HPLC ELUTION ORDER OF POLYCHLORINATED BIPHENYL CONGENERS WITH OCTADECYL-, PYRENYLETHYL-, AND NITRATED NAPHTHYLETHYL-SILYLATED SILICA GEL

Elizabeth R. Barnhart*, Nobuo Tanaka**, Kazuhiro Kimata**, Ken Hosoya**, Shigeru Terabe***, Louis R. Alexander*, and Donald G. Patterson, Jr.*
*Division of Environmental Health Laboratory Sciences
Centers for Disease Control, Atlanta, GA 30333
**Kyoto Institute of Technology, Kyoto 606, Japan
***Kyoto University, Kyoto 606, Japan

ABSTRACT

Polychlorinated biphenyls have been separated by HPLC on three different stationary phases (octadecyl, pyrenylethyl, and nitrated naphthyethyl-silylated silica gel). All of the PCBs can be separated by a combination of two different columns.

KEYWORDS: HPLC, PCBS

INTRODUCTION

Polychlorinated biphenyls (PCBs) have been employed extensively in industry. Now the compounds are widespread in the environment and food chain. The chemicals have been shown to be toxic to animals [1] and man [2], although the mechanism of toxicity is not known. Toxicity varies with the position and number of chlorine atoms on the biphenyl ring (209 PCB congeners are possible).

Analysis of environmental and biological samples for PCB content has in the past utilized packed column gas chromatography (GC) and the levels reported as total PCBs [3]. Recently, methods have been published which identify individual congeners using capillary column GC [4,5]. Individual PCBs for reference standards must often be synthesized by the analyst because many are unavailable commercially. HPLC can be used as a clean-up method for synthesis products [6,7] as well as for identification and quantification of PCBs in environmental samples [8]. Reversed phase HPLC is well-suited to the separation of PCB isomers (hydrophobic compounds) and the separation depends on the pattern of substitution, biplanarity of rings, dipole moments, and steric congestion [7], as well as water solubility [9]. Combinations of stationary phases, with reversed-phase HPLC, have been successful in optimizing the separation of isomers of tetrachlorodibenzo-p-dioxin [10]. Studies of relationships between receptor-binding/toxicity mechanisms and stationary phase retention mechanisms by HPLC on well characterized stationary phases might aid in the development of structure-activity relationships for the chlorinated aromatics. We will report on the separation of PCBs eluted from octadecyl- (C18), pyrenylethyl- (PYE), and nitrated naphthylethyl- (NE-NO2) silylated silica gel stationary phases with aqueous methanol using the hexachlorobiphenyls (HxCBs) as examples for this short abstract.

EXPERIMENTAL

Samples. The PCB congeners were obtained from Ultra Scientific (Hope, RI 02831, USA) and dissolved in toluene (5 mg/mL). Injections consisted of approximately 2 μL each.

Columns. Stainless steel columns were packed with silica gel bonded with an octadecyl, -pyrenylethyl-, or nitrated naphthylethyl-siloxane [11].

HPLC. The HPLC system included a pump (Waters 6000A), injector (Rheodyne), a column (150 mm x 4.6 mm I D), and a detector (Waters 990 Photodiode Array, 200-400 nm). The mobile phase consisted of aqueous methanol (85 or 90%) at a flow rate of 1.0 mL/min.

Procedure. Duplicate injections of solutions of each of the 12 HxCBs listed in Table 1 were made on each of the columns within a three week period. Values for k' of the HxCBs were calculated from the retention times recorded for the biphenyls and toluene(t_o), according to the equation:

$k'=(t_r-t_o)/t_o$.

RESULTS AND DISCUSSION

The Ballschmitter numbers [12], structures, and k' values (by stationary phase) for the HxCBs are given in Table 1. The plots of log k' for the HxCBs on the various columns are given in Figure 1 and the number of chlorine substituents ortho to the ring bridge is indicated by a different symbol.

TABLE 1. HPLC of Hexachlorobiphenyl Isomers on Three Stationary Phases

Ballschmitter ID Hexachlorobiphenyl	Structure	Values of k' on Stationary Phases		
		C18-85% MeOH	PYE-Methanol	NE-NO2, 85%
136 2,2',3,3',6,6'		1.2	0.7	1.1
151 2,2',3,5,5',6		2.6	1.8	2.2
155 2,2',4,4',6,6'		3.0	0.9	1.6
128 2,2',3,3',4,4'		3.15	1.8	2.5
138 2,2',3,4,4',5		3.6	2.2	2.2
129 2,2',3,3',4,5		3.7	2.05	2.8
139 2,2',3,4,4',6		4.0	1.45	3.1
154 2,2',4,5,5',6		4.3	1.6	2.9
137 2,2',3,4,4',5		4.4	2.4	3.45
141 2,2',3,3',4,5		4.5	2.3	3.3
158 2,3,3',4,4',6		4.7	3.4	3.5
169 3,3',4,4',5,5'		8.7	10.5	10.3

The data show that no single column is able to completely separate all of the 12 HxCBs, but their separations can be facilitated by combining two of the three columns tested (see Figure 1). PCB 136, with four ortho chlorine substituents elutes first on all three columns, probably because of its greater solubility in the aqueous mobile phase [9] and its twisted non-planar conformation. PCB 169, without ortho substituents, elutes last on all three columns, probably because of its planar conformation [7]. Hydrophobicity of the stationary phases increases in the order, NE-NO2<PYE<C18 [13]. PYE and NE-NO2 are expected to participate in more specific interactions like charge transfer [13].

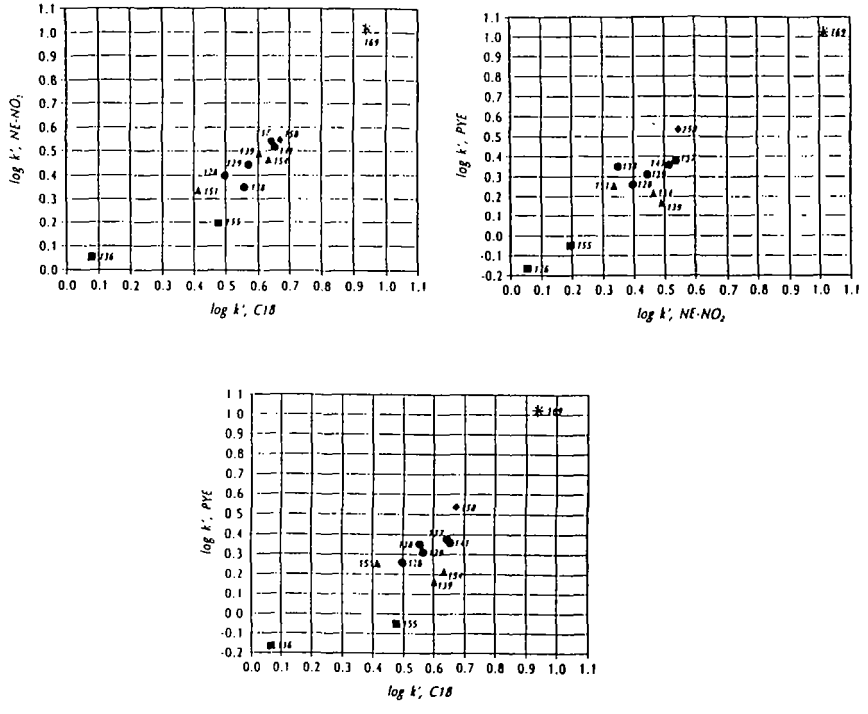

Figure 1. Graph of log k' of the hexachlorobiphenyls on the various columns.
(Mobile phase, aqueous methanol (90%) at 1.0 mL/min)
* = no ortho (to the ring) chlorine substituents.
◊ = 2 ortho substituents (on same ring)
○ = 2 ortho substituents (one on each ring).
△ = 3 ortho substituents.
□ = 4 ortho chlorine substituents.

The practical utility of a combination of stationary phases with aqueous methanol elution for separation of the environmentally important HxCBs is demonstrated. Toxicity data are not available on every congener, but there is evidence suggesting that PCBs with chlorines in lateral positions (meta and para to the ring bridge), having none or one ortho chlorine atom are more toxic [14]. In general, congeners with such substituents have higher retention indices.

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