

SEPARATION OF POLYCHLORINATED NAPHTHALENE ISOMERS AND DIOXIN-LIKE COMPOUNDS BY TWO DIMENSIONAL HPLC SYSTEM WITH POROUS GRAPHITIC CARBON AND PYRENYL SILICA COLUMN

Yuichi Horii¹, Kazuyo Kaminaka², Kousuke Ono², Makoto Okada¹
Takashi Imagawa² and Nobuyoshi Yamashita²

¹ Department of Science and Engineering, Ibaraki University, 2-1-1 Bunkyo, Mito, Ibaraki 310-0056, Japan; ² National Institute of Advanced Industrial Science and Technology, 16-1 Onogawa, Tukuba, Ibaraki 305-8569, Japan

Introduction

Polychlorinated naphthalenes (PCNs) are halogenated aromatic chemical mixture of 75 theoretically possible isomers with varying physico-chemical properties. PCNs were used in variety of industrial products including capacitor dielectrics, cutting oils, engine oil additives, die casting, ship insulation, wood, paper, and fabric preservatives and wire insulation¹ etc. Global production of technical PCNs such as Halowaxes, Nibren waxes has been estimated to be approximately 150,000 metric tons². Technical formulation of PCN, PCB^{3,4} and other thermal processes of industry (e.g. incinerator, chloro-alkali plant)⁵ are considered as major sources for environmental contamination by this compound. Chemical structures of PCNs are planar, similar to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), and therefore, some PCN isomers elicit toxic potency similar to PCDD/Fs⁶. Due to significant variation of toxic potency among the PCN isomers, it is essential to use isomer-specific determination for the purpose of reliable risk assessment⁷. However, PCN analysis in environmental and biological matrices is tedious and challenging, similar to PCDD/Fs. Separation from high amount of interferences, including lipid, pigments, pesticides will not suffice to obtain reliable data. It is necessary to solve co-elution phenomenon due to bulk of PCBs, PCDD/Fs and unknown interferences. Our recent report elucidated co-elution problem prevail even among the PCN isomers⁴.

In this study, we report an optimized analytical method to separate all PCN isomers, planar PCBs and PCDDs/DFs in technical PCN preparations (Halowax) and sediment core. The analytical method is constituted of accelerated solvent extractor (ASE), two-dimensional HPLC system with porous graphitic carbon column (Hypercarb) and pyrenyl-silica (PYE) column, preparative GC and HRGC-HRMS. Hypercarb is well known to separate planar structure compounds, such as planar PCBs and PCDDs/DFs effectively⁸. PYE-HPLC, which is selective for π electron donor or acceptor bounding, have been successfully applied to separate some of PCN isomers⁹.

Material and Methods

Halowax equivalent mixture (1000:1001:1014:1051 = 1:1:1:1) was prepared, and the mixture was used to standardize separation and recovery of individual isomer of PCNs through entire analytical procedure. Long sediment core samples were taken from Lake Kitaura (35°59'N and 140°35'E) by using a piston corer with inner tube (7cm i.d. and 4m long) made of polycarbonate in December 2000. Several short core samples were collected by diver and automated core sampler using acrylic

resin tube (11cm i.d. and 120cm long) in April 2001 from the same location. Samples were transported to laboratory immediately after the collection and sectioned using a stainless steel slicer. Each layer was freeze-dried and stored at -30 °C until analysis. Detailed procedure of sample preparation was described elsewhere¹⁰. The freeze-dried sediments were extracted using accelerated solvent extractor (ASE-200, DIONEX Co.)¹¹. An extraction condition of ASE is shown in Table 1. Bottom of extraction cells (33ml) were packed with 5g of alumina to remove interfering matrices. The extract was replaced into *n*-hexane and concentrated up to 2 mL. This solution was applied for a multi-layer acidic silica gel column and eluted with 200 mL of *n*-hexane. Eluate was concentrated up to 100ul and injected into two-dimensional HPLC system. This HPLC system was used for the purpose of separation of PCNs, PCBs, PCDDs/DFs. Hypercarb column (i.d. 4.6mm x 100mm) was used as the first fractionation to separate di-, mono-*ortho* PCBs and most PCB isomers from PCNs, non-*ortho* PCBs and PCDDs/Fs. Separation method for Hypercarb is shown in Table 2. In the first fractionation, 50% dichloromethane (DCM) in hexane (forward flow direction) and toluene (reverse flow direction) were used as mobile phase. The second fractionation was carried out by use of PYE column (i.d. 4.6mm x 250mm, Cosmosil 5-PYE, 5µm, Nacalai Co.) to separate each isomer of PCNs, non-*ortho* PCB and PCDDs/DFs. For elution of fraction I to III, 10% DCM in hexane was used and fraction IV was eluted by DCM only. Flow rate was kept at 2.5ml/min for Hypercarb and 1ml/min for PYE-HPLC.

Table 1. Extraction methods for accelerated solvent extractor (ASE-200)

step	extraction time	mobile phase	oven temp. (°C)	flush vol. (%)
1	5 min	50% acetone / hexane	100	60
2	10 min	toluene	175	60

Pressure inside of extraction cell was kept at 2000psi.

Table 2. Separation methods for PCNs, PCBs and PCDDs on Hypercarb-HPLC.

step	time (min)	mobile phase	flow direction
1 (fraction I)	0 – 8	50%DCM / hexane	forward
2 (fraction II)	8 -- 28	toluene	reverse
3 (wash)	28 – 48	toluene	reverse
4 (wash)	48 – 58	50%DCM / hexane	forward

fraction I : most of PCBs (include di-, mono-*ortho* PCBs) are eluted.

fraction II : non-*ortho* PCBs, PCNs and PCDDs/Fs are eluted.

Results and Discussion

Separation methods of PYE-HPLC and eluate composition are shown in Tabel 3. It is possible to separate 124568-, 124578-, 123457-, 123568-H6CN, non-*ortho* PCB and 1368-T4DD as a single peak on HRGC-HRMS by use of this fractionation program. Figure 1 shows elution profile of each isomers of T3CNs to O8CNs, non-*ortho* PCB, 1368-T4CDD and O8CDD on PYE-HPLC. It is worth to mention that the most of dioxin-like toxic isomers⁵, namely 123567-, 123568-, 123578-H6CN and 1234567-H7CN are eluted after O8CN. Some of tri-chlorinated isomers, 123467-H6CN and 123567-H6CN are separable by the use of additional preparative GC and HPLC.

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We found that the combination of porous graphitic carbon and pyrenyl-silica HPLC methods is Table 3. Separation methods for PCN isomers, non-ortho PCB and PCDDs on PYE-HPLC

fractions	time (min)	eluate compounds
I	0 - 10	T3CNs, T4CNs, 12458-P5CN non-ortho PCBs
II	10 - 12	12345-, 12357-, 12358-, 12346-, 12457-, 12467-, 12468- 12478-P5CN, 123458-, 124578-H6CN, 1368-T4CDD
III	12 - 24	12346-, 12356-, 12357-, 12367-, 12378-, 12456-, 12467-P5CN 123457-, 123578-, 124568-H6CN, 1234568-H7CN, O8CN
IV	24 - 70	123456-, 123467-*, 123567-*, 123568-H6CN, 1234567-H7CN O8CDD

*These isomers are separable by second fractionation using hexane.

effective to separate individual isomer of PCNs, PCBs and PCDDs/Fs from the environmental matrix. The analytical methods using two-dimensional HPLC, preparative GC and HRGC-HRMS enable high resolution and sensitive measure of dioxin-like compounds including PCNs with no co-elution, which is a fundamental problem, even in commercial standard preparations⁷.

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References

1. Kannan, K., Yamashita, N., Imagawa, T., Decoen, W., Kim, J.S., Day, R.M., Summer, C.L. and Giesy, J.P. (2000) *Environ. Sci. Technol.* 34, 566-572.
2. Falandysz, J. (1998) *Environ. Pollut.* 101, 77-90.
3. Haglund, P., Jakobsson, E., Asplund, L., Athanasiadou, M., Bergman, X. (1993) *J. Chromat.*, 634, 79-86.
4. Yamashita, N., Kannan, K., Imagawa, T., Miyazaki, A. Giesy, J.P. (2000) *Environ. Sci. Technol.* 34, 4236-4241.
5. Kannan, K., Imagawa, T., Blankenship, A.L., Giesy, J.P. (1998) *Environ. Sci. Technol.* 32, 2507-2514.
6. Blankenship, A. L., Kannan, K., Villalobos, S, A., Villeneuve, D, L., Falandysz, J., Imagawa, T., Jakobsson, E., Giesy, J, P. (2000) *Environ. Sci. Technol.* 34, 3153-3158.
7. Kodavanti, P., Kannan, N., Yamashita, N., Derr-Yellin, E., Ward, T., Burgin, D., Tilton, H., Birnbaum, L. (2001) *Environ. Health Perspect.* in press.
8. Echols, K., Gale, R., Tillitt, D., Schwartz, T., O'Laughlin, J. (1997) *Environ. Toxicol. Chem.* 16, 1590-1597.
9. Williams, D, T., Kennedy, B., LeBel, G, L. (1993) *Chemosphere.* 27, 795-806
10. Yamashita, N., Kanan, K., Imagawa, T., Villeneuve, D, L., Hashimoto, S., Miyazaki, A., Giesy, J, P. (2000) *Environ. Sci. Technol.* 34, 3560-3567.
11. Bandh, C., Björklund, E., Mathiasson, L., Näf, C., Zebühr, Y. (2000) *Environ. Sci. Technol.*

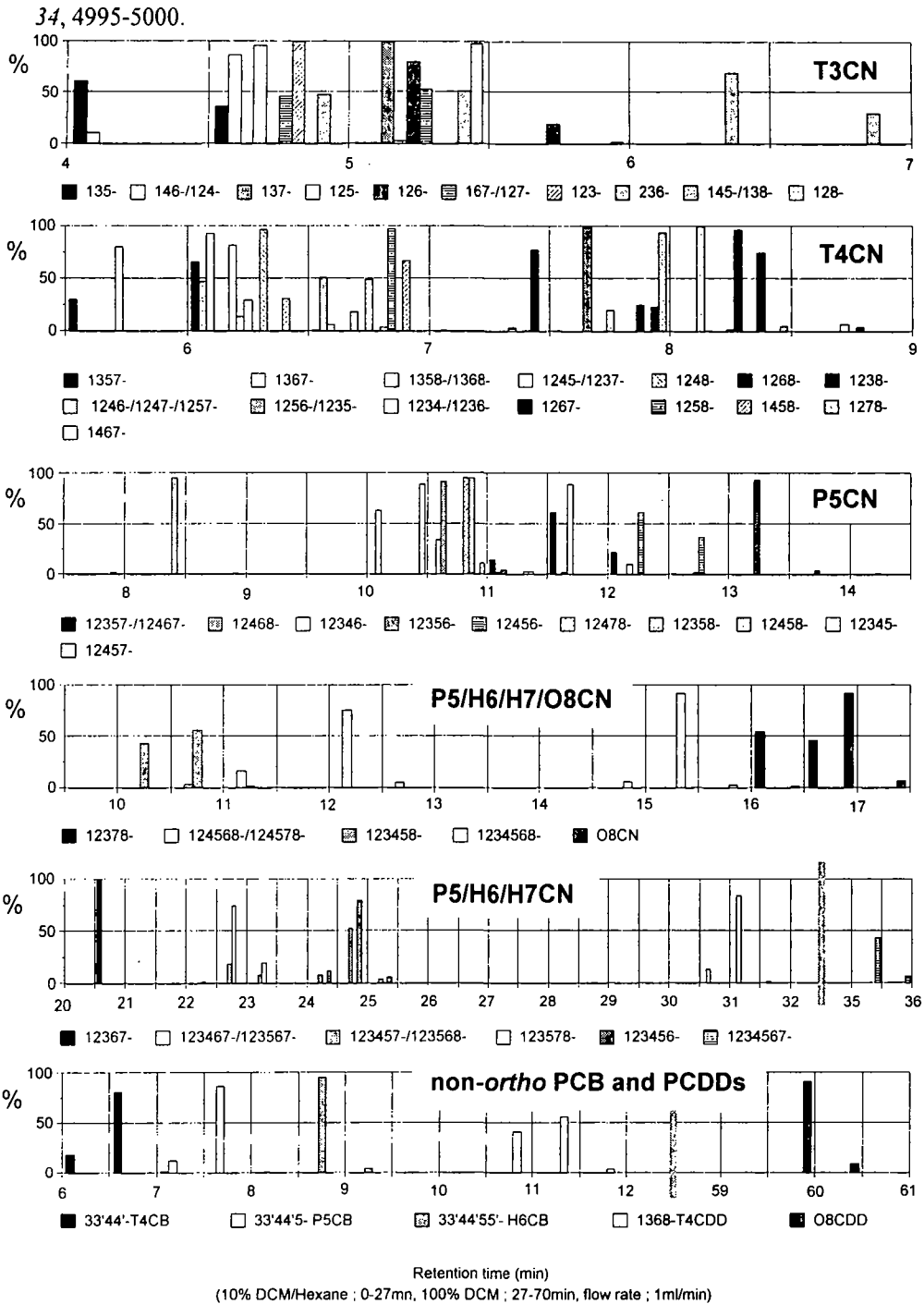


Figure 1.

Separation profiles for PCN isomers, non-ortho PCB and PCDDs on PYE- HPLC.
 #Y axis values are represented percentages of compounds in each fraction (0.5ml).

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