Separation of non-*ortho* PCB congeners on pre-packed carbon tubes. Application to analysis in sewage sludge and soil samples.

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INTRODUCTION

During the last years increasing attention has been focused on the study of non-ortho and mono-ortho polychlorinated biphenyls (PCBs) because they show the same type of toxicity as polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF). These compounds are usually present at lower concentrations compaired to the bulk of PCBs in environmental matrices. Therefore, it is necessary a fractionation step in order to analyse them without interferences of the other isomers.

Several methods are described in literature to achieve this fractionation: open-chromatographic columns filled with activated carbon, Carbopack C/Celite, Florisil, alumina, etc. (1, 2), and porous graphitic carbon (3) or pyrenil-silica columns (4, 5), both used in preparative HPLC. An exhaustive review of these methods has been published by Hess *et al.* (6). Each of them have advantages and disadvantages. Methods with HPLC columns (PYE and porous graphitic column) show a good separation and their automation is easy but they are very sensitive to the presence of lipids. On the other hand, open-chromatographic columns with alumina or Florisil do not achieve a good separation easily and it is not possible its automation. Although the separation in charcoal columnns can be good, there are usually high batch-to-batch variabilities and automation is a problem.

In this work we have developed a new method to separate PCB in three fractions (non-*ortho*, mono-*ortho* and the rest of PCBs) with SPE tubes pre-packed with Carbopack B. This is a simple and rapid method that presents a good separation with low amounts of solvent and that can be easily automated.

EXPERIMENTAL

Reagents and Materials

Supelclean ENVI-Carb SPE Tubes (3 ml, 0.25 g) were from Supelco (Bellefonte, PA, USA). Hexane (Pestipur) was obtained from SDS (Peypin, France) and toluene (glass distilled grade) was supplied by Rathburn (Walkerburn, Scotland). Isooctane (Suprasolv) was from Merck (Darmstadt, Germany).

A 20 PCB mixture (PCB 28, 52, 101, 149, 123, 77, 118, 153, 114, 105, 138, 167, 126, 128, 180, 156, 157, 170, 169 and 189) was prepared in isooctane from the individual solid congeners, supplied by The Community Bureau of Reference (Brussels, Belgium) and Cambridge Isotope Laboratories (Woburn, MA, USA). Aroclor 1254, used in several dilutions, was from Chem Service (Birkenhead Merseyside, United Kingdom). 1,2,3,4-tetrachloronaphtalene (TCN), supplied by ICN (Costa Mesa, CA, USA), was used as injection standard for PCB determination by HRGC-ECD.

PCB fractionation and samples analysis

Previously to the fractionation, carbon tubes were cleaned with 20 ml toluene and 20 ml hexane. The sample was applied to the stationary phase and the separation was carried out with the following eluents under vacuum: 15 ml hexane (Fraction 1, di-, tri- and tetra-*ortho* PCB), 20 ml hexane/toluene (99:1) (Fraction 2, mono-*ortho* PCB) and 20 ml toluene (Fraction 3, non-*ortho* PCB). All the fractions were evaporated under reduced pressure up to 1-2 ml and concentrated under stream of nitrogen until 15 µl. The injection standard was added to the concentrated fractions and analysed by HRGC-ECD.

Sewage sludge was obtained from a WWTP in Girona (Spain) and soil samples were from Burgos (Spain) and they were polluted by wire burning. The samples were analysed by isotopic dilution method as described elsewhere (7). After Florisil clean-up column, PCBs were fractionated in carbon tubes. Fraction 1 and fraction 2 were analysed by HRGC-ECD and fraction 3 by HRGC-HRMS.

HRGC-ECD conditions

A HP-5890 Series II with ECD detector and a DB-DIOXIN capillary column (60 m x 0.25 mm, 0.25 µm) from J&W Scientific (Folsom, CA, USA) was used. The temperature programme was 120°C (held for 3.0 min), increased at 20°C/min to 180°C and increased at 2°C/min to 270°C (held for 35 min). Injector and detector temperatures were 250°C and 350°C respectively. Injection was made on splitless mode (3 min).

HRGC-HRMS conditions

The samples were analysed in a CE 8000 gas chromatograph coupled to an AutoSpec-Ultima (Micromass, Manchester, UK) mass spectrometer, operating in EI ionization (32 eV) at 10,000 resolving power. A SPB-5 capillary column (60 m x 0.25 mm, 0.25 µm) from Supelco (Bellefonte, PA, USA) was used. The temperature programme was 120°C (held for 3 min), increased at 50°C/min to 170°C (held for 3 min), increased at 1°C/min to 221°C and

increased at 15°C/min to 280°C (held for 20 min). Injector temperature was 250°C and injection was made on splitless mode (1 min).

RESULTS AND DISCUSSION

The accuracy and precision of the fractionation has been validated using a 20 PCB mixture, as it is shown in Table 1.

Table 1.

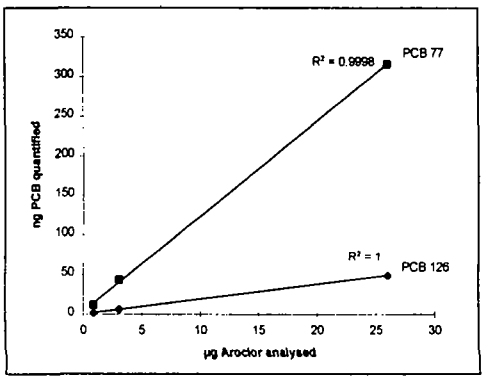
PCB	28	52	101	149	123	77	118	153	114	105
Fraction	I/II	I	I	I	II	III	II	I	I/II	II
Repeatability (RSD %)	5*	6.8	1.7	1.7	3.3	7.7	1.4	2.1	10*	2.5
Reproducibility (RSD %)	34*	13.5	2.8	1.4	14.7	19.0	15.8	6.4	27*	11.4
Recovery (%)	38*	89	98	105	71	113	70	101	47*	76
PCB	138	167	126	128	180	156	157	170	169	189
Fraction	I	II	III	I	I	II	II	I	III	II
Repeatability (RSD %)	2.4	3.9	9.2	2.1	3.5	8.0	2.2	4.0	9.9	6.3
Reproducibility (RSD %)	8.3	12.7	3.9	9.1	12.8	12.8	8.1	13.3	4.7	11.3
Recovery (%)	105	81	106	103	111	69	86	107	104	83

** These PCBs elute between the first and the second fraction and only the corresponding to the highest recoveries are shown..*

The recoveries are good for 18 PCBs and RSD are good in spite of considering changes of day, analyst and batch of carbon tubes.

To study the linearity of the separation, several amounts of Aroclor 1254 (57.7, 10.3 and 6.2 ng) have been analysed with these method. The graphic *ng PCB quantified vs µg Aroclor analysed* is shown in Figure 1.

Figure 1.



Therefore, it can be concluded that the method is reproducible and selective and that it shows a good linearity and good recoveries.

This fractionation has been successfully applied to the analysis of non-*ortho* PCBs in sewage sludge and soil samples by the isotopic dilution method. The concentration, expressed as ng PCB / g sample, and the recoveries of labeled standards (in parenthesis) are shown in Table 2.

Table 2.

PCB	Sludge	Soil1	Soil2	Soil3
77	1.06 (62%)	9.61 (64%)	70.2 (64%)	2.03 (56%)
126	0.064 (69%)	2.30 (65%)	19.0 (63%)	0.221 (67%)
169	0.010 (84%)	0.769 (78%)	3.19 (74%)	0.040 (50%)

Sewage sludges have a great amount of hydrocarbons that are co-extracted with PCBs and that are not removed in usual clean-up procedures. This fact can be a problem when the sample should be concentrated until very small volumes (mono and non-*ortho* PCB analysis). The developed fractionation removes hydrocarbons almost completely from the second and third fraction as can be observed if they are injected in a HRGC-FID system.

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