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Short communication

Fractionation of non-*ortho*-substituted toxic polychlorinated biphenyls on two nitro-containing liquid chromatographic stationary phases

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Abstract

Two liquid chromatographic stationary phases, dinitroanilinopropylsilica (DNAP) and tetranitrofluorenimino-propylsilica (TENF), are demonstrated to retain selectively the toxic non-*ortho*-chlorobiphenyl congeners CB-77, CB-126 and CB-169. On the DNAP column, these three congeners elute as a single peak. With hexane as mobile phase all eighteen tested chlorobiphenyl congeners are eluted within 13 min. Carryover effects of CBs in HPLC systems are discussed. To demonstrate the isolation of the three congeners, a sample of Aroclor 1254 was fractionated and analysed by GC with electron-capture detection.

1. Introduction

It is well known that the 209 theoretically possible congeners of chlorinated biphenyls (CBs) possess substantial differences in biological effects. The most toxic congeners are recognized as those which lack chloro substitution in the *ortho* position and in addition have chlorine substituents in both *para* and at least two *meta* positions. These configurations make it possible to obtain a planar structure that resembles the 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin and thus possess similar biological activity [1]. Determination of these congeners, CB-77 (3,4,3′,4′), CB-126 (3,4,5,3′,4′) and CB-169 (3,4,5,3′,4′,5′) [2], demands separation from the bulk of polychlorinated biphenyl (PCB), as they will otherwise be completely covered by the peaks of the more abundant congeners. Group separation is mostly performed by liquid chromatography, in open columns by gravitational flow or by high-performance liquid chromatography (HPLC) [3]. There are also some cases where multi-dimensional gas chromatography (GC) have been applied to achieve a separation [4–6]. The use of two-dimensional GC can be troublesome when there are large concentration differences between the solutes as in samples of PCB.

When liquid chromatography is applied, the use of different kinds of carbon material dominates the published methods for the fractionation of PCB into a non-*ortho* and sometimes a mono-*ortho* subsample of PCB. Those stationary phases include activated carbon such as Amoco

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PX-21 [7] and porous graphitic carbon [8]. Carbon materials often require strong solvents such as dichloromethane or toluene and exhibits retention times that can be more than 100 min. However, porous graphitic carbon has occasionally been used with pure hexane as mobile phase [9,10]. Aluminium oxide and Florisil [11,12] have also been shown to retain selectively the toxic non-*ortho*-substituted congeners. Disadvantages of aluminium oxide and Florisil are large batch-to-batch variations and the need for careful control of the adsorbent activity. Haglund et al. [13] showed that a column of 2-(1-pyrenyl)-ethyldimethylsilica (PYE) had a selectivity similar to that of carbon but with weaker adsorption of the solutes. The use of a stationary phase of C60–70 fullerenes bonded to a core of styrene–divinylbenzene to enrich non-*ortho*-substituted CBs has been demonstrated [14]. Reviews of methods for the separation of non-*ortho*-substituted CBs have been published by Creaser et al. [3] and De Voogt et al. [15], who also included biological activity and the occurrence of non-*ortho*-, mono-*ortho*- and di-*ortho*-chlorobiphenyls.

In this paper we present methods for the selective fractionation of non-*ortho*-substituted CBs on two stationary phases, dinitroanilinopropylsilica (DNAP) and tetranitrofluoreniminopropylsilica (TENF). Nitrophenylpropylsilica has been used previously for the clean-up of CBs and other chlorinated pesticides [16], but it does not separate the toxic non-*ortho*-substituted CBs from the bulk of PCB [17].

2. Experimental

2.1. Chemicals

The pure CB congeners were obtained from Ultra Scientific (Kingstown, RI, USA) and the commercial PCB mixture used was Aroclor 1254 (Monsanto, St. Louis, MO, USA).

2.2. Chromatographic conditions

The HPLC system consisted of a Waters (Milford, MA, USA) Model 590 pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector equipped with a 100-μl loop, a Crococil (France) column thermostat set at 25°C and an LKB (Bromma, Sweden) Model 2125 UV detector monitoring the eluent at 225 nm. The two HPLC columns of DNAP and TENF, both 25 cm × 4.6 mm I.D. with 5-μm particles, were obtained from ES Industries (Berlin, NJ, USA). The DNAP particles had a pore size of 60 Å and the TENF particles 100 Å. HPLC-grade hexane (Rathburn, Walkerburn, UK) was used as the mobile phase at a flow-rate of 1.0 ml/min. Capacity factors were determined in triplicate and the dead time was determined from the injection dip in the baseline caused by pentane (Rathburn), which was added to all samples. The volume injected was 30 μl, containing 10–30 ng of individual congeners and ca. 2.5μg of Aroclor 1254.

Gas chromatography was performed in the splitless injection mode on a Varian (Walnut Creek, CA, USA) Model 3400 gas chromatograph equipped with an electron-capture detector and a Jade (Austin, TX, USA) injector. The GC column was RT-5 (60 m × 0.32 mm, I.D., 0.25 μm film thickness) (Restek, Bellefonte, PA, USA). The temperature settings were as follows: injector, 250°C; detector, 300°C; column oven, 70°C held for 2 min and then raised at 10°C/min to 280°C, which was held for 10 min. Hydrogen was used as the carrier gas at a column head pressure of 11 psi and nitrogen was used as the detector make-up gas at a flow-rate of 35 ml/min. The split vent, which was opened after 2 min, was set to a flow rate of 23 ml/min. An ELDS Pro laboratory data system (Chromatography Data Systems, Svartsjö, Sweden) was used to register and process all UV and electron-capture detector signals.

Confirmation of the identity and a check of purity of the fractionated toxic non-*ortho*-PCB congeners were performed by gas chromatography–mass spectrometry (GC-MS). The GC column and the applied column oven temperature programme were the same as for the GC analysis with electron-capture detection (ECD). The transfer line to the mass spectrometer, an INCOS 50 quadropole mass spectrometer (Finnigan MAT, San Jose, CA, USA), was main-

tained at 300°C and the column outlet was directed into the mass spectrometer ion source, which had a temperature of 150°C. The instrument was operated in the electron impact (EI) mode with an electron energy of 70 eV utilizing both full range scan, 250–450 mass units and multiple ion detection monitoring the four most intense ions of the eluting non-*ortho*-CB and CBs with the next two higher degrees of chlorination. The latter was needed for detection of the toxic non-*ortho*-CB at the lowest concentration, CB 169.

2.3. HPLC fractionation

The fraction of toxic non-*ortho*-substituted CBs present in 2.5 μg Aroclor 1254 was separated on the DNAP column, collected between 10.80 and 12.60 min and was then carefully concentrated under a gentle stream of nitrogen to ca. 100 μl. A 2-μl volume of the concentrate was injected on to the gas chromatograph. A larger amount of Aroclor 1254, 15 μg dissolved in 300 μl of hexane, was injected into a 500-μl injection loop in order to check for memory effects due to overloading. A further investigation of memory effects was carried out by the injection of 100 ng of CB-105 dissolved in 50 μl of hexane on the HPLC–DNAP system. Eight fractions of 1 ml each, starting from 1 min before the beginning of the elution of CB-105, were collected. CB-103 (2,4,6,2′,5′) was added as an injection standard in different amounts according to the estimated concentrations of CB-105. The collected fractions were either concentrated or diluted in order to achieve similar absolute concentrations prior to analysis by GC.

3. Results and discussion

3.1. Retention of CB congeners

The capacity factors of eighteen CBs that have been proposed for determination by the Nordic Council of Ministers [1] are shown in Table 1. On both columns the toxic non-*ortho* compounds are the most retained, preceded by a group of mono-*ortho*-substituted congeners and the di-*ortho*-substituted compounds which have the weakest retention. This rule is followed by all tested compounds except CB-170 on the TENF column and CB-170 and CB-138 on the DNAP column, where they elute in the mono-*ortho* fraction. All CB congeners have higher or equivalent capacity factors on the DNAP compared with the TENF stationary phase. Further, it is remarkable that the three toxic non-*ortho*-CB congeners have the same retention time on the DNAP column and hence elute as a single peak, a phenomenon not described in the literature for any other column. This property makes it possible to collect a small fraction for subsequent analysis that should have a potential to contain less non-PCB interferences than the fractions collected after back-flush elution, or collection of the wide non-*ortho* heart-cut fraction obtained from, e.g., graphitized carbon or PYE. When the back-flush technique is utilized, all the components with stronger retention than the CBs will also be trapped in the same fraction.

Table 1
Capacity factors of CBs on the DNAP and TENF stationary phases using hexane as mobile phase

CB No.	Structure	DNAP	TENF
28	2,4,4′	0.85	0.80
47	2,4,2′,4′	0.67	0.65
52	2,5,2′,5′	0.69	0.74
77	3,4,3′,4′	2.43	1.74
101	2,4,5,2′,5′	0.68	0.62
105	2,3,4,3′,4′	1.59	1.10
114	2,3,4,5,4′	1.08	0.75
118	2,4,5,3′,4′	1.12	0.76
122	3,4,5,2′,3′	1.36	1.00
126	3,4,5,3′,4′	2.42	1.55
138	2,3,4,2′,4′,5′	0.96	0.73
153	2,4,5,2′,4′,5′	0.70	0.50
156	2,3,4,5,3′,4′	1.35	0.80
157	2,3,4,3′,4′,5′	1.57	0.96
167	2,4,5,3′,4′,5′	1.11	0.64
169	3,4,5,3′,4′,5′	2.43	1.44
170	2,3,4,5,2′,3′,4′	1.11	0.82
180	2,3,4,5,2′,4′,5′	0.81	0.54

The relative standard deviation in retention time was less than 1.0% on the DNAP column and less than 1.3% on the TENF column ($n = 3$).

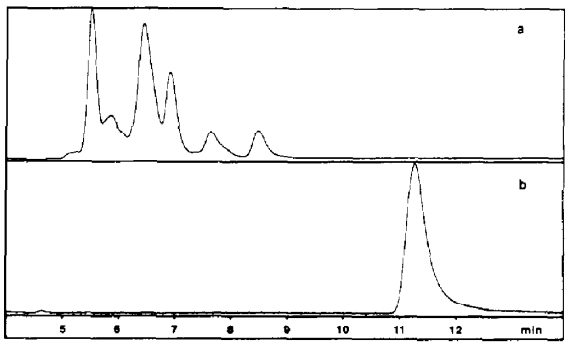

Fig. 1. Chromatograms of (a) Aroclor 1254 and (b) a standard of CB-77, CB-126 and CB-169 separated on a DNAP column. Hexane was used as the mobile phase at a flow-rate of 1.0 ml/min and the UV detector was set at 225 nm.

3.2. Isolation of toxic non-ortho-CB congeners

To illustrate the practical use in clean-up, a solution of the technical product Aroclor 1254 was fractionated on the DNAP column. The HPLC trace is shown in Fig. 1a and that of a standard sample containing CB-77, CB-126 and CB-169 in Fig. 1b. A good separation between the bulk of PCBs and the non-*ortho* congeners is demonstrated. The two groups, bulk and non-*ortho* components, are less resolved on the TENF column (Fig. 2a and b). Hence DNAP is to be preferred to TENF as long as the latter is not shown to have superior separation capabilities for matrix components or other interfering substances. In Fig. 3a and b the GC–ECD traces of Aroclor 1254 and the non-*ortho* fraction from DNAP are shown. Their relative peak heights are roughly in the orders corresponding to the amounts found in Aroclor 1254 [18] or Clophen A50 [13,19]. As it has been shown that on slightly polar GC columns with a 5% diphenyl content, only CBs with higher degrees of chlorination elute close to the toxic non-*ortho*-PCB congeners [20], their identity and purity can be checked by GC–MS. No interfering PCB congeners were found in the peaks of CB-126 and CB-169. For CB-77, a minor amount of a pentachlorobiphenyl, CB-110 [20], was detected in the peak. The detector signal from the pentachlorobiphenyl was 10% of that from CB-77. Hence columns with different polarities or detection by MS using only individual masses, not ranges, should be used for the quantification and identification of CB-77.

3.3. Carryover effects in HPLC systems

It is obvious that there are residues of bulk PCBs in the non-*ortho* chromatogram (Fig. 3b), predominantly from the mono-*ortho*-substituted congeners. This is obtained despite the good separation monitored on the UV detector. Neither rinsing the column outlet with hexane or acetone immediately before collection nor add-

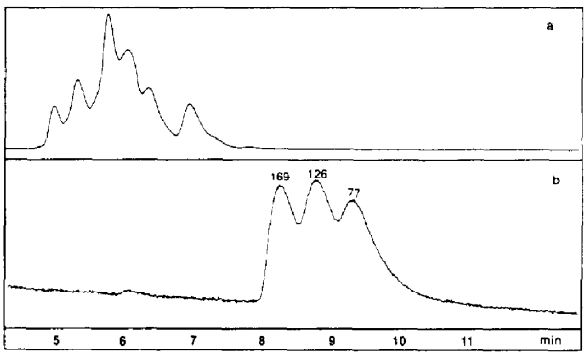

Fig. 2. Chromatograms of (a) Aroclor 1254 and (b) a standard of CB-77, CB-126 and CB-169 separated on a TENF column. Hexane was used as the mobile phase at a flow-rate of 1.0 ml/min and the UV detector was set at 225 nm.

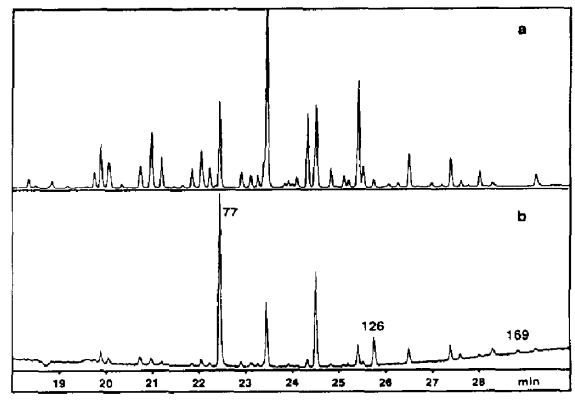

Fig. 3. GC–ECD of (a) Aroclor 1254 and (b) toxic non-*ortho* congeners fractionated from Aroclor 1254 on a DNAP column. A Restek RT-5 GC column (60 m × 0.32 mm I.D.) was used with hydrogen as carrier gas.

ing a modifier such as methyl-*tert.*-butyl ether to reduce adsorption eliminated the problem. The effect remained also after shortening the connecting tubing length to the minimum. There were no signs of overloading as two fractionation experiments with different amounts of Aroclor 1254 yielded the same relative peak heights in the subsequent GC analysis.

Hence, the effect is attributed to peak tailing due to interaction with the stationary phase. This problem becomes particularly evident because of the large concentration differences between the target compounds, i.e., CB-77, CB-126 and CB-169, and the more abundant components of Aroclor 1254. The major peaks of the remaining bulk PCB in the chromatogram of the non-*ortho*-CB congeners (Fig. 3b) correspond to the mono-*ortho* congeners CB-118 at a retention time of 23.5 min and CB-105 at 24.5 min. Of the individual congeners tested, CB-105 elutes closest to the non-*ortho* fraction (Table 1). CB-118 has a capacity factor less than half those of the non-*ortho* congeners on the DNAP column, but it is the second most abundant congener in Aroclor 1254 [20].

Fig. 4 shows the HPLC trace of CB-105 injected on to a DNAP column where eight collected fractions have been marked. The relative distribution of CB-105 in each fraction is listed in Table 2. A much longer tailing is demonstrated by the data in Table 2 compared with the tailing observed on the HPLC–UV trace. Still, in fraction eight, collected about 6 min after the peak apex, 0.2% of the total detected amount of this non-polar compound is present. The non-*ortho* fraction, which corresponds to the second half of fraction 4, fraction 5 and a small part of fraction 6 (10.80–12.60 min), is estimated to contain 1–1.5% of the total amount CB-105. For the purpose of determining CB-105, it can be concluded that collection of fractions 1 and 2 provides a yield of more than 97% of the detected amount.

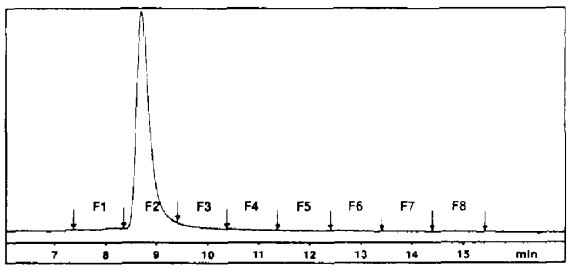

Fig. 4. Chromatogram of CB-105 injected on to a DNAP column. Fractions collected at 1-min intervals are marked. The relative distribution of CB-105 in the different fractions is listed in Table 2. Hexane was used as the mobile phase at a flow-rate of 1.0 ml/min and the UV detector was set at 225 nm.

Table 2
Relative distribution of CB-105 in different fractions as shown in Fig. 4

Fraction No.	Relative distribution (%)
1	0.03
2	92.5
3	5.0
4	1.2
5	0.6
6	0.3
7	0.2
8	0.2

The fractions were analysed by GC as described under Experimental.

The problem with overlap from tailing peaks seems to be present to a larger or smaller extent on all applied liquid chromatographic stationary phases when solutes with concentration differences of the orders found in PCB samples are to be separated [6,8,13,14]. However, as MS with single or multiple ion monitoring is frequently used for detection when determining CB-77, CB-126 and CB-169, residues of CB congeners with masses other than that currently being monitored will not be detected or will yield only small peaks from fragments with the same mass-to-charge ratio.

When the purity of the non-*ortho*-PCB fractions of Aroclor 1254 from a DNAP column and Clophen A50 from a PYE column are compared, it can be noted that the PYE column yields lower residual amounts of CB-105 and CB-118 than the DNAP column. On the other hand, those are the only "bulk PCB" peaks higher than the peak of CB-126 in the collected fraction from the DNAP

column, whereas the fraction collected from the PYE column has at least seven bulk-PCB peaks higher than that of CB-126 [13].

4. Conclusions

Two new alternatives for the selective separation of toxic non-*ortho*-chlorobiphenyls from the bulk PCB have been presented, dinitroanilinopropylsilica and tetranitrofluoreniminopropylsilica. Both stationary phases differ in functionality from the previously used materials based mainly on pure carbon or hydrocarbons. The nitro functionality is expected to provide alternative selectivity also to non-PCB components, a characteristic that might improve clean-up. The unusual retention pattern of CB-77, 126 and 169 on the DNAP column, all in a single peak, provide the possibility of collecting a small fraction for subsequent analysis by GC or on-line coupled LC–GC. These nitro stationary phases can thus be regarded as adequate alternatives for the selective clean-up of toxic non-*ortho*-CBs.

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