and additionally, between toxic, planar and less toxic, non-planar PCBs [8,16–18]. PYE provided higher efficiency in the class separation than carbon packing materials that are more commonly applied [6,8,16–21], even though the retention of PCBs on PYE was relatively small and the separations of planar non-*ortho*-substituted PCBs from the other *ortho*-substituted ones was not complete [6,16,18,19]. Some mono-*ortho*-substituted PCBs account for more than 50% of the total effect of halogenated aromatic hydrocarbons in terms of toxic equivalency [22–24]. Thus the development of a stationary phase which allows more complete separation based on the planarity of solutes is desirable [16–20,25].

In this study, two electron-acceptor-bonded, dipolar stationary phases, NPE and NPO, an electron-donor-bonded, dipolar phase, 3-(N-carbazolyl) propylsilyl (CZP) and two electron-donor-bonded, non-polar phases, PYE and 5-coronenylpentylsilyl (COP) silica particles were examined in polar and non-polar eluents; (i) for the separation of isomeric PCDDs and HxCNs coproduced during synthesis and (ii) for the separation of non-*ortho*-substituted and mono-*ortho*-substituted PCBs of high toxicity from the other more prevalent PCBs.

2. Experimental

2.1. Materials

2.1.1. Stationary phase

The alkyldimethylchlorosilane silylating agents employed in the synthesis of bonded phases were prepared from their corresponding alkenes by a reaction with dimethylchlorosilane in the presence of chloroplatinic acid catalyst. Surface modification of silica particles was carried out as previously described [26]. The NPE phase was prepared by the nitration of 2-phenylethylsilylated silica particles [27]. The structures of stationary phases are shown in Fig. 1. All columns are 15 cm×4.6 mm I.D., unless noted otherwise. A monomeric C_{18} phase (C_{18}(I), Cosmosil 5C_{18}-MS), a polymeric C_{18} phase (C_{18}(III), Cosmosil 5C_{18}-AR), PYE (Cosmosil 5PYE) and NPE (Cosmosil 5NPE) are commercially available (Nacalai Tesque, Kyoto, Japan).

2.1.2. Chemicals

PCDDs were prepared at Centers for Disease Control and Prevention as previously described [13]. The twelve pairs of isomers coproduced during their synthesis were employed as samples in this study. Thirty-three tetrachlorodibenzofuran (TCDF) isomers out of the possible thirty-eight structures were prepared at Daiichi College of Pharmacy [28]. The chromatographic properties of all the TCDFs were examined except 1,2,3,9-, 1,2,6,8-, 1,2,6,9-, 1,2,8,9- and 2,3,7,8-TCDF. A mixture of 1,2,3,4,6,7-/1,2,3,5,6,7-HxCNs was prepared at Stockholm University [10]. PCBs were purchased commercially (AccuStandard, New Haven, CT, USA). LC-grade water, methanol, acetonitrile, hexane and isooctane were used. Sample concentrations were 0.5 mg/ml (in toluene) in most cases, and less than 0.5 μl was injected.

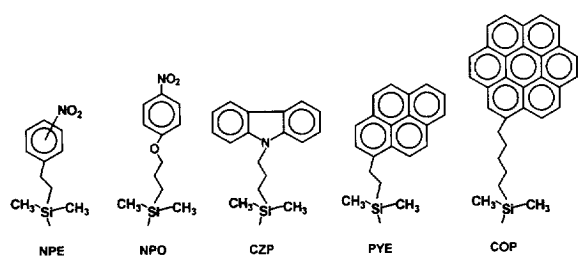

Fig. 1. Structures of bonded phases.

2.1.3. Instrumentation

Chromatographic separation was carried out at CDC. A Beckman 126 pump and 168 detector with NUVEAU software (Beckman, Fullerton, CA, USA) and a Shimadzu LC-9A (Shimadzu, Kyoto, Japan) system were employed for HPLC experiments with detection at 254 nm. The columns were kept at 30°C by using a water bath.

2.1.4. Safety

Special care should be taken when handling the highly toxic substances. They should be handled in a chemical hood in a well ventilated area with protective clothing. Solid materials and concentrated solutions should be handled in a specially constructed laboratory.

3. Results and discussion

3.1. Properties of stationary phases

Surface coverages and retention properties of the stationary phases are shown in Table 1. The hydrophobic selectivity $\alpha(CH_2)$ for each stationary phase is expressed as the separation factor between toluene and benzene in methanol–water (60:40, v/v) and was found to be in the following order, C_{18}(I)> COP>C_{18}(III)>PYE>NE>CZP~NPE~PE>NPO. The dipolar stationary phases were found to be less hydrophobic than the non-polar stationary phases. The selectivity of each stationary phase for solute planarity, α(T/O), was examined by using triphenylene (T) and *ortho*-terphenyl (O), which have similar hydrophobic properties, but a difference in planarity [15,29,30]. The selectivities of the stationary phases for solute planarity were also examined with planar benz-(*a*)-pyrene (BaP) and non-planar tetrabenzonaphthalene (TBN) [31] to show the selectivity α(BaP/TBN). The α(TBN/BaP) values are also listed in Table 1, because these values have been used to describe the selectivities of various C_{18} phases [31]. The COP phase possessing large planar

Table 1
Properties of electron-donor- and electron-acceptor-bonded silica packing materials

Stationary phase	C (%)	Surface coverage (μmol/m^2)	$\alpha(CH_2)$[a]	α(T/O)[b]	α(BaP/TBN)[c,d]	α(TBN/BaP)[c,d]
C_{18}(I)	18.3	3.2	1.96	1.92[d]	0.61	1.65
C_{18}(III)	16.4	2.9	1.88	2.65[d]	0.82	1.22
PE[e]	11.5	3.4	1.60	1.21[d]	0.51	1.97
NE[f]	14.1	3.1	1.71	1.81[d]	0.50	1.99
PYE	18.4	3.0	1.83	3.50[d]	0.54	1.85
COP	22.0	2.6	1.91	5.86[d]	0.77	1.30
CZP	13.2	2.0	1.62	2.96[g]	0.65	1.53
NPE	8.8	2.6	1.61	3.69[g]	0.63	1.59
NPO	10.5	2.9	1.57	4.34[g]	0.73	1.37

[a] k'(toluene)/k'(benzene) in 60% methanol.
[b] k'(triphenylene)/k'(*ortho*-terphenyl).
[c] BaP: benz[*a*]pyrene, TBN: tetrabenzonaphthalene.
[d] In methanol.
[e] 2-phenylethyldimethylsilyl bonded silica.
[f] 2-(1-naphthyl)ethyldimethylsilyl bonded silica.
[g] In 80% methanol.

aromatic moieties as well as polymeric C_{18}(III) showed clear preference toward planar solutes. NPE and NPO also showed high selectivity due to the favorable interaction between the electron-deficient phenyl groups and a large, planar polynuclear aromatic hydrocarbon. Although the polymeric C_{18} phase showed high selectivity based on the planarity of solutes in methanol, the selectivity cannot be utilized in non-polar solvents due to the lack of retention, as shown later for the separation of PCB congeners.

3.2. Selectivity for PCDD isomers

3.2.1. C_{18}(I) phase

Some PCDDs are produced as a mixture of two isomers due to Smiles rearrangement during their preparation from chlorocatechols and chloronitrobenzenes [12,13]. We divided these isomer pairs into two groups according to their chromatographic behavior. The PCDDs in Table 2 (PCDDs in group I) represent the PCDD isomer pairs which can be easily separated with common stationary phases for RPLC and GC, while the six pairs of PCDDs in Table 3 (PCDDs in group II) represent the isomer pairs that are particularly difficult to separate, even by HRGC. Previously the isomers in these pairs have only been separated by GC with a liquid crystal stationary phase [14] or by RPLC with PYE or NPO stationary phases [3]. The RPLC work has yielded tentative peak identification for the isomer pairs which is in general agreement with the identifications that were based on spectroscopy [13,32], with some contradictions in the case of 1,2,4,6-/1,2,4,9-TCDDs and 1,2,4,7-/1,2,4,8-TCDDs [33]. Tables 2 and 3 list the separation factors (α) for the PCDD isomer pairs in the three solvent systems that were based on methanol, acetonitrile and hexane.

The retention time for PCDDs in the reversed-phase mode was found to increase with the higher degree of chlorination. The PCDDs in group I were easily separated with the monomeric C_{18}(I) phase and polar mobile phases indicating a clear difference in hydrophobic properties between the isomers in each of these pairs (Table 2). It is interesting to note that in the separations of these PCDD isomer pairs, the minor isomer (smaller peak) was always eluted first [3]. Relative peak size was then used for tracing the elution order. During the preparation process, more symmetrically substituted isomers are usually produced to a greater extent than the less symmetrically substituted isomers because of greater thermodynamic stability with less steric congestions [34]. These more symmetric isomers therefore should

Table 2
Effect of solvent and stationary phase structure on the separation factors for PCDD isomer pairs (group I)

Isomer pair	$\alpha^a = k'_{(PCDD\text{-}I)}/k'_{(PCDD\text{-}II)}$					
(PCDD-I/PCDD-II) Mobile phase[b]	C_{18}(I)	PYE	COP	CZP	NPE	NPO
1,6-/1,9-DCDD						
Methanol	1.09	1.10	1.13	NS	0.80	0.76
Acetonitrile	1.07	1.08	1.28	NS	NS	NS(S)
Hexane	–	NS	NS	NS	0.67	0.61
1,2,6-/1,2,9-TrCDD						
Methanol	1.08	1.14	1.10	1.07	0.75	0.67
Acetonitrile	1.06	1.15	1.14	1.07	0.93	0.89
Hexane	–	NS	NS	NS	0.65	0.58
1,2,6,7-/1,2,8,9-TCDD						
Methanol	1.05	1.20	1.08	1.07	0.69	0.58
Acetonitrile	1.05	1.18	1.15	1.08	0.92	0.83
Hexane	–	1.06	NS	0.78	0.63	0.56
1,3,6,8-/1,3,7,9-TCDD						
Methanol	1.11	1.21	1.08	1.05	NS	0.96
Acetonitrile	1.05	1.28	1.13	NS	NS	NS(S)
Hexane	–	0.96	NS	0.90	0.76	0.76
1,2,3,6,7-/1,2,3,8,9-PnCDD						
Methanol	1.07	1.13	NS	1.07	0.83	0.74
Acetonitrile	1.06	1.16	1.07	1.08	0.95	0.88
Hexane	–	NS	NS	0.95	0.71	0.65
1,2,3,6,7,8-/1,2,3,7,8,9-HxCDD						
Methanol	1.08	1.09	NS	1.06	0.90	0.82
Acetonitrile	1.10	1.12	1.04	1.06	0.93	0.90
Hexane	–	1.08	NS(S)	0.93	0.75	0.70

[a] Separation factor for the indicated pair; NS: not separated; NS(S) indicates a partial separation as a shoulder.
[b] Mobile phases are as follows when mixed solvents were used. C_{18}(I): 90% Methanol and 80% acetonitrile for all congeners.
PYE: Methanol for DCDD–TCDD, ethanol–CH_2Cl_2 (1:1, v/v) for PnCDD and HxCDD.
COP: Methanol for DCDD and TrCDD, ethanol–CH_2Cl_2 (1:1, v/v) for TCDD–HxCDD, acetonitrile for DCDD–TCDD, acetonitrile–CH_2Cl_2 (1:1, v/v) for PnCDD and HxCDD. Hexane for DCDD and TrCDD, hexane–CH_2Cl_2 (1:1, v/v) for TCDD–HxCDD.
CZP: 90% Methanol for DCDD–TCDD, methanol for PnCDD and HxCDD, 80% acetonitrile for all congeners.
NPE: 80% Methanol for DCDD–TCDD, 90% methanol for PnCDD and HxCDD, 70% acetonitrile for all congeners.
NPO: 80% Methanol for DCDD–TCDD, 90% methanol for PnCDD and HxCDD, 70% acetonitrile for all congeners.

appear as larger UV peaks in the chromatographic profile and should (as has been reported) have the greater k' values on C_{18} phase, reflecting the greater hydrophobic surface area of the molecule [14,35,36]. The hydrophobic selectivity was not greatly affected by changing the organic solvent in the RPLC mobile phase from methanol to acetonitrile. The solutes were not retained on the C_{18} phases in hexane. No separation was achieved with the C_{18} phases (monomeric or polymeric) for any of the PCDDs in group II regardless of mobile phase, indicating very little difference in hydrophobic properties of each isomer in these pairs.

3.2.2. PYE phase and COP phase

The PYE phase also showed greater retention for the more symmetrically substituted PCDD isomers with increased separation factors compared to those on C_{18}(I) in reversed-phase mode. This is presumably due to the more favorable donor–acceptor interactions provided by the electron-deficient aromatic rings of these isomers and the favorable hydrophobic

Table 3
Effect of solvent and stationary phase structure on selectivity for PCDDs (group II) and HxCNs

Isomer pair (PCDD-I/PCDD-II) Mobile phase[b]	α[a]=$k'_{(PCDD\text{-}I)}/k'_{(PCDD\text{-}II)}$ (k': last peak)			
	PYE	CZP	NPO	NPO[c]
1,2,4,6-/1,2,4,9-TCDD				
Methanol	NS (17.6)	1.03 (9.81)	0.87 (11.4)	
Acetonitrile	NS (4.52)	1.03 (3.52)	0.96 (4.17)	
Hexane	NS (4.20)	NS (1.44)	0.90 (0.65)	0.90 (1.19)
1,2,4,7-/1,2,4,8-TCDD				
Methanol	1.05 (16.5)	NS (9.24)	0.94 (10.2)	
Acetonitrile	NS (4.66)	NS (3.68)	NS (4.20)	
Hexane	NS (3.31)	NS (1.27)	NS (0.46)	NS (0.79)
1,2,4,6,7-/1,2,4,8,9-PnCDD				
Methanol	NS (2.51)	NS (5.87)	0.87 (4.81)	
Acetonitrile	NS (11.5)	NS (6.89)	0.94 (6.19)	
Hexane	NS(S) (10.2)	NS (2.83)	0.91 (0.82)	0.90 (1.49)
1,2,4,6,8-/1,2,4,7,9-PnCDD				
Methanol	NS (2.98)	NS (7.08)	0.94 (4.34)	
Acetonitrile	1.03 (13.9)	1.02 (8.19)	NS (6.09)	
Hexane	1.05 (10.7)	NS (2.37)	NS(S) (0.50)	0.95 (0.86)
1,2,3,6,7,9-/1,2,3,6,8,9-HxCDD				
Methanol	NS (7.21)	NS (14.6)	0.91 (6.50)	
Acetonitrile	NS (39.1)	NS (16.5)	0.96 (8.99)	
Hexane	NS (29.4)	NS (5.09)	0.94 (0.74)	0.94 (1.32)
1,2,4,6,7,9-/1,2,4,6,8,9-HxCDD				
Methanol	1.04 (7.61)	NS (19.8)	0.97 (6.17)	
Acetonitrile	1.04 (39.0)	NS (19.0)	NS (8.40)	
Hexane	1.06 (32.0)	NS (6.43)	NS (0.67)	NS (1.22)
1,2,3,4,6,7-/1,2,3,5,6,7-HxCN				
Methanol	–	1.07 (9.25)	NS (4.60)	
Acetonitrile	0.95 (44.4)	1.04 (12.7)	NS (8.24)	
Hexane	0.97 (10.8)	1.03 (2.98)	NS (0.32)	NS (0.49)

[a] Separation factor for the indicated pair, according to the previous assignment [3,10]. NS: Not separated. NS(S) indicates a partial separation as a shoulder.
[b] Mobile phases are as follows when mixed solvents were used. 30 cm column was used for NPO in hexane and isooctane.
PYE: Methanol for DCDD–TCDD, ethanol–CH_2Cl_2 (1:1, v/v) for PnCDD and HxCDD.
CZP: 90% Methanol for DCDD–TCDD, methanol for PnCDD, HxCDD and HxCN, 80% acetonitrile for all congeners.
NPO: 80% Methanol for DCDD–TCDD, 90% methanol for PnCDD, HxCDD and HxCN, 70% acetonitrile for all congeners.
[c] At 10°C in isooctane.

interactions due to the greater molecular surface areas. The use of acetonitrile instead of methanol resulted in little changes in the separation factors. The separation of the PCDD isomers in group I that have clearly different hydrophobic properties was much poorer in hexane than in typical reversed-phase mode. This, in part, is due to the lack of hydrophobic selectivity in non-polar solvents which works together with donor–acceptor interactions in polar solvents to favor the more symmetrically substituted isomer [3,7]. The increased retention seen with PCDD congeners with increased chlorine substitution is understandable based on the increase in donor–acceptor interactions. We do not currently understand the elution order reversal for 1,3,6,8-/1,3,7,9-TCDDs in group I, or the loss of resolution for 1,2,4,7-/1,2,4,8-TCDDs in group II with hexane (Table 3). Interestingly, the separations of 1,2,4,6,8-/1,2,4,7,9-

PnCDDs and 1,2,4,6,7,9-/1,2,4,6,8,9-HxCDDs with similar hydrophobic properties were notably better in hexane than in the other solvents. The COP phase showed similar retention characteristics as the PYE phase, providing separation for 1,2,4,6,8-/1,2,4,7,9-PnCDDs in hexane. The resolution was poorer on COP than on the PYE phase because of the lower column efficiency due to poor column packing and possibly due to strong electronic interactions. PYE can provide best separation in acetonitrile for the PCDDs in group I, and in hexane or in methanol for some isomer pairs in group II.

3.2.3. Nitrophenyl-type stationary phases

NPE and NPO eluted the major isomer (larger peak) first followed by the smaller peak of the less symmetrically substituted isomer for all the PCDD isomer pairs in group I. The selectivity of the nitrophenyl stationary phases can be explained by the contribution of dipole–dipole interaction which overrides the contribution of hydrophobic interaction in the reversed-phase mode [3,7]. The dipole–dipole interaction is more favorable with the more dipolar isomers which have the chlorine atoms closer together. Both NPE and NPO showed similar retention properties for PCDDs, but NPO always gave the better separation. This could be because the NPE phase possesses a mixed stationary phase of ~70% *para*- and ~30% *ortho*-nitro substitution [27], while the NPO phase has only *p*-nitrophenoxy groups. The use of acetonitrile in the mobile phase drastically reduced the selectivity of both the NPE and NPO phases for the separation of PCDD isomers. Dipolar solvation of the stationary phase and the solutes with the dipolar solvent molecules can explain these results. Therefore alcohols should be used as an organic solvent with dipolar stationary phases for maximum selectivity in RPLC.

The k' values on NPE and NPO in hexane are small but in a similar range for all the PCDDs from dichlorodibenzo-*p*-dioxin (DCDD) to HxCDD. This is in contrast to the results found in the RPLC mode where an increase in k' was observed with increasing numbers of chlorine substituents. NPE and NPO resulted in the greater separation factors, even with the much smaller k' values, for the PCDDs in group I with hexane compared to methanol, as shown in Fig. 2 and Table 2. This can be explained by the lack of contribution based on hydrophobic selectivity in non-polar eluents. Hydrophobic interactions, which should be more favorable for the more symmetrically substituted isomers, work against dipolar selectivity

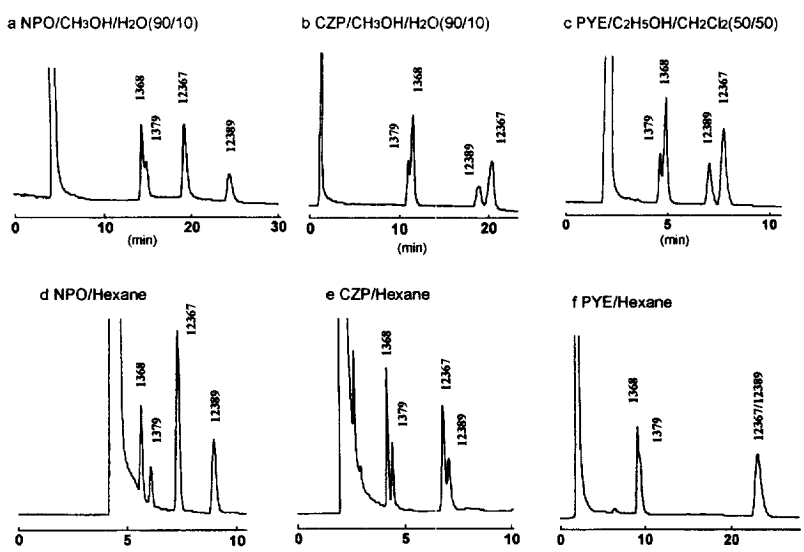

Fig. 2. Separation of 1,3,6,8-/1,3,7,9-TCDD and 1,2,3,6,7-/1,2,3,8,9-PnCDD. (a) NPO, column: 30 cm×4.6 mm I.D., mobile phase: 90% methanol, flow-rate: 1 ml/min; (b) CZP, 15 cm×4.6 mm I.D., 90% methanol, 2 ml/min; (c) PYE, 15 cm×4.6 mm I.D., ethanol–dichloromethane (50:50, v/v), 1 ml/min, (d) NPO, hexane, 1 ml/min, (e) CZP, hexane, 1 ml/min, (f) PYE, hexane, 2 ml/min.

in the reversed-phase mode [3,7]. The separation factors for the PCDDs in group I on the nitrophenyl-bonded stationary phases are in the order, hexane> methanol>acetonitrile.

The correlation between log k' values and dipole moments for the PCDDs is much better in hexane than in methanol [7]. Fig. 3 shows the plots between the calculated dipole moments [34] and log k' values of TCDDs, PnCDDs, HxCDDs and TCDFs obtained with the NPO phase in methanol–water and in hexane. In RPLC the k' values are dependent on the number of chlorine substitutions. The contribution of the number of the chlorine atoms on k' seen in RPLC, nearly disappeared in hexane, indicating both the dominant contribution of the dipolar interactions and the absence of hydrophobic interactions in non-polar eluents. Greater slopes were obtained in hexane and is more clearly seen with TCDFs, in spite of the much smaller k' values in hexane. Deviations in the plots of isomers having very small dipole moments such as 1,2,6,7-TCDD and 1,2,3,6,7-PnCDD in Fig. 3 can be explained by the contribution of the local dipoles to retention [7]. The TCDFs coproduced during preparation (1,2,6,7-/2,3,6,7-, 1,2,7,9-/1,3,7,8-, 1,6,7,8-/2,3,4,7-, 2,3,6,8-/1,2,6,8-, 1,3,4,9-/1,3,4,7-, 1,2,4,9-/1,2,4,7-TCDFs) were easily separated, whereas some peak overlapping has been observed in GC [14].

Three PCDD isomer pairs of group II were separated with NPO in hexane but with slightly smaller separation factors than in methanol. This is in contrast to the greater contribution of the dipolar interactions seen with the PCDDs of group I in hexane. The use of isooctane at 10°C provided ~70%

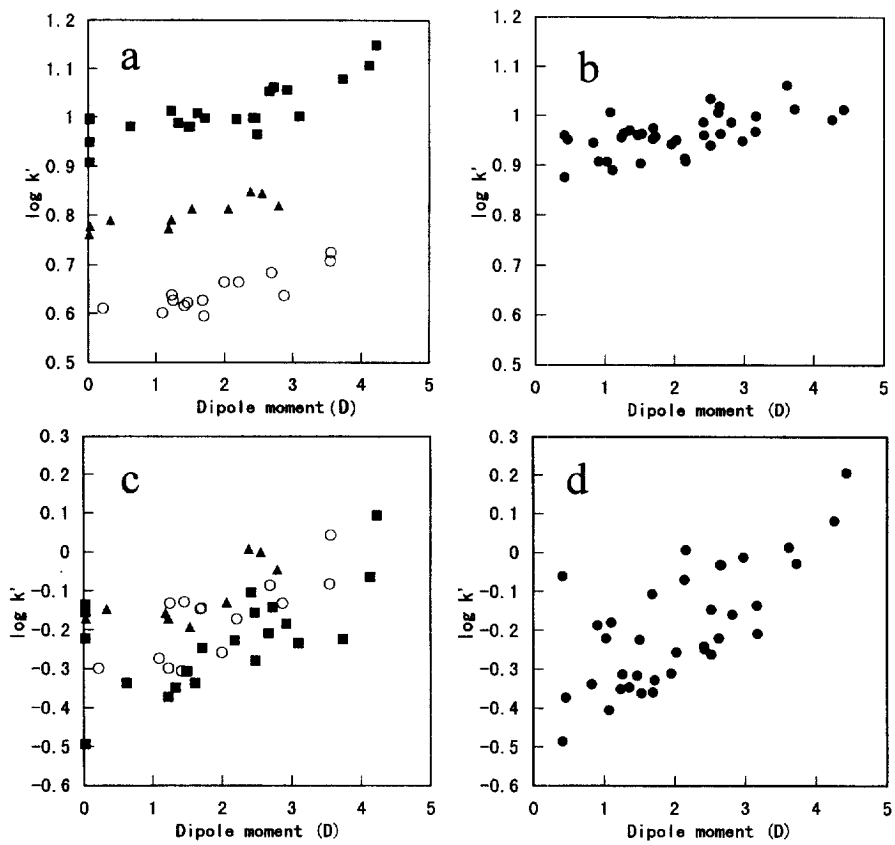

Fig. 3. Plots of log k' values on the NPO phase against dipole moments [34] of PCDDs (a and c) and TCDFs (b and d) in methanol–water (80% for TCDDs, 90% for PnCDDs and HxCDDs in (a) and 80% in (b)) and in hexane (c and d). ■=TCDD, ○=PnCDD, ▲=HxCDD, ●=TCDF.

greater retention than in hexane at 30°C but with similar separation factors. The hydrophobic interaction in a RPLC system seems to help the separation of PCDD isomer pairs in group II possibly by providing a tight association which leads to a greater dipolar interaction between the solute and the stationary phase, although the hydrophobic interaction itself does not show any preference for the dioxin congeners of this group. The combination of NPO and PYE phases can separate PCDD isomer pairs in group II in hexane except for the 1,2,4,7-/1,2,4,8-TCDD pair, which needed a methanol system.

3.2.4. CZP stationary phase

The separation of PCDD isomers on CZP was generally poorer than on the other donor–acceptor phases. It is interesting to note that an elution order reversal on CZP phase occurred between reversed phase and normal phase mode (Fig. 2b and e and Table 2). The CZP phase possesses dipolar property due to the presence of a nitrogen in the five-membered ring (dipole moment of pyrrole, $\mu=1.84$ D [37]), though much smaller than that of nitrobenzene ($\mu=4.22$ D [37]). The hydrophobic property is also smaller than that of C_{18} or PYE phase (Table 1). Longer retention of the more symmetrically substituted isomer is expected if the hydrophobic interaction makes a dominant contribution, while dipolar interaction can lead to the longer retention of the more dipolar isomer. These two factors work against each other on the CZP phase in RPLC mode. The hydrophobic interaction seems to dominate, however, since the less symmetrical PCDD isomer was eluted first. A dominant contribution of the dipolar interaction resulted the reversed elution order in hexane, which was similar to the separation with the NPO phase but with much lower selectivity.

3.3. Separation of HxCN isomers

1,2,3,4,6,7-/1,2,3,5,6,7-HxCNs are coproduced during synthesis [9–11], and also known to be difficult to separate. The partial separation of these isomers was reported using PYE phase in hexane [9–11]. C_{18}, NPE and NPO phases did not provide separation in any mobile phase tested. We found that the HxCNs could be separated by the electron-donor bonded phases, more easily in polar solvents than in hexane.

As shown in Table 3 and Fig. 4, the HxCNs were separated on PYE and CZP with the opposite elution order. The minor isomer (smaller peak) eluted first followed by the larger peak of the major isomer on PYE. The larger peak was assigned to be the more symmetrical 1,2,3,5,6,7-HxCN [10,11] as with PCDD isomer pairs. The contribution of donor–acceptor type interactions and possibly hydrophobic interaction provided the separation on PYE in polar solvents. CZP phase provided separation presumably based on the dipolar property according to the elution order.

The results are of interest for the following

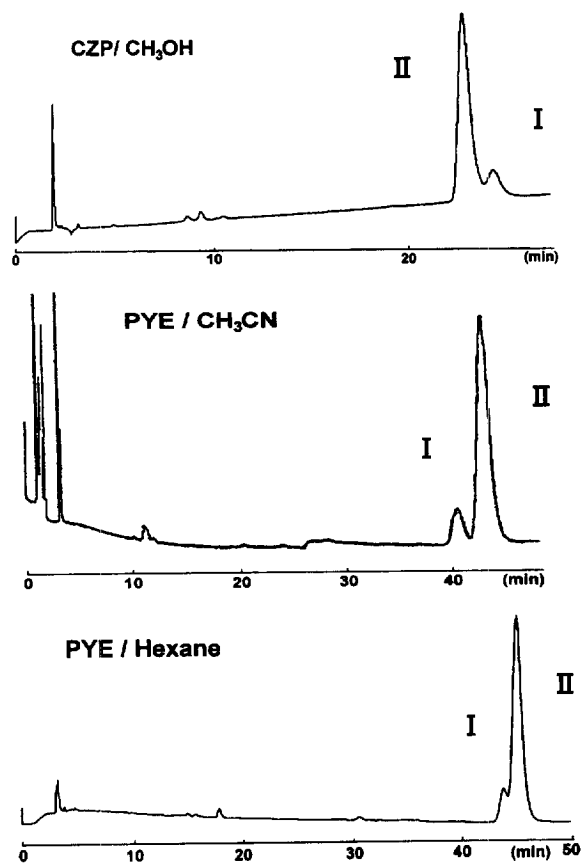

Fig. 4. Separation of 1,2,3,4,6,7-HxCN (I)/1,2,3,5,6,7-HxCN (II) on (a) CZP (15 cm×4.6 mm I.D.) in methanol (1.5 ml/min) and (b) on PYE (15 cm×4.6 mm I.D.) in acetonitrile (2 ml/min) and (c) on PYE (25 cm×4.6 mm I.D.) in hexane (1 ml/min).

reasons; (1) the CZP phase, which was not very selective for the PCDD isomers of group II, successfully separated the HxCNs while the NPO phase failed (2) the more dipolar isomer was retained longer and with a greater separation factor in reversed phase mode than in hexane on CZP phase in spite of the possible contribution of hydrophobic interactions which show the opposite preference. The latter observation is similar to that seen with the PCDD isomer pairs of group II on NPO phase where the better separation factors were obtained in methanol–water than in hexane. Hydrophobic interactions seem to help the dipolar recognition and enhance the separation in methanol for the isomers having little difference in the hydrophobic properties, whereas hydrophobic interaction reduces the selectivity based on the dipolar interaction with the PCDD isomers of group I which have a clear difference in hydrophobic properties [3,7].

3.4. Separation of non-ortho-substituted and mono-ortho-substituted PCBs of high toxicity from multi-ortho-substituted PCBs

Group (class) separation among various chlorinated aromatic compounds is an important step in environmental analysis. PYE phase has been employed for this purpose. Several authors have reported the use of PYE for the isolation of one type of chlorinated aromatic compounds and also non-*ortho*-substituted and mono-*ortho*-substituted PCBs from mixtures [8,16–18]. The retention on PYE phase in non-polar mobile phase was not large enough, however, to completely separate non-*ortho*-substituted and mono-*ortho*-substituted PCBs (which are of high toxicity) from the others PCBs (of lower toxicity) [6,16,18,19]. In order to examine the effect of the aromatic ring size in the stationary phase on the retention of PCBs in hexane, COP phase with its larger aromatic system was examined. As shown in Table 1, this stationary phase preferentially retained planar compounds. NPE and NPO also showed high selectivity based on solute planarity for PAHs in reversed-phase mode, but showed small retention and poor selectivity for PCBs in normal phase mode, as shown in Table 4. Polymeric C_{18}(III) did not provide significant retention for the PCBs in non-polar solvents.

Table 4 shows the recognition of planarity for planar and non-planar PCBs and Fig. 5 shows the elution profile of non-*ortho*-substituted PCBs, 77, 126, 127 and 169, and mono-*ortho*-substituted PCBs of high toxicity, 105, 118 and 157, which accounts for considerable portions of the total dioxin-like toxicity of halogenated aromatic compounds [22–24], in hexane together with the elution profile of the PCBs commonly found in human adipose tissues. COP phase provided greater recognition based on planarity of PCBs, and will make it possible to fractionate the toxic PCBs at the tail of the other PCBs more easily than with the PYE phase which showed more overlap. Better column packing of COP phase is expected to improve the peak shape. A solvent gradient or a column back-flush will be necessary, however, if TCDDs and TCDFs or higher chlorinated PCDDs and PCDFs are to be collected

Table 4
Recognition of planarity of PCBs

Mobile phase solvent	$\alpha^a(k'$, last peak)							
	CZP		PYE		COP		NPO	
	77/66	169/155	77/66	169/155	77/66	169/155	77/66	169/155
n-Octane	1.8 (0.51)	– –	2.2 (1.16)	20 (2.04)	2.8 (1.91)	60 (4.7)	1.4 (0.81)	4.5 (0.85)
Hexane	1.7 (0.68)	16 (0.92)	2.2 (1.45)	18 (2.71)	2.8 (2.59)	50 (7.1)	1.5 (0.94)	4.6 (1.02)
Isooctane	1.8 (0.84)	14 (1.13)	2.3 (1.90)	20 (3.53)	3.1 (4.18)	69 (11)	1.5 (1.05)	4.7 (1.09)

[a] Separation factor $\alpha = k'$(planar PCB)/k'(non-planar PCB). Structures of PCBs are as follows. No. 66: 2,3′,4,4′-tetrachlorobiphenyl (TCB), No. 77: 3,3′,4,4′-TCB, No. 155: 2,2′,4,4′,6,6′-hexachlorobiphenyl (HxCB) and No. 169: 3,3′,4,4′,5,5′-HxCB.

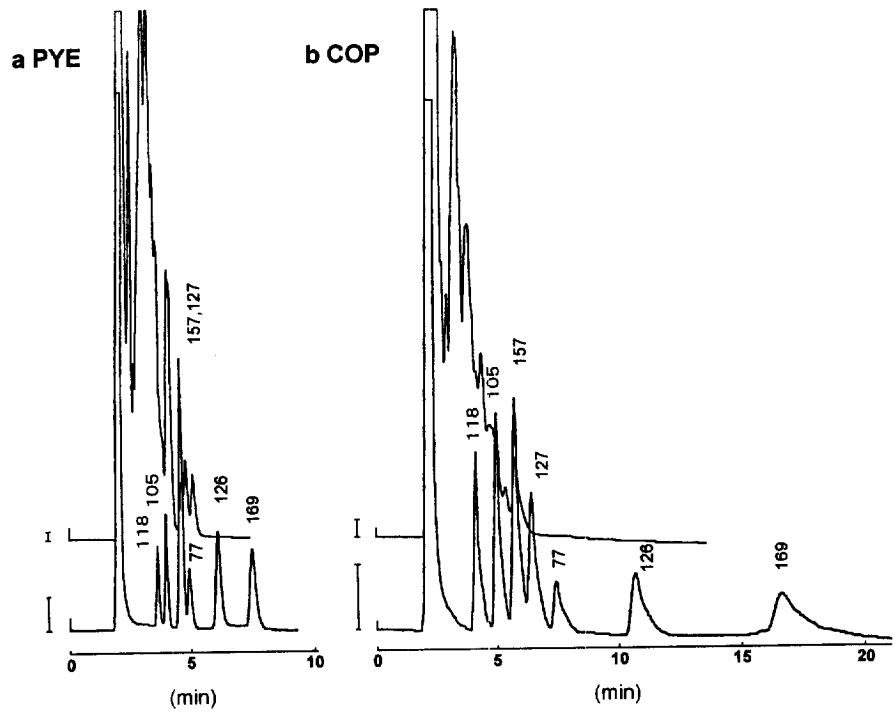

Fig. 5. Elution profile of PCBs usually found in human adipose tissues (upper chromatogram; PCBs No. 22, 28, 30, 33, 37, 41, 44, 47, 49, 52, 56, 60, 66, 74, 87, 99, 101, 105, 110, 114, 118, 122, 130, 137, 138, 146, 151, 153, 154, 156, 157, 158, 167, 170, 172, 174, 177, 178, 180, 182, 183, 187, 189, 191, 193, 194, 195, 196, 197, 201, 203, 204, 206, 209 at equal concentrations) and PCBs of high toxicity (lower chromatogram; non-*ortho*-substituted PCBs 77, 126, 127, 169 and mono-*ortho*-substituted PCBs 105, 108, 157) on (a) PYE and (b) COP in hexane. The scale bars indicate 0.001 AU.

[8], since COP provides much longer retention for PCDF and PCDD compared to PYE.

4. Summary

The combination of dipolar and non-polar bonded phases is very effective for the separation of isomer pairs of polychlorinated aromatic compounds with close structural similarities. Isomer pairs of PCDDs having significant differences in hydrophobic properties were most easily separated by NPO phase in hexane and by PYE phase in acetonitrile, while those with similar hydrophobic properties were better separated on NPO phase in methanol–water or on PYE phase in either hexane or methanol–water. Hydrophobic selectivity seems to help dipolar recognition for the dioxin isomers of group II and the HxCNs with similar hydrophobic properties, whereas hydrophobic selectivity works against the dipolar selectivity for the dioxin isomers in group I with clear difference in hydrophobic properties. Although no single stationary phase was universally selective for all isomer pairs which are produced as mixtures during synthesis and are particularly difficult to separate, all the isomer pairs of the PCDDs and HxCNs tested in this study can be separated in unmixed organic solvents by NPO/PYE and CZP/PYE combination, respectively. COP phase, which has a larger aromatic system than PYE phase, provided the greater separation based on the planarity of PCBs. Various donor–acceptor type stationary phases are useful for providing separations which would otherwise be very difficult to achieve. Isomer separations are to be examined with dipolar and non-polar stationary phases in polar (alcoholic) and non-polar solvents to optimize conditions, while the

dipolar stationary phases showed poor selectivity in acetonitrile.

Acknowledgements

This work was supported in part by a Monbusho International Joint Research Program and a Grant-in-Aid for Scientific Research funded by the Ministry of Education, Science, Sports, and Culture, Japan. The authors thank Beckman Instrument Co. for the loan of the equipment, 126 pump and 168 photodiode array detector with NUVEAU software.

References

[1] D.G. Patterson Jr., C.R. Lapeza Jr., E.R. Barnhart, D.F. Groce, V.W. Burse, Chemosphere 19 (1989) 127–134.
[2] D.G. Patterson Jr., S.G. Isaacs, L.R. Alexander, W.E. Turner, L. Hampton, J.T. Bernert, L.L. Needham, in: C. Rappe, H.R. Buser (Eds.), Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls, WHO, IARC:Lyon, Vol. 11, 1991 pp. 299–342.
[3] K. Kimata, K. Hosoya, T. Araki, N. Tanaka, E.R. Barnhart, L.R. Alexander, S. Sirimanne, P.C. McClure, J. Grainger, D.G. Patterson Jr., Anal. Chem. 65 (1993) 2502–2509.
[4] L. Nondek, J. Chromatogr. 373 (1986) 61–80.
[5] G. Felix, A. Thienpont, P. Dentraygues, Chromatographia 34 (1992) 177–181.
[6] E. Grimvall, C. Ostman, J. Chromatogr. A 675 (1994) 55–64.
[7] U. Pyell, P. Garrigues, G. Felix, M.T. Rayez, A. Thienpont, P. Dentraygues, J. Chromatogr. 634 (1993) 169–181.
[8] U. Pyell, P. Garrigues, J. Chromatogr. A 660 (1994) 223–229.
[9] E. Jakobsson, L. Asplund, P. Haglund, A. Bergman, Organohalogen compounds, 10th International Meeting, Dioxin'90, Bayreuth, Vol. 4, 1990, pp. 414–418.
[10] E. Jakobsson, E. Eriksson, A. Bergman, Acta Chem. Scand. 46 (1992) 527–532.
[11] D.T. Williams, B. Kennedy, G.L. LeBel, Chemosphere 27 (1993) 795–806.
[12] A.S. Kende, M.R. DeCamp, Tetrahedron Lett., 1975, 2877–2880.
[13] L.T. Gelbaum, D.G. Patterson Jr., D. Ashley, D.F. Groce, Chemosphere 17 (1988) 551–558.
[14] J.J. Ryan, H.B.S. Conacher, L.G. Panopio, B.B.Y. Lau, J.A. Hardy, Y. Masuda, J. Chromatogr. 541 (1991) 131–183.
[15] N. Tanaka, Y. Tokuda, K. Iwaguchi, M. Araki, J. Chromatogr. 239 (1982) 761–772.
[16] P. Haglund, L. Asplund, U. Jarnberg, B. Jannson, J. Chromatogr. 507 (1990) 389–398.
[17] P. de Voogt, P. Haglund, L.B. Reutergardh, C. de Wit, F. Waern, Anal. Chem. 66 (1994) 305A–311A.
[18] D.E. Wells, I. Echarri, C. McKenzie, J. Chromatogr. A 718 (1995) 107–118.
[19] E. Grimvall, C. Ostman, J. Chromatogr., A 685 (1994) 338–343.
[20] A. Kocan, J. Petrik, J. Chovancova, B. Drobna, J. Chromatogr. A 665 (1994) 139–153.
[21] K.P. Feltz, D.E. Tillitt, R.W. Gale, P.H. Peterman, Environ. Sci. Technol. 29 (1995) 709–718.
[22] A. Parkinson, S. Safe, L.W. Robertson, P.E. Thomas, D.E. Ryan, L.M. Reik, W. Levin, J. Biol. Chem. 258 (1983) 5967.
[23] D.G. Patterson Jr., G.D. Todd, W.E. Turner, V. Maggio, L.R. Alexander, L.L. Needham, Environ. Health Perspectives Suppl. 102 (1994) 195–204.
[24] U.G. Ahlborg, G.C. Becking, L.S. Birnbaum. A. Brouwer, H.J.G.M. Derks, M. Feeley, G. Golor, A. Handberg, J.C. Larsen, A.K.D. Liem, S.H. Safe, C. Schlatter, F. Waern, M. Younes, E. Yrjanheikki, Chemosphere 28 (1994) 1049–1067.
[25] D.L. Stalling, C. Guo, K.C. Kuo, S. Saim, J. Microcol. Sep. 5 (1993) 223–235.
[26] K. Jinno, S. Shimura, N. Tanaka, K. Kimata, J.C. Fetzer, W.R. Biggs, Chromatographia 27 (1989) 285–291.
[27] K. Kimata, K. Hosoya, N. Tanaka, T. Araki, D.G. Patterson, J. Chromatogr. 595 (1992) 77–88.
[28] H. Kuroki, K. Haraguchi, Y. Masuda, Chemosphere 13 (1984) 561–573.
[29] K. Jinno, T. Nagoshi, N. Tanaka, M. Okamoto, J.C. Fetzer, W.R. Biggs, J. Chromatogr. 392 (1987) 75–82.
[30] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721–728.
[31] L.C. Sander, S.A. Wise, Critical Rev. Anal. Chem. 18 (1987) 299–414.
[32] J. Grainger, L.T. Gelbaum, Appl. Spectrosc. 41 (1987) 809–820.
[33] J. Grainger, P.C. McClure, Z. Liu, B. Botero, S. Sirimanne, D.G. Patterson Jr., M. Sewer, C. Gillyard, K. Kimata, K. Hosoya, T. Araki, N. Tanaka, S. Terabe, Chemosphere 32 (1996) 13–23.
[34] C.J. Koester, R.A. Hites, Chemosphere 17 (1988) 2355–2362.
[35] M.L. Taylor, T.O. Tiernan, B. Ramalingam, D.J. Wagel, J.H. Garrett, J.G. Solch, in: L.H. Keith, C. Rappe, G. Choudhary (Eds.), Chlorinated Dioxins and Dibenzofurans in the Total Environment, Butterworth, Boston, MA, Vol. II, Ch. 2, 1985, pp. 17–35.
[36] T.J. Nestrick, L.L. Lamparski, R.H. Stehl, Anal. Chem. 51 (1979) 2273–2281.
[37] R.C. Weast (Ed.), CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 61st edn., 1980–1981, p. E-66.