PYE [2-(1-pyrenyl)ethyldimethylsilylated silica] Column HPLC and HR-GC-(micro) ECD in the Accurate Determination of Toxic Co-planar PCBs and Polybrominated Diphenyl Ethers (PBDEs)

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Measurement of toxicologically relevant polychlorinated biphenyl (PCB) congeners such as non-*ortho* (IUPAC#) 77, 81, 126, 169 and mono-*ortho* 105, 114, 118, 123, 156, 157, 189 and di-*ortho* 170, 180 and polybrominated diphenyl ethers (PBDEs) such as 47, 66, 85, 99, 100, 138, 153, 154 in environmental samples become almost mandatory in several countries now. However, most of the available methods involve expensive instrumentations such as HRGC-HRMS or ECNI-LRMS, apart from expensive extraction and clean-up (with large volume of solvents) steps. A method has been devised combining the analytical separation power of PYE [2-(1-pyrenyl)ethyldimethysilylated silica] column HPLC and high-resolution gas chromatographic techniques including micro-electron capture detection (ECD) and two dimensional gas chromatograpy-ECD techniques to determine these eco-toxic substances at parts-per-trillion (ppt) levels. This combination resolves co-elution of congeners that occur in disproportionate ratios (*e.g.* CB-110 and -77) and allows accurate congener-specific determination of target compounds. This method is cost effective as it requires only hexane, that in small quantities (10 mL) and GC-ECD. The elution and analysis time are optimized to less man hours. This method is effectively utilized in the analysis of co-planar PCBs and PBDEs from archived solvent extracts of samples previously analyzed for pesticides and PCBs. Structure based separation of contaminant classes improves GC-ECD determination at ppt levels.

Key Words : PYE [2-(1-pyrenyl)ethyldimethylsilylated silica] column HPLC, Coplanar PCBs, MDGC-ECD, PBDEs, Coastal monitoring

Introduction

Polychlorinated biphenyls (PCBs) are ranked as one of the most important environmental pollutants of modern times. Polybrominated diphenyl ethers (PBDEs) with similar environmental behavior (hydrophobic, lipophilic, thermally stable) represent another class of important contaminant to monitor. Growing evidences suggest that they are wide-spread global environmental pollutants like PCBs that are capable of bioaccumulation in food chain.[1,2]

Environmental analysis of PCBs in the last decades has seen growth and perfection. Technical advancement in environmental analytical chemistry such as high resolution gas chromatography, high precision liquid chromatography has greatly improved the congener-specific determination of polychlorinated biphenyls (PCBs) at trace levels in the environmental matrices. An attempt was made in 1980s to determine hitherto undetermined ultra-trace level co-planar PCB congeners, 33'44-tetra (77); 33'44'5-penta (126) and 33'44'55'-hexa (169) in various environmental matrices such as sediments, trophic organisms, humans and commercial PCB mixtures using charcoal chromatography and HRGC-ECD, HRGC-MS.[3-7] Another major development in 80s is the application of multidimensional high resolution gas chromatography for PCB analysis.[8] This technique enabled separation of pre-selected unresolved (co-eluting) compounds from a non-polar high resolution capillary column to a second column of different polarity without any sample loss. This technique has helped greatly to i) understand the accurate composition of commercial PCB mixtures such as Aroclor and Clophen;[9] Kanechlor, Phenoclor and Sovol,[10] ii) identify impurities in technical grade (99% pure) PCB standards as well as environmental interferences in the analysis of open ocean water[11] and iii) verify the separation efficiency of several charcoal methods employed in the determination of toxic non-*ortho* PCBs.[12]

Reliable analysis of PCBs in environmental samples using this sensitive technique was aided by a Nucleosil HPLC clean-up method developed for this purpose.[13] These methods together have shown that proper distillation of commercial solvents, proper storage of solvents and sample extracts, minimal usage of solvents in the analysis are the essential steps in avoiding co-contaminants.[14] This is especially true i) in the analyses of water and suspended particulate matter where the concentration of Chlorinated biphenyls are rather very low and ii) in the determination of non-*ortho* chlorinated biphenyls which are at least two to three orders below the most persistent PCBs such as 22'344'5'-(PCB-138), and 22'44'55'-(PCB-153).

Almost all the procedures on toxic PCBs use large volume of organic solvents such as dichloromethane, benzene, ethyl acetate etc. there by introducing co-contaminants in the analysis. Environmental samples as such have enormous co-contaminants coming from different environmental matrices.

Additionally, there is a measurable difference in the content of lower chlorinated biphenyls, non-*ortho* PCBs and other congeners in the environmental samples, for example, sea water (solution) contains more of lower chlorinated biphenyls whereas biological samples-depends on their trophic level-contain more of higher chlorinated biphenyls (lower chlorinated biphenyls are metabolized in higher trophic organisms).

MDGC-ECD analysis of commercial PCBs has shown that among the theoretically possible 209 CB congeners only 132 congeners are quantifiable.[9] Co-elution of PCBs is a common phenomenon and surprisingly only 56 congeners elute as baseline separated single peaks in high resolution capillary column such as SE-54. Several of the toxic non-*ortho*, mono-*ortho* and di-*ortho* PCBs elute not as base-line separated peaks and hence their determination needs special separation methods such as multi dimensional gas chromatography (MDGC-ECD).

Several charcoals and graphite are in use to enrich and separate the toxic PCBs from the rest of PCBs and other co-contaminants.[15] Indeed, these methods enhance the determination of non-*ortho* PCBs greatly. However, they do not offer 100% separation.[12] Problems of co-elution persist, for example, for the following congeners (co-eluting congeners in brackets): CB-77 (-110); -126 (-129, -178); -81 (-87, -97, -115); -105 (-132, -153); -118 (-123, -149); -156 (-171, -202).

These observations suggest that structure-dependent separation of PCB before final determination will greatly enhance the congener-specific measurement in an accurate and unambiguous way.

2-(1-Pyrenyl)ethyldimethylsilylated silica (PYE) column has been shown to separate non-*ortho* PCBs from other PCBs using only hexane.[16] This column has been experimented with some environmental samples as well.[17] This stationary phase separates PCBs with different π electron densities and chiral properties. An attempt was made in Germany at the Institute for Marine Research (IFM), University of Kiel to incorporate electron donor-acceptor (EDA) high-performance liquid chromatography (HPLC) with MDGC-ECD.

This scheme involves minimum solvent volume and less analytical time in separating i) early eluting, less concentrated lower chlorinated biphenyls from ii) dominant and persistent congeners and iii) all the toxic mono- and non-*ortho* chlorinated biphenyls. We checked the efficiency of this column (first author at the above mentioned Institute) using low concentration (at pg levels) spikes and real environmental samples that were previously determined using MDGC-ECD. The PYE column separation of PCBs on the basis of structural properties added an additional dimension to the already existing multidimensional gas chromatography. This technique, originally developed in Germany at the Institute of Marine Research, University of Kiel has been adapted with slight modification in Korea (Korea Ocean Research and Development Institute-KORDI) to incorporate PBDEs. PBDEs have been measured successfully from archived extracts that were primarily analyzed for PCBs in Korean coastal samples. PYE column HPLC in combination with GC-micro ECD technique offers an alternative, yet most accurate and cost effective method for the measurement of toxic PCBs and PBDEs.

Materials and Methods

PCB studies (IFM, Kiel). Commercially available hexane was distilled using a special distillation unit with 130-150 cm column. The distillation occurred in a pure nitrogen atmosphere and the distilled solvent was stored in −20 °C solvent compartments in inert atmosphere (N^2). 100 mL batch of solvent was tested every time before analysis for any background interference (this in practice is ~2000 fold concentration; the interfering peaks should have < 0.1 pg equivalent of PCB in 2 μL injection)

HPLC was performed with Pump-Constametric III with Rheodyne injector and the flow rate was 1 mL of hexane per minute on Cosmosil 5-PYE column [2-(1-pyrenyl) ethyldimethyl silylated silica gel], 250 × 4.6 mm, particle size 5 mm, Nacalai Tesque, Kyoto, Japan. The eluate was collected in 4 fractions totaling 16 mL. The dead volume was 3.5 mL. The first fraction: 3.5-5.0 mL; second fraction 5-7.5 mL; third fraction 7.5-16 mL. These fractions were subsequently cooled (0 °C) and concentrated using vacuum flash evaporator (using N_2) and analyzed in high resolution MDGC-ECD.

This was performed using Fison 8000 GC-ECD with moving capillary stream switching (MCSS) technique. The gas chromatograph was equipped with an on column injector, two columns in two independent ovens and two ^{63}Ni electron capture detectors. Apolar SE-54 (50 m, 0.25 mm i.d.) was placed in the first oven, and a more polar OV-210 (30 m, 0.32 mm i.d.) in the second oven. Gas pressure (H_2) 1.1 bar and 0.6 bar. Temperature programming conditions were: first column from 50 °C (1 min) to 160 °C (at 25 °C min^{-1}) and up to 250 °C at 5 °C min^{-1} and second column kept at 100 °C until 25 min after injection, and increased to 180 °C at 25 °C min^{-1} and then to 250 °C at 5 °C min^{-1}.

Polychlorinated biphenyl congeners co eluting from the first column can be separated on the second column. Quantitation was carried out on the basis of a special mixer of CB standard (99.9% pure, Promochem, FRG) that contained 45 individual PCBs ranging from 4-50 pg/mL belonging to all structural classes *i.e.* non-*ortho* to tetra-*ortho* chlorine substituted PCBs (Table 1).

PBDE studies (KORDI, Korea). To characterize the elution profile of persistent PBDEs using PYE column HPLC, we purchased a certified reference material mix of 9 PBDE congeners recommended in Lake Michigan Study from AccuStandard Inc., USA. They are 244'-tri (28), 22'44'-tetra (47), 23'44-tetra (66), 22'344'-penta (85), 22'44'5-penta (99), 22'44'6-penta (100), 22'344'5'-hexa (138), 22'44'55' (153), 22'44'56'-hexa (154) polybrominated diphenyl ethers at equal concentrations (approximately 10 μg/mL). The PYE column was characterized

anew to accommodate all the co-planar PCBs and the 9 persistent PBDE congeners in one fraction. The new elution profile consisted of three fractions: 0-6 mL; 6-16 mL; and 16-26 mL hexane. All the four co-planar PCBs, namely 33'44-tetra (77), 344'5-tetra (81), 33'44'5-penta (126), and 33'44'55'-hexa (169) biphenyls and 8 out of 9 PBDEs eluted in the second fraction (6-16 mL hexane).

Table 1. PCB standard mixture and the concentration of individual congeners grouped on the basis of their elution in PYE HPLC

IUPAC No.	Structure	Conc. pg/μL	No. of *ortho* Cl	Co-elution
	Fraction I category			
8	2,4'	17.5	1	5
18	22'5	12	2	17,15
28	244'	9.1	1	31
31	24'5	10.6	1	28
44	22'35'	10.7	2	
49	22'45'	12.1	2	
52	22'55'	17.3	2	
66	23'44'	11	1	95
92	22'355'	10.6	2	
99	22'44'5	9.7	2	
101	22'455'	18.5	2	90
129	22'33'45	9.7	2	126,178
141	22'3455'	9.6	2	179
149	22'34'5'6	12.1	3	123,118
183	22'3445'6	10.3	3	
187	22'34'55'6	16.1	3	
202	22'33'55'66'	10.6	4	156,171
	Fraction II category			
70	23'4'5	13.9	1	
74	244'5	9.7	1	
95	22'3'56	11.4	3	66
110	233'4'6	11.1	2	77
128	22'33'44'	14.2	2	
132	22'33'46'	11.1	3	105,153
137	22'344'5	12	2	176
138	22'344'5'	27.6	2	158,160
153	22'44'55'	16.7	2	105,132
174	22'33'456'	9.4	3	
177	22'33'4'56	9.5	3	
178	22'33'55'6	9.8	3	129,126
179	22'33'466'	9.3	4	141
180	22'344'55'	32.6	2	
199	22'33'4566'	9.3	4	
	Fraction III category			
77	33'44	10.3	0	110
81	344'5	10.6	0	
126	33'44'5	11	0	129,178
169	33'44'55'	9.2	0	
105	233'44'	11	1	153,132
118	23'44'5	17	1	123,149
123	2'344'5	11.8	1	149,118
156	233'44'5	9.4	1	171,202
157	233'44'5'	4.3	1	173,201
189	233'44'55'	3.4	1	
170	22'33'44'5	13.4	2	190
191	233'44'5'6	2.6	2	
194	233'44'55'6	12.6	2	

Sediment and mussels were collected from two major Bays in Southern Korea, namely, Phohang Bay and Busan Bay. Sediments were taken using a van Veen Grab sampler on board. Approximately the top 2 cm of sediment was taken by a stainless steel spoon and stored in pre-combusted amber glass jar. The samples were immediately frozen by dry ice and transferred to laboratory for analysis. Mussels from port areas were collected manually and transferred in frozen condition to the laboratory in clean glass jars. 30 individuals were dissected and pooled as one sample for analysis. The sediment samples were prepared for PCBs and organochlorine analysis in accordance with previously reported method,[18] but with some minor modifications.[19]

Presence of PBDE and co-planar PCB congeners in samples after PYE HPLC was confirmed using GC-MS. A Gas chromatography (Shimadzu GC-2010)-mass spectrometry (Shimadzu MS QP-2010) system was used for this purpose with the following temperature program: the oven temperature was programmed from 120 °C (1 min) to 160 °C at the rate of 3 °C/min, held for 2 min., then increased to 250 °C at a rate of 5 °C/min, hold for 3 min., and finally increased to 300 °C at the rate of 10 °C/min and held for 5 min. The same temperature program was applied in GC-micro ECD for co-planar PCBs and PBDEs determination as well. The ions that were selected for monitoring (SIM) are given in Table 2.

Results

PCB studies. In the PYE-column HPLC, PCBs eluted after 3.5 mL and a total of 17 PCBs eluted in the first

Table 2. Target ions and confirmation ions for the determination of selected PBDE and PCB congeners in Shimadzu GCMS-QP2010

PBDEs		
Analyte	Target Ion	Confirmation Ion
PBDE 28	406	408; 246
PBDE 47	485.7	483.7; 487.7; 326
PBDE 66	485.7	483.7; 487.7: 326
PBDE 85	563.6	565.6; 561.6; 404; 406
PBDE 99	563.6	565.6; 561.6; 404; 406
PBDE 100	563.6	565.6; 561.6; 404; 406
PBDE 138	643	485; 483; 481
PBDE 153	643	485; 483; 481
PBED 154	643	485; 483; 481
PCBs		
Analyte	Target Ion	Confirmation Ion
CB-77	290	292; 294; 220
CB-81	290	294; 220; 292
CB-126	324	326; 328
CB-169	358	360; 362

fraction *i.e.* 3.5 to 5.0 mL (Table 1). This included all the lower chlorinated biphenyls and congeners with 1-4 *ortho* Cl substitution in the biphenyl rings (Fig. 1). The second fraction (5.0-7.5 mL) contained most frequently occurring persistent congeners, usually with high concentrations in the environmental samples such as CB-110, -128, -138, -153, -174, 180 etc. The toxic mono- and non-*ortho* PCBs eluted exclusively in the 7.5-16 mL fraction (III fraction). All the toxic PCBs with TCDD toxic equivalent factors (TIFs) given by WHO/IPCS eluted in this fraction (Fig. 1).

The recovery of PCBs was in the range of 85 to 120%. The standard mixture contained 27 PCBs with co elution problem. PYE column separated 18 of them in to distinct fractions. The most important among them are PCBs 77/110; 126/129/178; 156/171/202. PYE column achieved where charcoals failed in the separation of PCBs 77/110. Our previous experiment[12] showed that nearly 2% of CB-110 co-eluted with CB-77 after charcoal separation and that would interfere seriously with the determination of CB-77. This problem was solved with PYE column that achieved complete separation of CB-110 from CB-77. The following pairs were not included in the test solution: 137/176; 141/179; 157/173/201. However, it was presumed from the observed efficiency of the PYE column that these PCBs would be separated effectively based on their structural properties.

A few congeners such as 8/5; 18/17/15; 28/31; 101/90; 138/158/160 were not separated in the PYE column because they had the same number of *ortho* Cls. Their accurate determination, however, needed MDGC-ECD or HRGC-HRMS.

We tested the separation efficiency and the overall recovery using actual environmental samples in which PCBs have been measured previously. These belong to three different environmental matrices namely, sea water (solution), sea water particulate matter and kidney from a bird (Fig. 2). The recovery of PCBs was high (85-110%) and separation was not affected by any difference in the environmental matrix. In the sea water solution, only the soluble portion of PCBs, namely, lower chlorinated biphenyls with low K_{ow} existed. By fractionating these congeners in the first fraction PYE HPLC proved useful in the water analysis. The particulate material in the sea water, instead, accumulate lipophilic, persistent CB congeners such as CB-138, 177 and 180. Figure 2 shows that PYE column separates these congeners in the second fraction, thus, aiding water analysis. Non-*ortho* co-planar PCBs occurs in an order or two lower than other dominant congeners and hence a complete

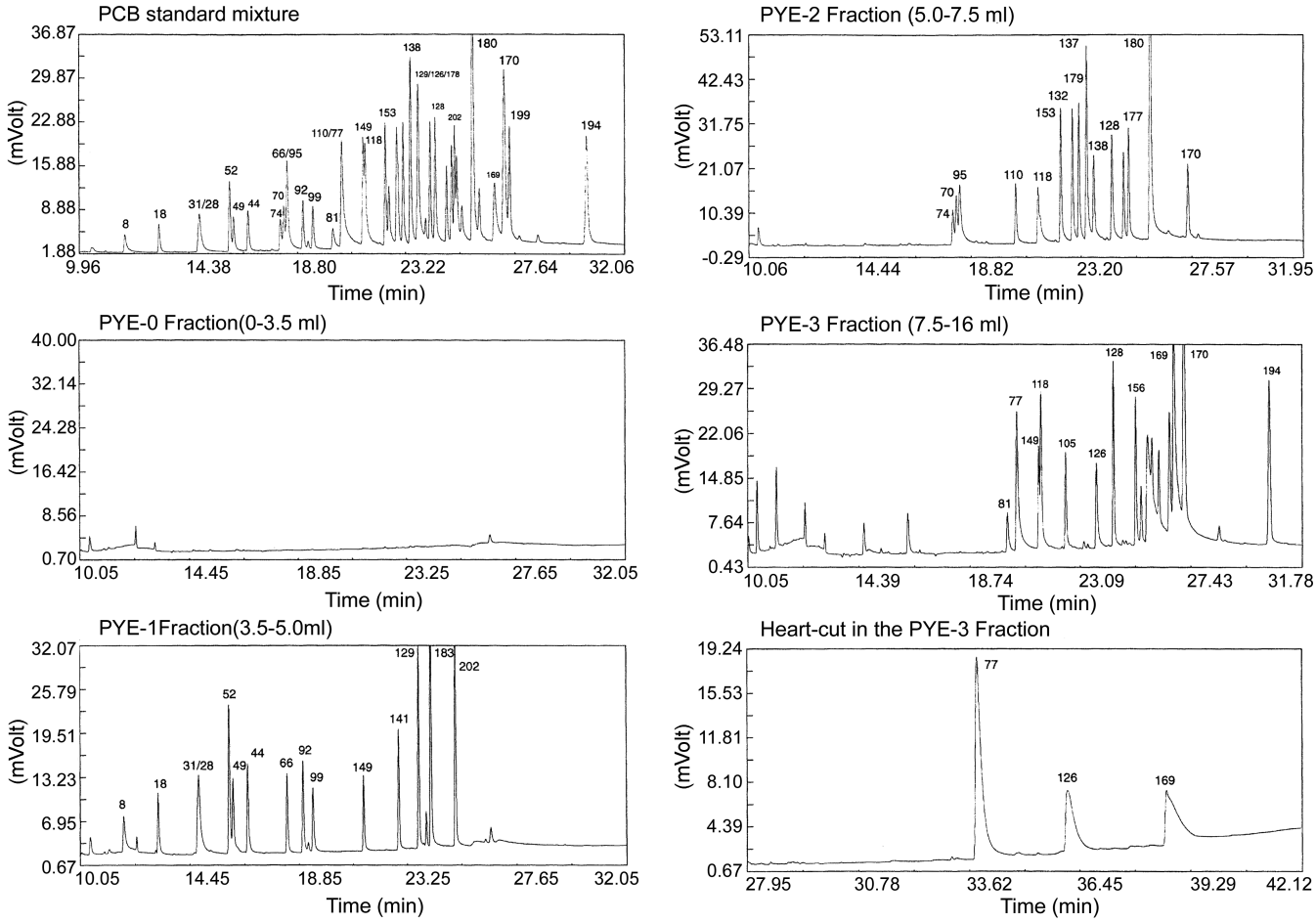

Figure 1. GC-ECD chromatograms of a test solution. 0 fraction (0-3.5 mL); 1 fraction (3.5-5.0 mL); 2 fraction (5.0-7.5 mL); 3 fraction (7.5-16 mL) and main ECD chromatogram of a heart-cut for PCBs-77, -126 and -169. No co-elution from PCBs-110, -129 and -178 was noted.

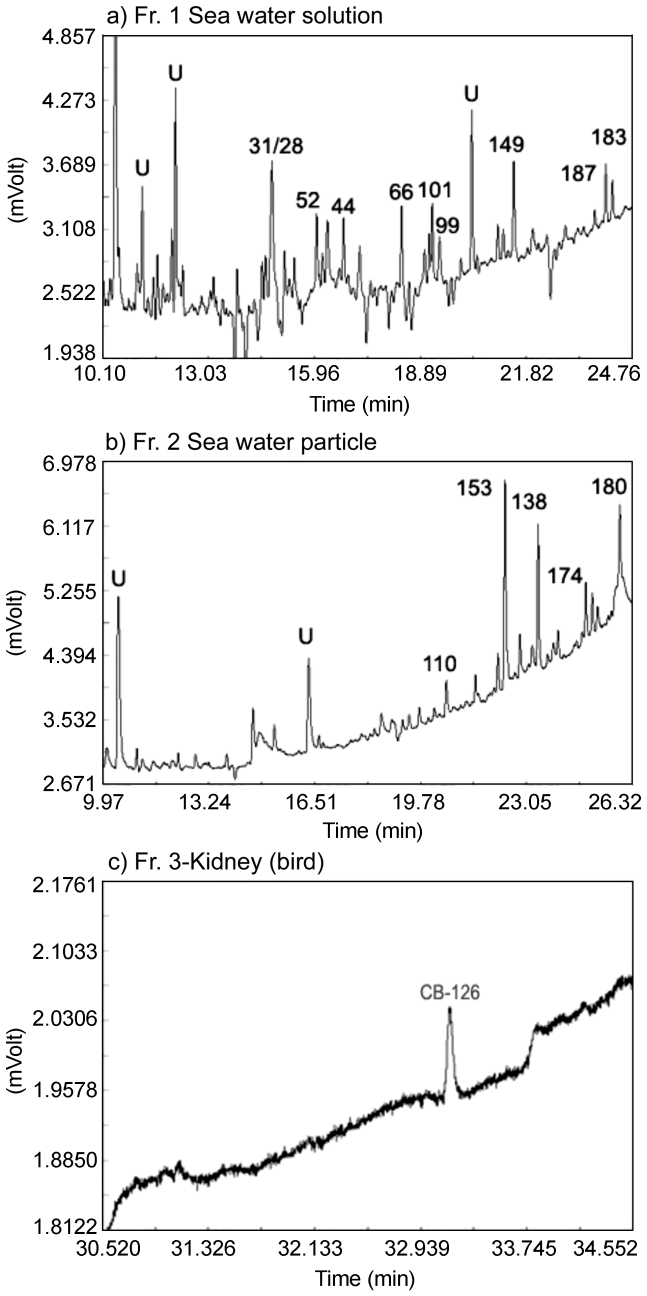

Figure 2. GC-ECD chromatograms of environmental samples. Portions from a sensitive range. a) an extract from German coastal water (solution). U-represent non-PCB peaks of unknown environmental origin, b) suspended particulate matter from German coastal zone and, c) chromatogram after a heart-cut of Aoks (Alkan).

separation of these congeners from the rest aids in the accurate measurement of these toxic PCBs.

PBDE studies. PBDEs and PCBs are similar chemicals. In fact, PBDE congener identification by IUPAC follows PCB numbering system. Hence, it is likely that PBDEs behave in a similar way like PCBs in PYE HPLC. However, by molecular design PBDEs elute later than PCBs in a chromatographic column. In fact, 8 out of 9 PBDEs tested eluted along the 4 co-planar PCBs, *i.e.* later than most other PCBs. The new elution profile was chosen to eliminate most of the PCBs-unlike in IFM study where PCBs were separated into various structural classes-from co-planar PCBs and PBDEs. PCBs were measured in those samples earlier. We used the same extract to obtain co-planar PCBs and PBDEs. PYE HPLC gave us the needed separation of bulk of PCBs in one fraction leaving only co-planar PCBs and target PBDEs with a few mono-*ortho* PCBs that did not interfere in our analysis (Fig. 3). Repeated trials with PBDE (9 congeners) and PCBs (4 co-planar congeners) standards yielded high recovery ranging from 90% to 110%. Environmental samples (sediments and mussels) from two Korean Bays with heavy ship trafficking and industrial inputs were tested with PYE HPLC. GC-MS confirmed the presence of both co-planar PCBs and PBDEs in those samples (see Fig. 4 and 5). 33'44'55-hexachloro biphenyl (169) was added as internal standards in all samples before extraction. Though the original intention was to purchase PCB-196 (22'33'-44'5'6) - that does not occur in appreciable quantities in environmental samples - by serendipity, we got CB-169-a target compound in this analysis - from the company. Hence, it was easy for us to check the recovery and loss of CB-169 in our samples. The typical recoveries of CB-169 in the environmental samples were in the range of 80% to 95%. We also analyzed the commercial PBDE mixture called Bromkal DE-71 using our method. Most interestingly, the GC-ECD chromatogram of mussels resembled closely that of Bromkal DE-71 (see Fig. 6 and 7). The composition of PBDEs in mussels and sediments from Busan Bay was compared with an American study[20] where commercial penta PBDE formulation, bio-solids, flame retardant foam and channel cat fish were studied. There was a striking resemblance between the composition of PBDE in Busan Bay and US environmental samples as seen in Figure 8.

Discussion

PYE column separates the lower chlorinated biphenyls from predominant congeners that occur in environmental samples such as CB-153, -138, -128, -110. This type of separation is rather convenient when scientific scrutiny is on the lower chlorinated PCBs, either in studying their biotransformation or partition behavior in the environment.

This method is especially useful in the determination of non-*ortho* PCBs that occur at ultra-trace level quantities (*i.e.* between 10^{-15} to 10^{-18}). The desired micro-concentration ($\times$ 100 times) of the final extract is now possible because the bulk of other PCBs is conveniently separated out. This applies equally well to the determination of lower chlorinated biphenyls in the environmental samples as well. These PCBs usually have poor response factors due to low detection limits when chromatographed with higher chlorinated biphenyls. This can be avoided now, using a pre-GC separation of extracts using PYE column HPLC.

Thus, PYE column HPLC is very efficient in i) separating less concentrated lower chlorinated biphenyls from more concentrated persistent congeners, ii) in separating co

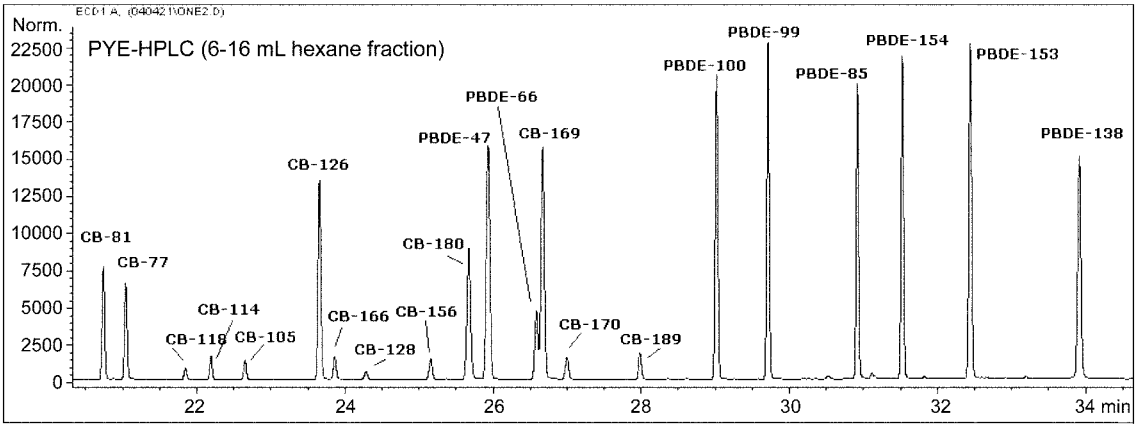

Figure 3. A typical chromatogram of PBDE and co-planar PCB elution, in a slightly modified PYE column chromatography GC-micro ECD method. Peaks are identified based on their IUPAC numbers.

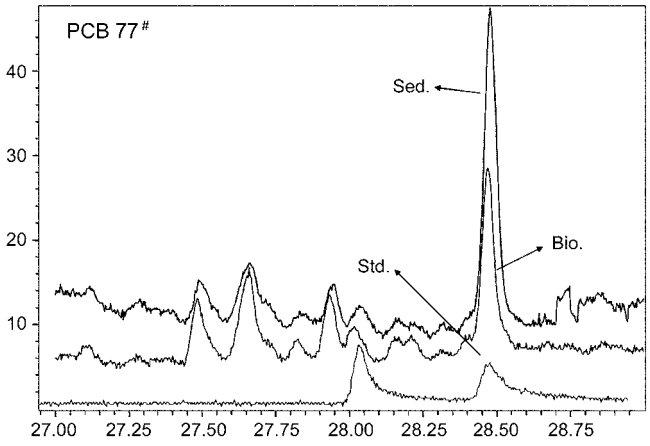

Figure 4. GC-MS mass spectrograms of 33'44-tetra chlrobiphenyl (77) in standard, sediment and mussel.

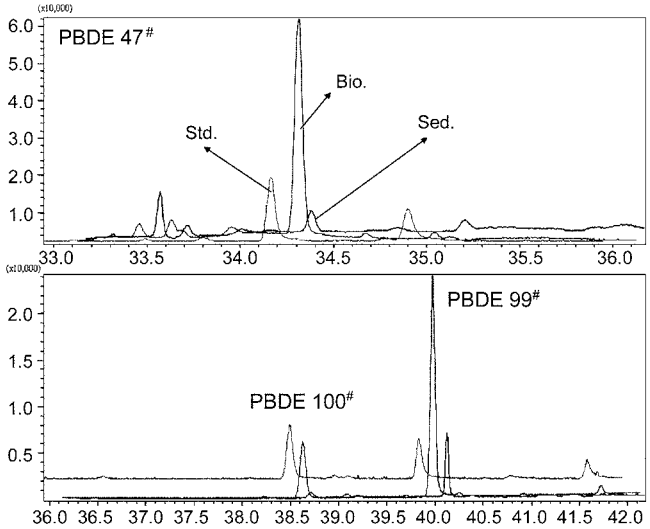

Figure 5. GC-MS mass spectrograms of standard, biological tissue and sediment of PBDEs 47, 99 and 100. The chromatograms are slightly displaced for clarity.

eluting congeners with different structural properties, and iii) the efficiency of PYE column separation is not affected by environmental matrix. Though this method has been effectively used in the analysis of two lots of Aroclor 1254[21] and in coastal sediments, mussels, fish, bird and marine mammal[22] it has never been published in detail as seen in this communication.

The selectivity of the PYE may be explained by a charge-transfer mechanism, in which electron-density acceptor and

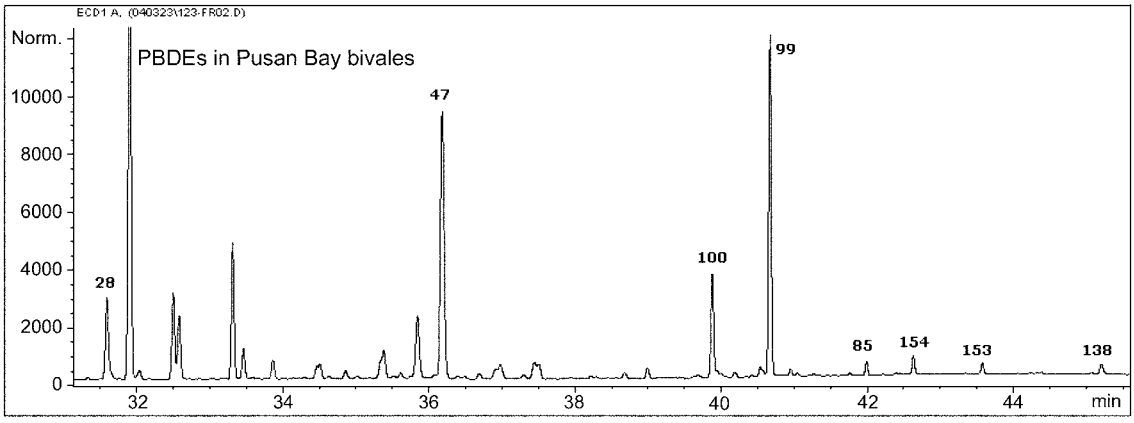

Figure 6. PBDEs in Busan Bay, Korea. The dominant PBDEs such as 99, 47, 100, 153, 154, 85 (in that order) that are present in Bromkal DE-71 commercial mixture (see next Fig.) are determined in mussels as well.

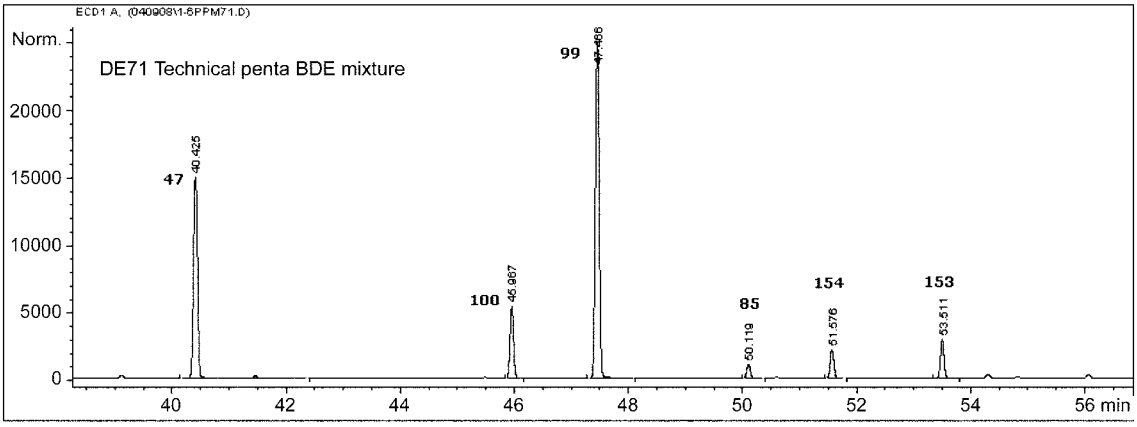

Figure 7. Typical PBDE congener pattern of Bromkal DE-71 commercial mixture (note: elution profile remains similar to chromatogram in Figure 6, inspite of different temperature program applied here).

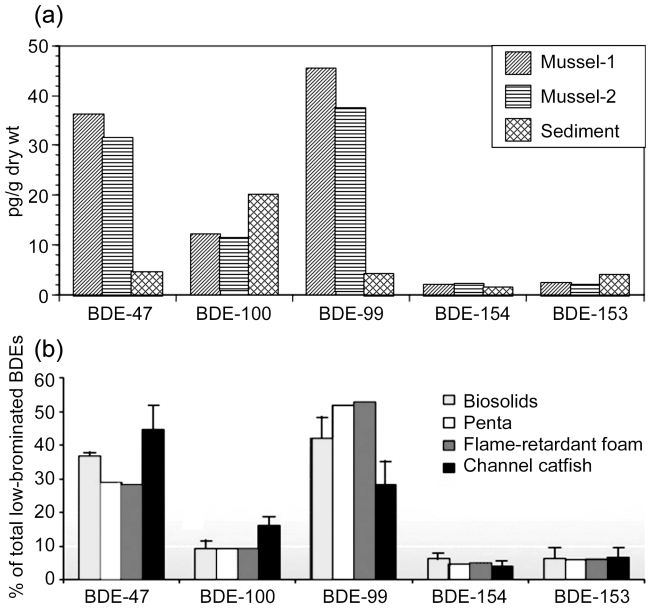

Figure 8. PBDE congener pattern in the biota and sediment from Busan Bay is compared with that of a study in USA.[20] Note the striking similarity.

donor regions (EDA) of the PCBs and PBDEs induce a change in the localization of the π-electron cloud of the pyrene moieties of the phase so that an EDA complex is formed. This type of mechanism could account for the observed retention behavior in the following three ways:

(1) Highly chlorinated/brominated biphenyl substances would be expected to form strong EDA complexes with the PYE column because halogenated compounds are very good electron-density acceptors and polycyclic aromatic hydrocarbons, such as pyrene, are among the most effective electron donors known.

(2) PCBs with many *ortho* chlorines should be less retained owing to steric interaction between *ortho* chlorines (or between *ortho* chlorines and *ortho* hydrogens), leading to twisting of the biphenyl δ-bond, an increase in the distance between the biphenyl and the pyrene moieties, and thus to a weaker EDA complex.

(3) PCBs or PBDEs with half-ring structures with the chlorines/bromines close together offer naturally better acceptor pockets for EDA complexing than those that have half-ring structures with the chlorines/bromines spread over the rings, and are therefore more retained.

With PBDE determination, GC-ECD is seldom used, as ECNI LCMS offers the best sensitivity and selectivity. However, Agilent technologies reported PBDE determination using GC-micro ECD in their on-line journal.[23] We tested our standards with varying temperature programs that offered optimal separation efficiency for a particular analysis. Micro ECD is quite sensitive to target PBDEs at femtogram to picogram quantities. PYE column HPLC is rather helpful in situations where archived sample extracts are re-analyzed for PBDEs and co-planar PCBs. This method is efficient, accurate, cost-effective and relatively simple. We have standardized this technique for routine analysis of PBDEs and co-planar PCBs in environmental samples. 21 sediment samples from Masan Bay were analyzed so far using this method. In general, environmentally persistent PBDEs in Korea are very similar to samples from US and they resemble the commercial penta formulation to a great extent. PBDEs have been measured in a few Korean labs recently but samples were sent to US and Canada for analysis. To our knowledge, this is the first report on PBDEs from a Korean laboratory using their own resources.

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References

1. Boon, J. P.; Lewis, W.; Tjoen-a-choy, M.; Al lchin, C.; Law, R.; Deboer, J.; Tenhallers-Tjabbes, C.; Zegers, B. *Environ. Sci. Technol.* **2002**, *36*, 4025-4032.
2. Rahman, F.; Langford, K. H.; Scrimshaw, M. D.; Lester, J. N. *Sci. Total Environ.* **2001**, *275*, 1-17.
3. Kannan, N.; Tanabe, S.; Wakimoto, T.; Tatsukawa, R. *Chemosphere* **1987**, *16*, 1631-1634.
4. Kannan, N.; Tanabe, S.; Wakimoto, T.; Tatsukawa, R. *J. Assoc. Off. Anal. Chem*. **1987**, *70*, 451-454.
5. Kannan, N.; Tanabe, S.; Tatsukawa, R. *Arch. Environ. Health* **1988**, *43*, 11-14.
6. Kannan, N.; Tanabe, S.; Okamoto, T.; Tatsukawa, R.; Phillips, D. J. H. *Environ. Pollut.* **1989**, *62*, 223-235.
7. Kannan, N.; Tanabe, S.; Ono, M.; Tatsukawa, R. *Arch. Environ. Contam. Toxicol*. **1989**, *18*, 850-857.
8. Duinker, J. C.; Schulz, D. E.; Petrick, G. *Anal. Chemi*. **1988**, *60*, 478-482.
9. Schulz, D. E.; Petrick, G.; Duinker, J. C. *Environ. Sci. Technol.* **1989**, *23*, 852-859.
10. Kannan, N.; Schulz-Bull, D. E.; Petrick, G.; Duinker, J. C. *Inte. J. Environ. Anal. Chem*. **1992**, *47*, 201-215.
11. Schulz-Bull, D. E.; Patrick, G.; Kannan, N.; Duinker, J. C. *Mar. Chem.* **1995**, *48*, 245-270.
12. Kannan, N.; Petrick, G.; Schulz, D.; Duinker, J.; Boon, J. P.; Arnhem, E. V.; Jansen, S. *Chemosphere* **1991**, *23*, 1055-1076.
13. Petrick, G.; Schulz, D. E.; Duinker, J. C. *Journal of Chromatography* **1988**, *435*, 241-248.
14. Anonymous *Intergovernmental Oceanographic Commission Manuals and Guides* **1993**, *27*, 1-34.
15. Creaser, C. S.; Krokos, F.; Startin, J. R. *Chemosphere* **1992**, *25*, 1981-2008.
16. Haglund, P.; Asplund, L.; Järnberg, U.; Jansson, B. J. *Chromatogra.* **1990**, *507*, 389-398.
17. Ramos, L.; Jométez, B.; Fernández, M.; Hernández, L.; González, M. *J. Organohalogen Compounds* **1996**, *27*, 376-381.
18. Sloan, C. A.; Adams, N. G.; Pearce, R. W.; Brown, D. W.; Chan, S. L. *Northwest Fisheries Science Center Organic Analytical Procedures*, NOAA Technical Memorandum NOS ORCA 71; NOAA: Mayland, 1993; pp 53-97.
19. Hong, S. H.; Yim, U. H.; Shim, W. J.; Oh, J. R.; Lee, I. S. *Mar. Pollut. Bull.* **2003**, *46*, 244-253.
20. Hale, R. C.; La Guardia, M. J.; Harvey, E. P.; Gaylor, M. O.; Mainor, T. M.; Duff, W. H. *Nature* **2001**, *412*, 140-141.
21. Kodavanti, P. R. S.; Kannan, N.; Yamashita, N.; Derr-Yellin, E. C.; Ward, T. R.; Burgin, D. E.; Tilson, H. A.; Birnbaum, L. S. *Environ. Health Perspect.* **2001**, *109*, 1153-1160.
22. Kannan, N.; Petrick, G.; Bruhn, R.; Schulz-Bull, D. E. *Chemosphere* **1998**, *37*, 2385-2393.
23. Kuhn, E.; Ellis, J.; Wilber, S. *Agilent Technologies Publications*; September 11, 2003 (http://www.agilent.com/chem).