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Separation for Subsequent Analysis of PCBs, PCDD/Fs, and PAHs According to Aromaticity and Planarity Using a Two-Dimensional HPLC System

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In this paper, an automated separation method is described for the subsequent GC/MS analysis of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polycyclic aromatic compounds (PAHs), and related compounds in complex environmental matrices. The high-performance liquid chromatography (HPLC) method utilizes a coupled column system consisting of a nitrophenylpropylsilica (nitro) column (Nucleosil, 5 μm, 250 × 4.6 mm) and a 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE) column (Cosmosil, 5 μm, 150 × 4.6 mm) and provides five well-defined fractions ready for injection on GC/MS. The fractions are as follow: aliphatic/monocyclic aromatic compounds, mono-tetra-ortho-PCBs, non-ortho-PCBs, PCDD/Fs (tetra-octa-chlorinated), and PAHs. The separation takes less than 40 min and consumes less than 70 mL of solvents. Evaluation of the HPLC system was performed with both environmentally collected samples and standard solution mixtures containing a wide range of PCBs, PCDD/Fs, and PAHs.

Introduction

During the last decades, the interest in hydrophobic organic contaminants such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), and polycyclic aromatic hydrocarbons (PAHs) has significantly increased. This is mainly due to their potentially toxic, carcinogenic, and/or mutagenic effects on animals and humans (*1*, *2*). Detection and quantitation of these compounds in environmental samples are in most cases intricate problems since they are often present in trace levels in complex environmental matrices.

Analysis of PCBs, PCDD/Fs, and PAHs usually involves extensive cleanup procedures that aim mainly at two things: to remove most of the co-extracted matter that is not the analytes and to separate the pollutants into groups due to their physical and chemical properties. Cleanup of extracts for subsequent analysis of PAHs often involves liquid−liquid partitioning procedures (*3*, *4*) and/or open column chromatography (e.g., aluminum oxide, silica gel, Florisil, and Sephadex LH-20) (*5*). Isomer-specific determination of PCBs and PCDD/Fs generally requires more extensive cleanup procedures, which often include liquid chromatography with combinations of several adsorbents such as modified silica gel, aluminum oxide (*6*), and activated carbon (*7*−*10*). In recent years, HPLC has become a more useful tool for cleanup and separation of these compounds. The main advantages of HPLC are the higher efficiency, reproducibility, and speed of this method compared to open column liquid chromatography. Other reasons are the lower solvent consumption, which reduces the risk of solvent-introduced contamination, and less manipulation of the sample by the operator.

The aim of the present work was to develop a rapid and effective HPLC separation method that provides well-defined fractions of mono-tetra-ortho-PCBs (substituted with one to four chlorine atoms in ortho positions), non-ortho-PCBs (not substituted in ortho position), PCDD/Fs, and PAHs (≥4 rings) ready for direct analysis on gas chromatography/mass spectrometry (GC/MS). There are many advantages in being able to analyze a wide range of priority substances separated from one and the same sample due to limited sample amounts, unique samples, and time-consuming and difficult sampling.

Experimental Section

Standard Mixtures and Samples. Standard mixtures as well as environmentally collected samples were run through the HPLC system for evaluation of the separation capacity, reproducibility, and accuracy of the system. The standard mixture contained 15 native PCBs (see Figure 2a), 8 native PCDD/Fs (see Figure 2b), and 16 native PAHs (see Figure 2c; Table 2). The amounts of the standard mixtures injected on the HPLC were approximately 1 ng/PCB congener, 450 pg/PCDD/F congener, and 20 ng/PAH compound. To further evaluate the HPLC method with a wider range of PCDD/Fs and PCBs, both an electrostatic filter precipitate extract from a municipal waste combustion incinerator (two different concentration levels differing with a factor of 10, e.g., 4.0 pg of 2,3,7,8-TCDD and 40 pg of 2,3,7,8-TCDD, respectively) and Aroclor 1254 were run through the system. Different abiotic and biotic sample matrices were also used, and these samples were two air samples (where 2200 m^3 of air has been filtered through a GF/C filter); three herring oil samples (0.1, 0.5, and 1 g of lipid weight); two harbor porpoise blubber samples (both of 500 mg of lipid weight); and finally five sediment samples (0.2−4 g dry weight). These samples were chosen since they represent different kinds of complexity. The air samples as well as the sediment samples were chosen due to their content of a large variety of different compounds while the herring oil and harbor

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porpoise blubber represent two different kinds of lipid-rich matrices.

Chemicals. All solvents used were of high purity. The toluene and the hexane used for extraction and Silica cleanup, respectively, were of glass-distilled quality (Burdick & Jackson, Fluka Chemie AG, Buchs, Switzerland), and the *n*-hexane and the dichloromethane used for the HPLC fractionation were of HPLC grade (Merck, Darmstadt, Germany). The silica (Merck, 63−200 μm) was activated at 500 °C for 24 h and then modified with water (3% or 10% w/w), 7.7 M potassium hydroxide (KOH) in methanol (35% w/w), or concentrated sulfuric acid (H_2SO_4) (40% w/w). All individual PCB congeners, native and ^{13}C-labeled, together with the ^{13}C-labeled PCDD/F congeners were purchased from Cambridge Isotope Laboratories (Woburn, MA). The technical PCB product, Aroclor 1254, was purchased from Dr. Ehrenstorfer (Augsburg, Germany), and the electrostatic filter precipitate was collected at a Swedish municipal waste combustion incinerator in 1987. The 20 PAHs used were purchased from Aldrich-Chemie (Steinheim, Germany), Radiant Dyes Chemie (Wermelskirchen, Germany), Cambridge Isotope Laboratories (Woburn, MA), and Supelco Inc. (Bellafonte, PA).

Extraction and Addition of Standards before HPLC Separation. All samples, except for Aroclor 1254, were extracted with toluene for 24 h using a Soxhlet apparatus. During all extractions, except the electrostatic filter precipitate, the Soxhlet apparatus was equipped with a Dean-Stark trap (*11*) for the removal of water. Prior to extraction of the herring oil samples, the harbor porpoise blubber samples, the air samples, and the sediment samples, eight ^{13}C-labeled PCB standards [2,2′,5,5′-TCB (IUPAC No. 52), 3,3′,4,4′-TCB (IUPAC No. 77), 2,2′,4,5,5′-PnCB (IUPAC No. 101), 2,3′,4,4′,5-PnCB (IUPAC No. 118), 3,3′,4,4′,5-PnCB (IUPAC No. 126), 2,2′,3,4,4′,5-HxCB (IUPAC No. 138), 3,3′,4,4′,5,5′-HxCB (IUPAC No. 169), and 2,2′,3,3′,4,5,5′-HpCB (IUPAC No. 180) were added [except for the air samples, where PCB no. 118 was exchanged for ^{13}C-labeled 2,2′,4,4′,5,5′-HxCB (PCB no. 153)] together with eight ^{13}C-labeled PCDD/F standards (2,3,7,8-TCDF, 2,3,7,8-TCDD, 2,3,4,7,8-PnCDF, 1,2,3,7,8-PnCDD, 1,2,3,4,7,8-HxCDF, 1,2,-3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD). To the air samples that were also analyzed for PAHs, three PAH internal standards (picene, perylene-d_{12}, and dibenzo[*a*,*i*]-pyrene) were added before Soxhlet extraction. The Aroclor 1254 solution was prepared by diluting Aroclor 1254 with *n*-hexane. Three ^{13}C-labeled non-ortho-PCBs (PCB no. 77, PCB no. 126, and PCB no. 169) were added to the Aroclor 1254 solution prior to HPLC fractionation since the corresponding native congeners are present in very low concentrations.

Cleanup. The toluene extracts of all samples extracted were volume reduced and eluted through different columns of modified silica (SiO_2) gel. The SiO_2 columns, which retain polar compounds as well as lipids, were eluted with *n*-hexane (4 mL/g of SiO_2). A total of 5 g of SiO_2, 10% deactivated with water, was used for the air samples as well as the sediment samples. For the herring oil samples, 20 g of SiO_2 was used, of which 10 g was modified with KOH and 10 g was deactivated with water (3%). To remove almost all lipids in the harbor porpoise blubber samples, three SiO_2 columns were used. The first contained 10 g of KOH-modified SiO_2 together with 10 g of neutral SiO_2 (3% water), and the last two contained 5 g of H_2SO_4-modified SiO_2 together with 5 g of neutral SiO_2 (3% water). For the analysis of the sediment samples, an additional cleanup step was introduced to remove most of the elementary sulfur, which otherwise could interfere during the HPLC separation. Five grams of fine granular copper (Mallinckrodt Special Chemicals Co., Kentucky) was therefore added to the sediment extracts dissolved in approximately 15 mL of *n*-hexane to reduce the sulfur content. The sediment extracts together with the copper were sonificated for 30 min, left overnight, and then sonificated for another 30 min before eluting the extract through some glass wool in a pasteur pipet to remove the copper granulates.

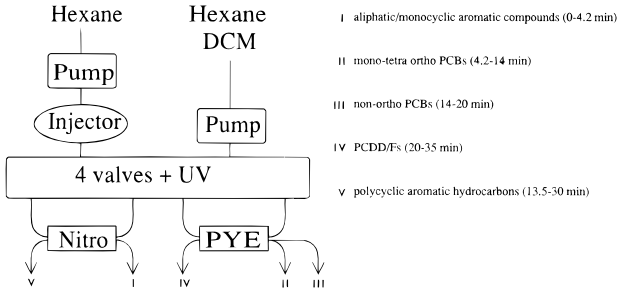

FIGURE 1. Schematic picture of the HPLC separation method. The minutes in parentheses refer to the elution intervals for the different groups of compounds (DCM, dichloromethane).

HPLC System. In the present study, a nitrophenylpropylsilica (nitro) column (Nucleosil, 5-μm particles, 250 × 4.6 mm, Macherey-Nagel, Germany) was used in combination with a 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE) column (Cosmosil, 5-μm particles, 150 × 4.6 mm, Nacalai Tesque, Kyoto, Japan) and provided five well-defined fractions. The fractions contained the following: aliphatic/monocyclic aromatic compounds (I), mono-tetra-ortho-PCBs (II), non-ortho-PCBs (III), PCDD/Fs (tetra-octachlorinated) (IV), and PAHs (V) (Figure 1). The system was used in a straight-phase mode with *n*-hexane and dichloromethane as mobile phases. When operated in a straight-phase mode, the nitro column shows the same retention properties as, for example, the earlier-studied aminopropylsilica column (according to the number of aromatic rings) (*12*, *13*) but is somewhat more reproducible regarding retention times than the aminopropylsilica column. The selectivity of the PYE column for planar aromatic compounds has been shown previously by Haglund et al. (*14*).

Apart from the two columns, the HPLC fractionation system consisted of two pumps: one Hitachi L-6200 Intelligent pump equipped with a gradient unit and one additional Hitachi L-6000 pump. The operation of the additional pump was controlled by the L-6200 pump microprocessor. Furthermore, the system was composed of a Hitachi L-4200 UV−VIS detector operating at 237 nm and a Rheodyne 7125 valve injector with a 180-μL loop. The column switching system was built of Rheodyne type 70 pneumatically-operated switching valves. This column switching system has been described earlier by Zebühr et al. (*15*).

The samples were injected into the HPLC system on the nitro column with *n*-hexane as mobile phase at a flow rate of 1.0 mL/min. Here, three fractions were obtained, of which one was further separated on the PYE column. The first fraction (containing aliphatic/monocyclic aromatic compounds) (I) eluted, with the two columns isolated from one another, directly from the nitro column to the sample vial within 4.2 min. The second fraction eluting from the nitro column (4.2−13.5 min) contained aromatic com-

pounds with two aromatic rings in their chemical structure (e.g., PCBs and PCDD/Fs), here referred to as the dicyclic aromatic fraction. This fraction was switched onto the PYE column for further separation into the final fractions II, III, and IV. After the dicyclic aromatic fraction had eluted from the nitro column, the two columns were again isolated from one another. The isolated nitro column was then eluted in the reverse-flow direction (13.5−30 min), and during this time the PAHs (fraction V) were eluted in one narrow peak within 25−29 min. Since two-ringed PAHs and also partially three-ringed PAHs elute in the dicyclic aromatic fraction (together with PCBs and PCDD/Fs), fraction V was analyzed for PAHs with four or more rings (see GC/MS analysis). The dicyclic aromatic fraction was further separated on the PYE column and provided three fractions (II, III, and IV). The first one, eluting with *n*-hexane within 4.2−14 min, contained the mono-tetra-ortho PCBs (II). Secondly, the non-ortho-PCBs (III) were eluted with *n*-hexane within 14−20 min. When all the PCBs had eluted, the PYE column was back-flushed with dichloromethane in order to elute the PCDD/Fs (fraction IV) (20−35 min). The flow rate was 1.0 mL/min during the separation, except during elution of the PCDD/Fs, when the flow rate was increased to 1.5 mL/min. The temperature of the PYE column was kept at 0 °C during the forward fractionation and then raised to 25 °C during back-flush of the PCDD/Fs. After the PCDD/F fraction had eluted, the PYE column was washed with 10 mL of dichloromethane and 10 mL of *n*-hexane in the reverse-flow direction, and the nitro column was washed with 15 mL of *n*-hexane in the reverse-flow direction immediately after the PAHs had eluted.

GC/MS Analysis. All fractions (except fraction I) obtained from the HPLC system were analyzed by GC/MS. Prior to the GC/MS analysis, the solvents were volume reduced, and the analytes were redissolved in 10−50 μL of toluene, containing the recovery estimation standards for the particular fraction.

Five ^{13}C-labeled mono-di-ortho-PCBs (nos. 52, 101, 118, 138, and 180) and three ^{13}C-labeled non-ortho-PCBs (nos. 77, 126, and 169) were added to fractions II and III of the standard mixture for recovery estimations. To fractions IV and V obtained from injection of the standard mixture, ^{13}C-labeled 2,3,4,7,8-PnCDD and pyrene-d_{10}, respectively, were added for recovery estimations. The recoveries of a selection of PCBs in fraction II of the Aroclor 1254 solution were estimated after the addition of five ^{13}C-labeled mono-di-ortho-PCBs mentioned above. To fraction III (containing the previously added ^{13}C-labeled non-ortho-PCBs) of the Aroclor 1254 solution, ^{13}C-labeled 2,3,3′,4,4′-PCB (IUPAC No. 105) was added prior to recovery estimations. The recoveries of a range of tetra-octa-chlorinated PCDD/Fs in the electrostatic filter precipitate solution were determined after the addition of eight ^{13}C-labeled PCDD/Fs (listed previously) to fraction IV obtained from the HPLC system. To the herring oil samples, the harbor porpoise blubber samples, the air samples, and the sediment samples, ^{13}C-labeled PCB no. 153/(no. 118 to air samples) and PCB no. 105 were added to fractions II and III, respectively, for recovery estimations of the internal standards previously added to these environmental samples. The recoveries of the labeled PCDD/F standards in fraction IV of the herring oil samples and the harbor porpoise blubber samples were estimated after the addition of ^{13}C-labeled 2,3,4,7,8-PnCDD. To fraction V of the air samples, pyrene-d_{10} was added for recovery estimations of the earlier added PAH standards. The concentrations of some selected PCB congeners and PCDD/F congeners (see Figure 3) in the Aroclor 1254 solution and the electrostatic filter precipitate solution, respectively, were established after the addition of eight ^{13}C-labeled PCBs and eight ^{13}C-labeled PCDD/Fs mentioned above.

The analyses of mono-tetra-ortho-PCBs and PAHs were performed on a Hewlett-Packard (HP) 5890 Series II coupled to a HP 5971A low-resolution mass selective detector. The samples were injected (1 μL) splitless into the GC at an injector temperature of 280 °C. A CPSil8 CB fused-silica column (Chrompack, 25 m × 0.25 mm i.d., 0.12-μm film thickness) was used temperature-programmed from a starting temperature of 100 °C, kept for 2 min, followed by an increase with 7 °C/min to 290 °C, and kept for 10 min for the PAHs and from a starting temperature of 100 °C, kept for 2 min, followed by an increase with 25 °C/min to 170 °C, then 3 °C/min to 230 °C, and finally 20 °C/min to 290 °C, and kept for 5 min for the PCBs. The samples were monitored in selected ion monitoring (SIM) mode, recording two isotopic molecular ions for the mono-tetra-ortho-PCBs and the molecular ion for the PAHs. A Fisons 8060 GC, equipped with the same column as mentioned above and connected to a Fisons MD 800 low-resolution mass spectrometer, was used for the analysis of the non-ortho-PCBs. The non-ortho-PCB fractions (III) were introduced by on-column injection at a temperature of 100 °C, kept for 2 min, and followed by the same column program as for the mono-tetra-ortho-PCBs. The analyses of the mono-tetra-ortho-PCBs, the non-ortho-PCBs, and the PAHs were all performed in the electron impact (EI) mode at an ionization voltage of 70 eV. GC/high-resolution MS (HP 5790A Series /VG 70E) was used to analyze the fractions containing the PCDD/Fs (IV). The analytes were injected splitless at an injector temperature of 270 °C and chromatographed on a SP-2331 capillary column (Supelco, 30 m × 0.25 mm, 0.20-μm film thickness) using a GC oven temperature program starting at 110 °C (2 min), followed by a 10 °C/min increase to 200 °C, and directly followed by a 4 °C/min increase to 265 °C (10 min). The VG 70E MS was operated at a resolution of 10 000 with perfluorokerosene lock masses for selected ion monitoring (SIM) of PCDD/Fs in four groups. Two isotopic molecular ions were monitored for each analyte. The MS was operating in EI mode at an ionization voltage of 28 eV.

Results and Discussion

The recoveries of the standared mixtures and the environmentally collected samples are summarized in Figures 2−6. In all figures, the mean recoveries (%), the standard deviation (SD) and/or the range of the recoveries [minimum recovery (%), maximum recovery (%)] are presented. The concentration ranges of the analytes in the different fractions are also given for the samples that were analyzed (see Table 1).

The mean recoveries of the standard mixtures were generally good for all compounds and ranged from 76 to 96% for the PCBs (Figure 2a), from 83 to 97% for the PCDD/Fs (Figure 2b), and from 81 to 98% for the PAHs (Figure 2c). Among the PCB standard compounds, the mean recovery of 2,3,3′,4,4′,5-HxCB (IUPAC No. 157) was the lowest of all compounds and the SD was the highest, which is probably the result of the fact that this PCB is one of the last eluting mono-ortho-PCBs, which elute just before the non-ortho-PCBs are collected. The larger SD of the recoveries for the

TABLE 1

Concentration Ranges and Total Amount/Compound Injected on HPLC for Compounds Analyzed in Different Samples[a]

sample	no. of samples	compd	concn range	unit	total amount/congener injected on HPLC	unit
Aroclor 1254 solution	2	ortho-PCBs	160−4900	ng/g	11−340	ng
		non-ortho-PCBs	1.8−290		0.12−20	
electrostatic filter precipitate solution	2	PCDD/Fs	0.12−140	ng/g	50−5000	pg
herring oil	3	ortho-PCBs	22−160	ng/g lw	2.2−160	ng
		non-ortho-PCBs	0.12−1.5		12−1500	pg
harbor porpoise blubber	2	ortho-PCBs	110−8500	ng/g lw	55−4200	ng
		non-ortho-PCBs	27−380	pg/g lw	13−190	pg
		PCDD/Fs	0.79−1.8	pg/g lw	0.40−0.90	pg
air	2	ortho-PCBs	0.51−2.8	pg/m^3	1.1−6.2	ng
		non-ortho-PCBs	0.012−0.11		26−240	pg
		PAHs	32−550		70−1200	ng
sediment	5	ortho-PCBs	0.33−79	ng/g dw	0.82−280	ng
		non-ortho-PCBs	0.027−5.4		6.7−19000	pg

[a] lw, lipid weight; dw, dry weight.

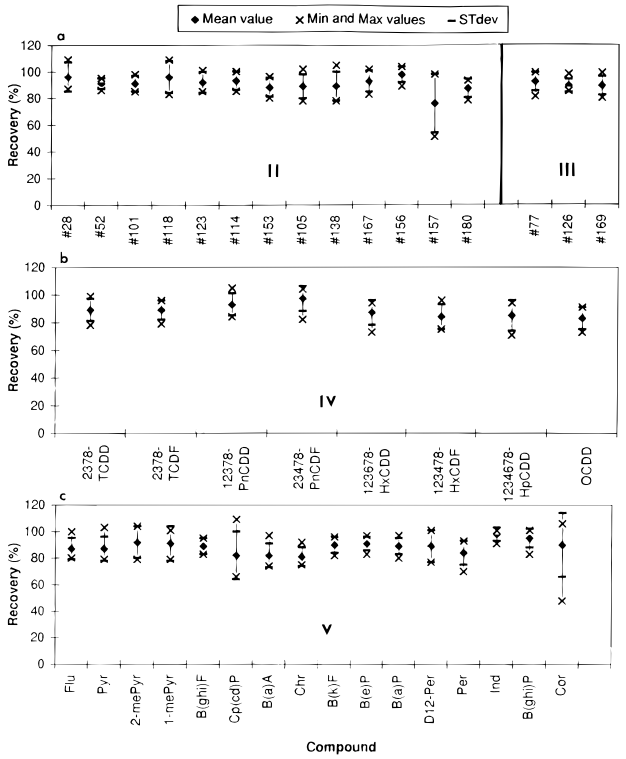

FIGURE 2. Mean recoveries (%), standard deviations (SD), minimum recoveries (%), and maximum recoveries (%) (min and max values) of (a) the 15 PCBs, (b) the 8 PCDD/Fs, and (c) the 16 PAHs in the standard mixture. PAH abbreviations are listed in Table 2. The roman numerals refer to the number of the particular HPLC fraction.

two PAH compounds, cyclopenta[*cd*]pyrene and coronene, are probably due to compound instability and discrimination in the GC inlet system, respectively, rather than discrimination in the HPLC fractionation.

In the Aroclor 1254 solution, a wide range of PCBs with different degrees of chlorination were selected for recovery estimation. The result showed a relatively low degree of discrepancy between the recoveries of the different PCB congeners, which varied between 66 and 95% (Figure 3a). The mean recoveries of the PCB congeners in the PCB standard mixture were generally higher than those of the Aroclor 1254 solution and varied less, which might be due to the much more complex content of PCBs in the Aroclor 1254 solution.

TABLE 2

PAH Abbreviations

Flu	fluoranthene
Pyr	pyrene
2-mePyr	2-methylpyrene
1-mePyr	1-methylpyrene
BghiF	benzo[*ghi*]fluoranthene
CpcdP	cyclopenta[*cd*]pyrene
BaA	benz[*a*]anthracene
Chr	chrysene
BkF	benzo[*k*]fluoranthene
BeP	benzo[*e*]pyrene
BaP	benzo[*a*]pyrene
Per	perylene
Ind	indeno[1,2,3,-*cd*]pyrene
BghiP	benzo[*ghi*]perylene
Cor	coronene

The 15 selected PCDD/F congeners, with different degrees of chlorination, in the electrostatic filter precipitate showed good mean recoveries that ranged from 50 to 114% (Figure 3b). However, the lower recoveries of the TCDFs (total) were probably due to some early eluting TCDFs in fraction III, i.e., the non-ortho PCB-fraction.

The recoveries of the labeled standards in the environmentally collected samples were also generally good. The mean recoveries of the ^{13}C-labeled PCBs in the herring oil, harbor porpoise blubber, air, and sediment samples varied between 74 and 94%, 67 and 88%, 77 and 91%, and 61 and 81%, respectively (Figure 4a−d), while the mean recoveries for the ^{13}C-labeled PCDD/Fs ranged between 69 and 99% in the herring oil samples and between 65 and 100% in the harbor porpoise samples (Figure 5a,b). In the air samples, the mean recoveries of the PAH internal standards ranged between 68 and 81% (Figure 6). It should be noted, however, that for the above-mentioned samples the standards were added prior to extraction, which implies that losses have occurred during both workup and HPLC separation.

By keeping the PYE column at 0 °C during the fractionation, the separation efficiency was considerably increased compared to keeping it at room temperature. Also, by raising the temperature to 25 °C during back-flush of PCDD/Fs, the recoveries were improved and the time of elution was shortened for these compounds. But still, to obtain optimum performance of the PYE column, lipids must be removed almost completely, or the separating properties of the column will be deteriorated. Residuals of

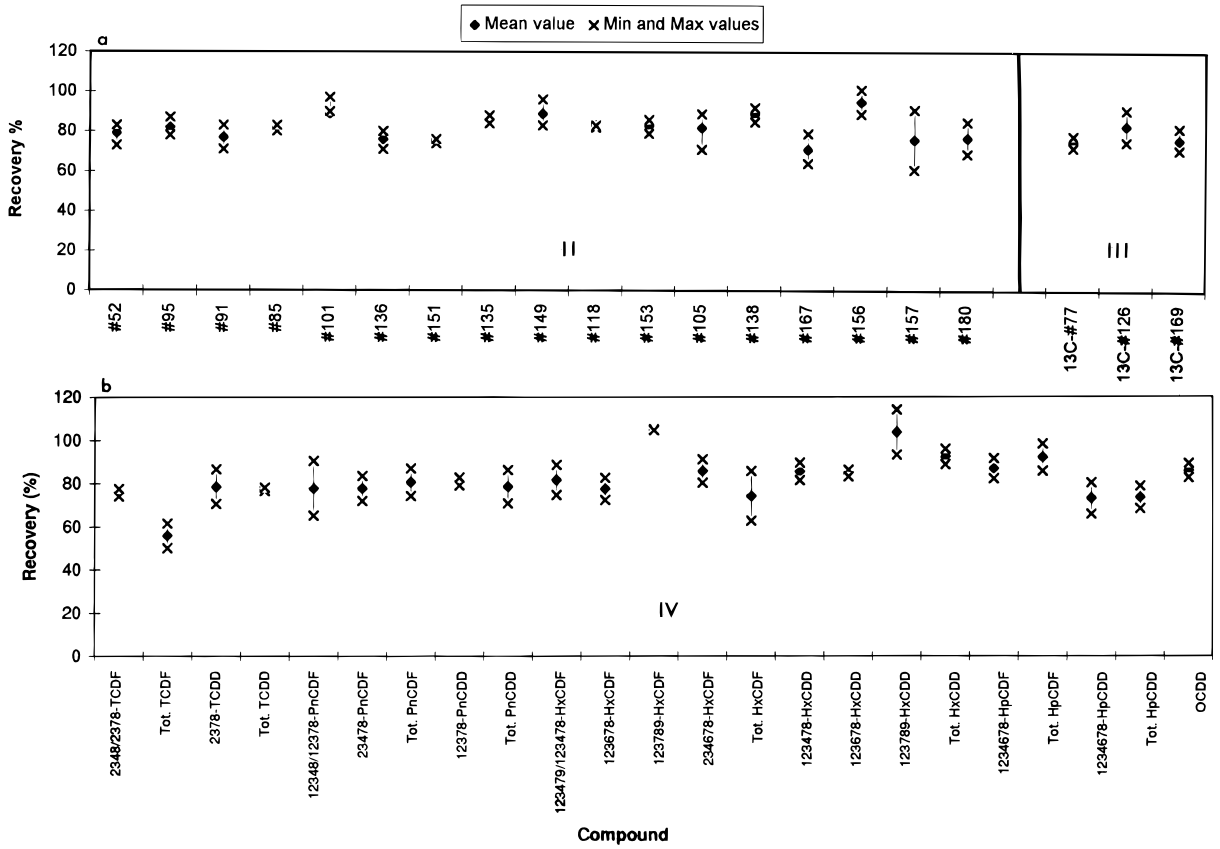

FIGURE 3. Mean recoveries (%), minimum recoveries (%), and maximum recoveries (%) (min and max values) of (a) 20 PCBs in the Aroclor 1254 solution and (b) 15 PCDD/Fs in the electrostatic filter precipitate solution. The concentration ranges of the congeners and the total amount/congener injected are given in Table 1. Two significant figures are valid.

lipids, affecting the retention of the PCBs on the PYE column, might explain the somewhat lower recoveries for non-ortho-PCB no. 77 (Figure 4b) and 2,3,7,8-TCDF (Figure 5b) in one of the harbor porpoise blubber samples. The similar sample matrix effects due to lipids have been reported earlier by Haglund et al. (*16*), who recommended gel permeation chromatography (GPC) as a useful tool to separate the lipids from the PCBs before separation on HPLC. The two different lipid-rich samples the herring oils, and the harbor porpoise blubber samples showed dissimilar behavior when applied to the SiO_2 columns. The lipids in the herring oil samples were relatively easy to remove, while the lipids in the harbor porpoise blubber samples were extremely hard to remove.

The first fraction (I) eluting from the nitro column was defined as the fraction collected from the start to the elution of toluene. Since this paper has focused on sample preparation for subsequent analysis of compounds like PCBs, PCDD/Fs, and PAHs, fraction I has not been evaluated further in terms of recovery of the compounds found in this fraction.

Interfering compounds such as PCBs and polychlorinated naphthalenes (PCNs) can be major obstructions to accurate quantitation of non-ortho-PCBs and PCDD/Fs. In many cases, even if the interferences are not totally removed, the problem can be solved with analysis by high-resolution GC/MS. If not, additional cleanup steps must be included in the method. In the present study, the non-ortho-PCB fractions were examined for interferences from other PCBs by comparing the isotopic patterns of the compounds and checking for co-eluting PCBs with higher chlorination. The peak areas of interfering PCBs, mainly PCB no. 110/136 with PCB no. 77, were less than 10% of the PCBs of interest in all samples analyzed, which makes the interfering contributions low enough to be disregarded. The results from the non-ortho-PCB fractions where interferences of PCNs could be suspected were confirmed by high-resolution GC/MS.

HPLC methods used for the isolation of planar non-ortho-PCBs and PCDD/Fs from nonplanar compounds often include an activated carbon column (*7*, *8*). This column has some drawbacks, such as low efficiency and broad elution profiles with severe tailing. An aromatic solvent is also often used to elute the compounds from the carbon column, which makes the evaporation time-consuming and UV detection impossible. The present HPLC method has taken advantage of earlier experiences obtained from an automated system, where an amino-propylsilica column was combined with an activated carbon column (*15*). The advantages of the new system are better long-term stability for the nitro column compared to the amino column and better chromatographic properties of the PYE column compared to the carbon column.

The sensitivity of the PYE column for sample matrix has to be considered when selecting a cleanup procedure for the samples prior to HPLC fractionation. The fact that the nitro and PYE columns are analytical columns must also be accounted for when determining the sample sizes to run on the system. Additionally, caution must also be taken if highly electronegative solvents are introduced to affinity columns (e.g., the PYE column) during separation since it may cause the column to act unpredictably (*17*), and hence the PYE column must be washed thoroughly before a new sample is entered into the system.

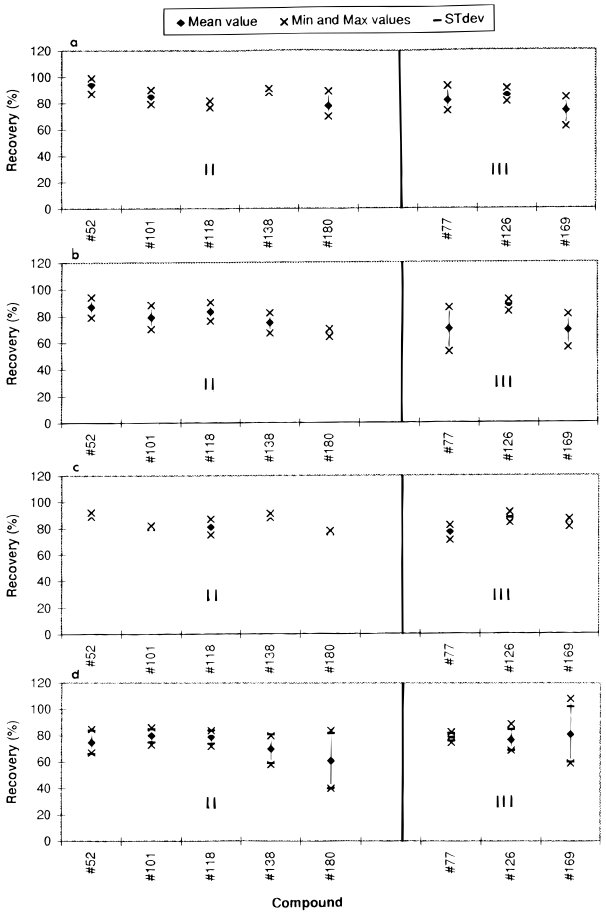

FIGURE 4. Mean recoveries (%), standard deviations (SD), maximum recoveries (%), and minimum recoveries (%) (min and max values) of the ^{13}C-labeled PCBs in fractions II and III in (a) the herring oil samples, (b) the harbor porpoise blubber samples, (c) the air samples, and (d) the sediment samples. The concentration ranges and the total amount/congener injected on the HPLC are given in Table 1.

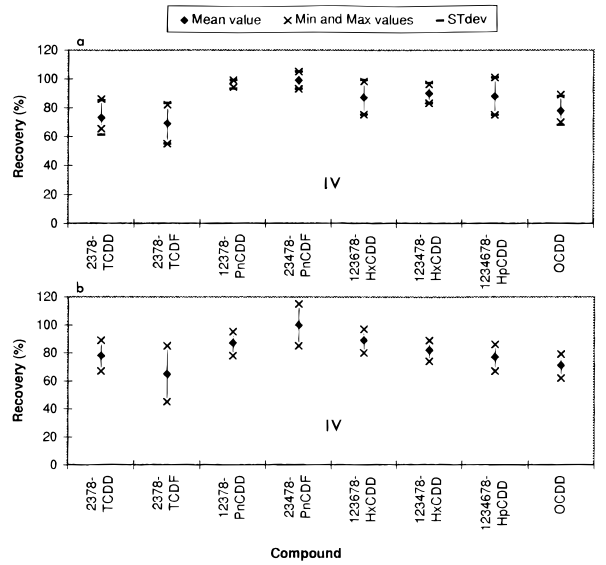

FIGURE 5. Mean recoveries (%), standard deviations (SD), maximum recoveries (%), and minimum recoveries (%) (min and max values) of the ^{13}C-labeled PCDD/Fs in fraction IV in (a) the herring oil and (b) the harbor porpoise blubber samples. The concentration ranges and the total amount/congener injected on the HPLC for the harbor porpoise blubber samples are given in Table 1.

The two-dimensional HPLC separation method presented here proved to be a rapid and effective fractionation method for the subsequent analysis of PCDD/Fs, PCBs, and PAHs. Five fractions (aliphatic/monocyclic aromatic compounds, mono-tetra-ortho-PCBs, non-ortho-PCBs, PCDD/Fs, and PAHs) were provided from the HPLC system, ready for injection on GC/MS. The separation was completed in 40 min and required less than 70 mL of solvents.

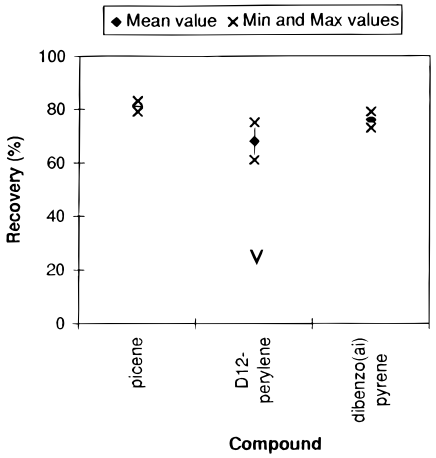

FIGURE 6. Mean recoveries (%), standard deviations (SD), maximum recoveries (%), and minimum recoveries (%) (min and max values) of the PAH internal standards in the air samples. The concentration ranges and the total amount/compound injected on the HPLC are given in Table 1.

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