한국환경분석학회지 제12권 (제2호) 130~135, 2009
J. of the Korea Society for Environmental Analysis

Separation Characteristics of Polychlorinated Biphenyls (PCBs) on Two Naphthalenecarboxamide Bonded Silica Stationary Phases

Seung-Ryul Hwang*†, Ki-Bong Song*, Sun Kyoung Shin*, and Myung-Ho Hyun**

**Organics Analysis Division, Environmental Measurement Standards Department, National Institute of Environmental Research, Environmental Research Complex, Incheon 404-708, Korea*
***Department of Chemistry, Pusan National University, Busan 609-735, Korea*

Received March 2009, accepted June 2009

In this study, we synthesized two new stationary phases based on naphthalenecarboxamide isomers and investigated the separation characteristics of 62 PCBs resulted from π-π interaction and steric repulsion. Two stationary phases(1- and 2-naphthalenecarboxamide) were simply prepared by coupling reaction of acids(1- and 2-naphthoic acid) and amionopropyl silica gel with EEDQ(2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) as coupling reagent. The fractionation of PCBs were performed using high performance liquid chromatography (HPLC) equipped with two new stationary phase columns and compared with commercially available 2-(1-pyrenyl)ethyldimethylsilyated (PYE) and octadecyl (C_{18}) stationary phase column. As a consequence, we could identify PCBs which had been eluted in the HPLC fractions using gas chromatography/mass spectrometer (GC/MS) and confirm that 2-naphthalenecarboxamide stationary phase was more efficient to separated PCBs than 1-naphthalenecarboxamide stationary phase. The retention capacity was increased according to the planarity of PCBs and especially 3,3',4,4',5-pentaCB (IUPAC No. 126) was eluted in the last fraction. In the case of stationary phase, the retention of PCBs was as follows; PYE > 2-naphthalenecarboxamide > 1-naphthalenecarboxamide > C_{18}. This result means that the difference of π-electrons conjugation and isomeric molecular structure of stationary phases may determine the separation characteristics of PCBs in HPLC fractionation.

Key wrods: PCBs, HPLC, Stationary Phases, Naphthalenecarboxamide

1. Introduction

Polychlorinated biphenyls (PCBs) is one of ubiquitous environmental pollutants and interesting molecules from the structural point of view. Internal rotation of PCBs depends on the π-electrons conjugation and the steric repulsion between the chlorines in *ortho* positions of two phenyl rings.[1] Non and mono-*ortho* substituted PCBs (especially coplanar PCBs) may have planar structure and considered as "dioxin-like" compounds due to the toxic similarity of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofuranes (PCDFs).[2]

For the analysis of dioxin-like PCBs, various analytical methods have been developed in the area of gas chromatography (GC) and liquid chromatography (LC). The stationary phase has an important role to separate target analytes from impurities in chromatographic process, so effective stationary phases such as activated carbon, alumina, 2-(1-pyrenyl)ethyldimethylsilyated (PYE) have been used in the cleanup step of dioxin-like PCBs analysis.[3,4] However, although these stationary phases have some advantages in separating dioxin-like PCBs, they require large amount of harmful solvent typically toluene, methylenechloride and/or longer cleanup time for the elution of π-electron rich aromatic compounds like PCDDs/PCDFs.[5-7]

The object of this study is to determine the separa-

†To whom correspondence should be addressed.
E-mail: komelong@korea.kr

tion characteristics of PCBs on the two new naphthalenecarboxamide stationary phases and apply to the analytical method development on the basis of intermolecular interaction such as the π-π interaction and steric repulsion.

2. Experimental

2.1 Reagents and standards

All solvents were pesticide residue analysis grade and obtained from WAKO Pure Chemicals Corporation (Osaka, Japan). For the preparation of stationary phases, 1-naphthoic acid, 2-naphthoic acid, EEDQ, butanoyl chloride were purchased from Sigma-Aldrich(St. Louis, MO, USA) and aminopropylsilica gel (Kromasil) from AZO NOBEL AB(Eka Chemicals, Bohus, Sweden). The mixture of 62 ^{12}C-PCBs(BP-MS) and ^{13}C-2,4,4'-TriCB (IUPAC No. 28) were purchased from Wellington Laboratories (Ontario Canada).

2.2 Preparation of Naphthalenecarboxamide silica stationary phases

Two stationary phases, 1-NAP(N-propyl-1-naphthalenecarboxamide ethoxysilylated silica gel) and 2-NAP (N-propyl-2-naphthalenecarboxamide ethoxysilylated silica gel), were prepared by coupling which 1- or 2-naphthoic acid 0.8 g (4.67 mmol) was dissolved into methylenechloride (100 mL), then 2.0 g (8.09 mmol) of EEDQ was added by stirring for 1 hour at room temperature. The resulting solution was dropped slowly to 4.0 g of aminopropylsilica gel (Kromasil 5 μm, 100 Å) in methylenechloride (100 mL) with stirring for 1 hour at room temperature and then reacted for 24 hours. The silica gel was separated by filtration, washed successively with methanol, ethanol, acetone, methylenechloride, toluene and *n*-hexane and then dried under vacuum. Based on the elemental analysis of 1-NAP (C, 10.37%; H, 1.37%; N, 1.66%) and 2-NAP (C, 12.02%; H, 1.43%; N, 1.57%), the surface concentration of the naphthalenecarboxamide group of 1-NAP and 2-NAP were calculated to be 1.16 μmol/m^2 and 1.59 μmol/m^2 respectively.

2.3 HPLC fractionation condition

The fractionation of PCBs was performed using an Waters 2690 Alliance System equipped with Waters 996

Table 1. Mean recoveries (as %) of the PCBs between stationary phases

Stationary phase	non-*ortho* PCBs	mono-*ortho* PCBs	di-*ortho* PCBs	tri-*ortho* PCBs	tetra-*ortho* PCBs
PYE	73.9~99.4	43.1~79.7	47.3~89.9	43.8~87.5	43.7~69.8
1-NAP	72.9~101.7	29.7~88.9	59.7~89.3	57.6~93.8	55.1~87.4
2-NAP	81.1~106.8	35.0~96.2	60.4~91.5	59.5~89.5	58.4~84.0
C_{18}	51.5~86.0	63.4~87.1	65.4~87.0	68.1~89.0	63.6~86.8

Table 2. Specification of HPLC columns

Column	Specification	Remarks
PYE	Cosmosil 5-PYE 2-(1-pyrenyl) ethyldimethylsilylated silicagel 250 mm×4.6 mm i.d., particle size 5 μm Nacalai Tesque, Promochem	commercially available
C_{18}	Xterra RP18 250 mm×4.6 mm i.d., particle size 5 μm Waters	commercially available
1-NAP	N-propyl-1-naphthalenecarboxamide silicagel 250 mm×4.6 mm i.d., particle size 5 μm	laboratory made
2-NAP	N-propyl-2-naphthalenecarboxamide silicagel 250 mm×4.6 mm i.d., particle size 5 μm	laboratory made

photodiode array detector (PDA) on the four HPLC columns: commercially available Cosmosil 5-PYE (2-(1-pyrenyl)ethyldimethylsilylated silica gel, Nacalai Tesque, Kyoto, Japan), Xterra RP18 (octadecyl silica gel, Waters Corporation, Milford, MA, USA), laboratory made 1-NAP and 2-NAP to compare separation characteristics (Table 2). The mobile phase was *n*-hexane and the flow rate was 0.5 mL/min. Injection volume of the mixture of PCBs was 20 μL and each fraction of 0.5 mL (1 minute) was collected. The fractionation experiment was repeated three times to confirm the precision.

2.4 GC/MS analysis

The PCBs of each fraction were determined using an Agilent 6890 series GC system interfaced with Agilent 5973 Network MSD. A J&W Scientific DB-5 (60 m fused silica capillary column, 0.25 mm i.d. and 0.1 μm film thickness) was used with helium as the carrier gas at a flow rate of 1.0 mL/min. Injection port was maintained at 280°C and 1 μL sample was injected in splitless mode. Column temperature was held at 100°C for initial 2.0 min, and then programmed at 30 °C/min to 170°C, ramped at 5 °C/min to 300°C and held for 5 min.

3. Results and Discussion

3.1 Separation characteristics

Two new naphthalenecarboxamide stationary phases have been designed to change the separation characteristics of PYE with an amide group as polar moiety and a naphthyl group as π-electron system instead of pyren group. Since two stationary phases are constitutional isomers, it will be interesting to compare the retention behavior of PCBs caused by subtle difference of molecular structure. In this study chromatographic behavior of PCBs on the four HPLC columns was investigated to consider the separation characteristics resulted from inter molecular interaction such as π-π interaction and steric repulsion between the selectors bonded on silica gel stationary phases and the analyte PCBs.

As previously reported, pyrene bonded PYE stationary phase has been used for the separation of PCBs, PCDDs/PCDFs and other aromatic compounds.[8,9] It was also considered that naphthyl group of 2-NAP was oriented out of silica surface (parallel to alkyl chain of silica gel) while 1-NAP was perpendicular as shown in Fig. 1. In the process of separation, when PCB molecule approaches to naphthalenecarboxamide stationary phase, it is predicted that the steric repulsion between PCB molecule and alkyl chain of 2-NAP will be decreased comparing to 1-NAP. In C_{18} column alkyl chain-bonded silica stationary phase has no π-electron, it is impossible to do π-π interaction with PCBs, so the retention behavior of PCBs can be comprehended by hydrophobic interaction of octadecyl group.[10]

On the three stationary phases except C_{18}, 62 PCBs were separated using *n*-hexane as a mobile phase according to their planarity caused by π-electrons conjugation. The separation results showed that non-*ortho* substituted PCBs such as IUPAC No. 77, 81, 126, 169 were retained longer than others. Considering steric and electronic effect of two phenyl ring, as shown in Fig. 2 and Fig. 3, non and mono-*ortho* substituted PCBs with tetra or more chlorines have coplanar conformation and relatively π-acidic property due to electronegativity of chlorines and enable to stronger interaction with π-basic stationary phases.

The retention of non and mono-*ortho* substituted PCBs were increased in the following order: PYE > 2-NAP > 1-NAP > C_{18}. This type of elution pattern indicates that π-basic stationary phases can retain π-acidic

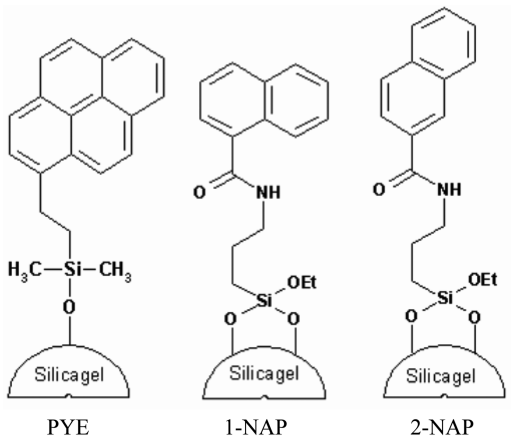

Fig. 1. Chemical structure of stationary phases.

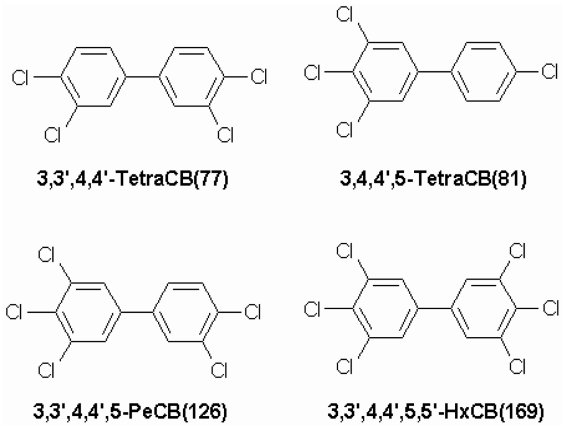

Fig. 2. Chemical structure of non-*ortho* substituted PCBs (IUPAC No. 77, 81, 126 and 169).

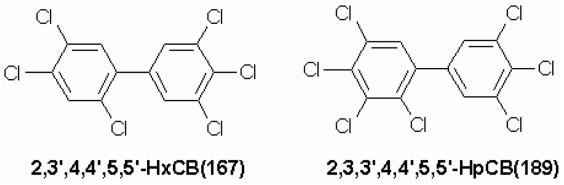

Fig. 3. Chemical structure of mono-*ortho* substituted PCBs (IUPAC No. 167 and 189).

PCBs to a significant extent by attractive π-π interaction, especially for non and mono-*ortho* substituted coplanar. In the case of C_{18} column, all the PCBs were eluted in one fraction (3.5~4.0 mL) and there was no selectivity. According to these results it is assumed that π-π interaction plays an important role in separation of PCBs and the selectivity is increased in proportion to π-electrons conjugation of stationary phase.

In the fractionation experiment, the total recoveries of PCBs were in the range 43.1~99.4% on the PYE, 29.7~101.7% on the1-NAP, 35.0~106.8% on the 2-NAP and 51.5~89.0% on the C_{18}, respectively (Fig. 1). The reported recoveries were the average value of the three repeated fractionation results.

3.2 Non and mono-ortho substituted PCBs

Among the 62 PCBs there are seven non-*ortho* substituted PCBs (IUPAC No. 3, 15, 37, 77, 81, 126, 169) which have no chlorine at *ortho* position and fifteen mono-*ortho* substituted PCBs (IUPAC No. 1, 8, 22, 28, 33, 70, 74, 105, 114, 118, 123, 156, 157, 167, 189) which have one chlorine at *ortho* position of phenyl ring. Non- or mono-*ortho* substituted PCBs have coplanar conformation and π-electrons of two phenyl groups are conjugated. It was observed that the retention was increased in proportion to substituted chlorines of PCBs on the PYE, 1-NAP and 2-NAP. The results are as follows: on the PYE, PCBs have been eluted in fraction 2 (3.5~4.0 mL, IUPAC No. 1, 3, 8), fraction 3 (4.0~4.5 mL, IUPAC No. 22, 28, 33), fraction 4 (4.5~5.0 mL, IUPAC No. 15, 70, 74), fraction 5 (5.0~5.5 mL, IUPAC No. 118, 123, 167), fraction 6 (5.5~6.0 mL, IUPAC No. 37, 105, 114), fraction 8 (6.5~7.0 mL, IUPAC No. 156, 157, fraction 9 (7.0~7.5 mL, IUPAC No. 77, 81, 189), fraction 10 (7.5~13.0 mL, IUPAC No. 126, 169).

On the 1-NAP phase, PCBs have been eluted in fraction 2 (3.5~4.0 mL, IUPAC No. 1, 3, 8, 15, 28, 74), fraction 3 (4.0~4.5 mL, IUPAC No. 22, 33, 37, 70, 81, 114, 118, 123,15 189), fraction 4 (4.5~5.0 m, IUPAC No. 77, 105, 126, 157, 169), fraction 5 (5.0~5.5 mL, IUPAC No. 167).

On the 2-NAP phase, PCBs have been eluted in fraction 2 (3.5~4.0 mL, IUPAC No. 1, 3), fraction 3 (4.0~4.5 mL, IUPAC No. 8, 15, 22, 28, 33, 70, 74, 114, 118, 123), fraction 4 (4.5~5.0 mL, IUPAC No. 37, 81, 156, fraction 5 (5.0~5.5 mL, IUPAC No. 77, 105, 126, 157, 167, 189), fraction 6 (5.5~6.0 mL, IUPAC No. 169).

As shown in Fig. 4~Fig. 9, on the 1-NAP and 2-NAP the elution results of non and mono-*ortho* substituted PCBs was similar to the results of PYE which demonstrated the selectivity for non and mono-*ortho* substituted PCBs. Coplanar PCB 2,3',4,4',5,5'-hexaCB (IUPAC No. 167) always has been eluted in fraction 5 (5.0~5.5 mL) except C_{18} and has the same π-π interaction on the PYE, 1-NAP and 2-NAP stationary phases. On the 1-NAP, 2,3',4,4',5,5'-hexaCB(IUPAC No. 167) is retained longer than any other PCBs even the elution of 3,3',4,4',5-pentaCB (IUPAC No. 126). However, on the PYE, 3,3',4,4',5-pentaCB (IUPAC No. 126) and 3,3',4,4',5,5'-hexaCB (IUPAC No. 169) were eluted in the final fraction 10 (7.5~13.0 mL) and on the 2-NAP 3,3',4,4',5,5'-hexaCB (IUPAC No. 169) was eluted in the final fraction 6 (5.5~6.0 mL).

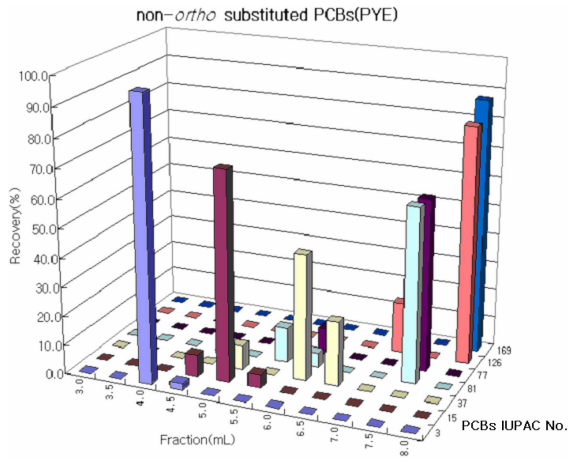

Fig. 4. The fractionation result of non-*ortho* substituted PCBs on the PYE stationary phase (mean recoveries as %).

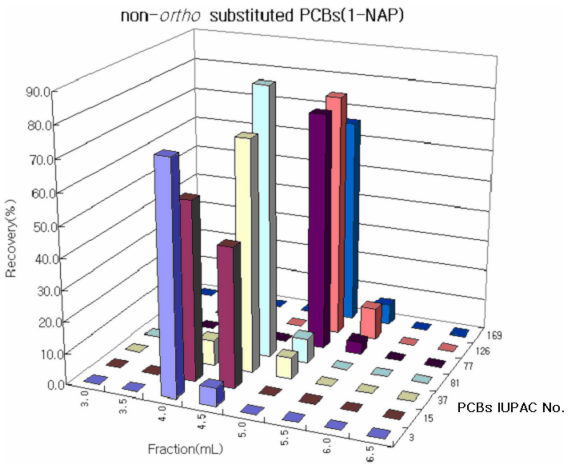

Fig. 6. The fractionation result of non-*ortho* substituted PCBs on the 1-NAP stationary phase (mean recoveries as %).

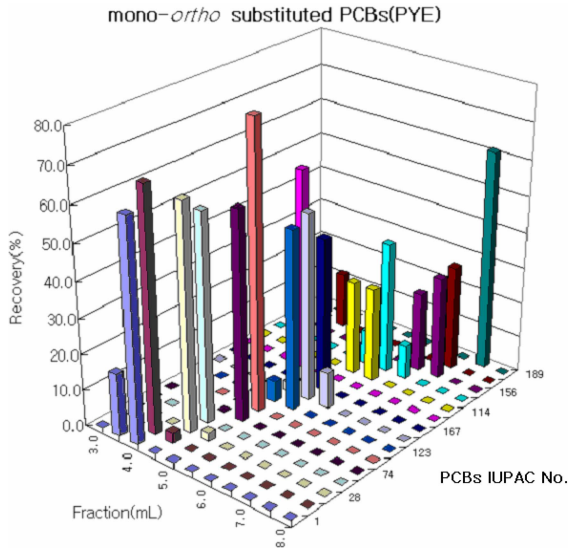

Fig. 5. The fractionation result of mono-*ortho* substituted PCBs on the PYE stationary phase (mean recoveries as %).

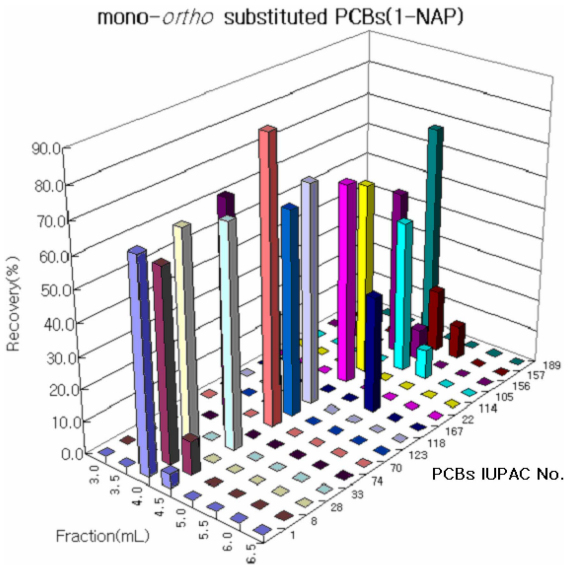

Fig. 7. The fractionation result of mono-*ortho* substituted PCBs on the 1-NAP stationary phase (mean recoveries as %).

Especially in the aspect of toxicity, the TEF (toxic equivalent factor) values of 3,3',4,4',5-pentaCB (IUPAC No. 126) and 3,3',4,4',5,5'-hexaCB (IUPAC No. 169) have been estimated 0.1 and 0.01 respectively, which are higher than other PCBs. So it is very critical to determine the concentration of PCB 126 and PCB 169, because the results play an important role to estimate the toxic equivalent (TEQ) of coplanar PCBs.[11)]

4. Conclusions

Two naphthalenecarboxamide stationary phases, 1-NAP and 2-NAP have been simply synthesized by one step coupling reaction using EEDQ as coupling reagent and applied to the separation of PCBs using HPLC. The separation characteristics of PCBs are similar to commercially available PYE but comparing to PYE, naphthalenecarboxamide stationary phases have a hydro-

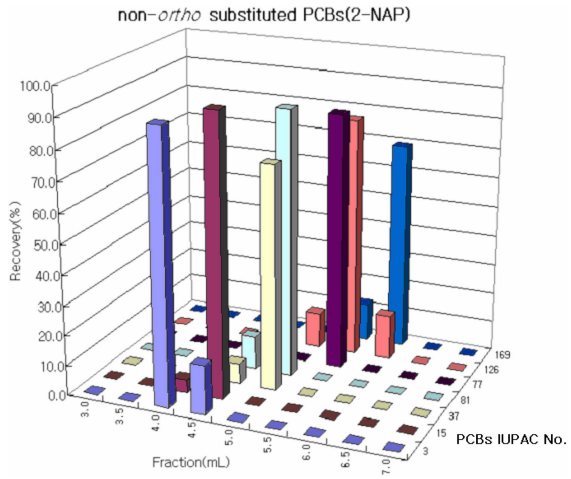

Fig. 8. The fractionation result of non-*ortho* substituted PCBs on the 2-NAP stationary phase (mean recoveries as %).

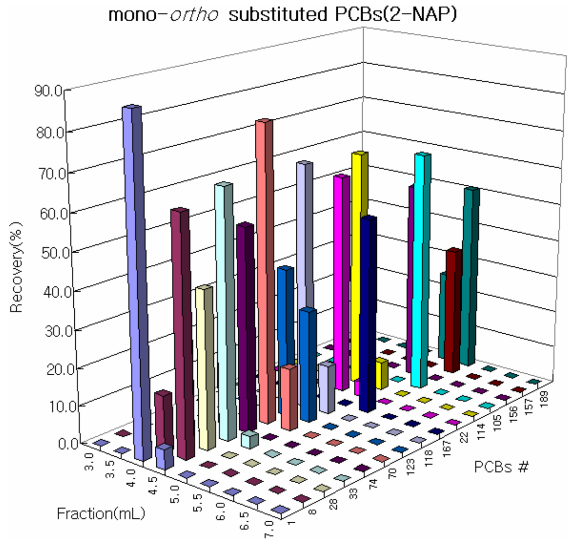

Fig. 9. The fractionation result of mono-*ortho* substituted PCBs on the 2-NAP stationary phase (mean recoveries as %).

philic carbonyl group and the results of PCBs fractionation have showed the difference between 1-NAP and 2-NAP caused by π-π interaction and steric repulsion of constitutional isomers.

In addition dioxin-like coplanar PCBs such as IUPAC No. 77, 81, 126, 169, 189 were separated efficiently from others in short time without harmful solvent such as toluene, dichloromethane etc. In future it needs to perform the theoretical calculation of molecular interaction to acquire more information for the separation mechanism of PCBs on the naphthalenecarboxamide stationary phases and on the basis of this research it is expected to develop more efficient stationary phase for GC or LC.

Acknowledgement

These stationary phases and their packed HPLC columns have been registered to Korean patent(Patent No. 10-0689779, 2007).

References

1. O. V. Dorofeeva, V. P. Novikov, N. F. Moiseeva, V. S. Yungman, *J. Mol. Struct.(Theochem).*, **2003**, 637, 137-153
2. S. Safe, *Cirt. Rev. Toxicol.*, **1990**, 21, 51-88
3. P. Hess, J. de Boer, W.P. Cofino, P.E.G. Leonards, D.E. Wells, *J. Chromatogr. A.*, **1995**, 703, 417-465
4. J.W. Cochran and G.M. Frame, *J. Chromatogr. A.*, **1999**, 843, 323-368
5. Kjell Lundgren, Bert van Bavel, Mats Tysklind, *J. Chromatogr. A.*, **2002**, 962, 79-93
6. L. Ramos, L. M. Hernández, M. J. Gonzáalez, *Anal. Chem.*, **1999**, 71, 70-77
7. M. Concejero, L. Ramos, B. Jiménez, B. Gómara, E. Abad, J. Rivera, M. J. Gonzáalez, *J. Chromatogr. A.*, **2001**, 917, 227-237
8. N. Hanari, Y. Horii, S. Taniyasu, J. Falandysz, I. Bochentin, A. Orlikowska, T. Puzyn, N. Yamashita, *Pol. J. Environ. Stud.*, **2004**, 2, 139-151
9. B. Gómara, C. García-Ruiz, M.J. González, M.L. Marina, *Analytica Chimica Acta,* **2006**, 565, 208-213
10. M. Turowski, T. Morimoto, K. Kimata, H. Monde, T. Ikegami, K. Hosoya, N. Tanaka, *J. Chromatogr. A.*, **2001**, 911, 177-190
11. P. H. Dyke and J. Stratford, *Chemospere*, **2001**, 47, 103-116