

SEPARATION OF POLYCHLORINATED POLYCYCLIC AROMATIC POLLUTANTS ON A HPLC CARBON COLUMN

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ABSTRACT

The purpose of this work was to find a method to separate PCBs and other environmental pollutants using HPLC carbon column chromatography. The HPLC carbon column separate compounds according to their planarity and polarity. The separation of a number of PCBs was investigated by this HPLC method. The separation was achieved by gradient elution with n-hexane/methylene chloride and toluene in the forward direction followed by reversed elution with toluene. The eluate was divided into seventeen fractions and each fraction was analysed for PCBs. Initial results indicate that this HPLC method is a useful method for the separation of PCBs according to the number of *ortho* chlorine atoms. The planar polychlorinated polycyclic aromatic compounds like PCDDs and PCDFs are collected in the final fraction.

INTRODUCTION

In recent years the interest for non-*ortho* PCBs as well as the mono-*ortho* PCBs and the di-*ortho* PCBs has increased because of the toxicity of these compounds (1). In human samples and samples collected in the Baltic Sea it has been shown that the toxic equivalents (TEQs) for the PCBs exceeds the TEQs for the PCDD/Fs (2). Nonpolar activated carbon columns are often used for the separation of planar aromatic compounds (e. g. non-*ortho* PCBs) from other non-planar aromatic compounds (e.g. tetra-*ortho* PCBs). The most common method used is open column liquid chromatography (3-5) but in recent years HPLC has received increasing attention because of its higher inherent reproducibility and its potential for automatization (6-8). Different types of carbons as well as different types of supporting materials have been used for the clean-up of environmental samples (3-8). Here we report on a HPLC carbon column method using activated carbon Amoco PX-21 dispersed on a common HPLC supporting material. The HPLC carbon column was kindly provided by Ted Schwartz, U. S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, Columbia, MO, USA.

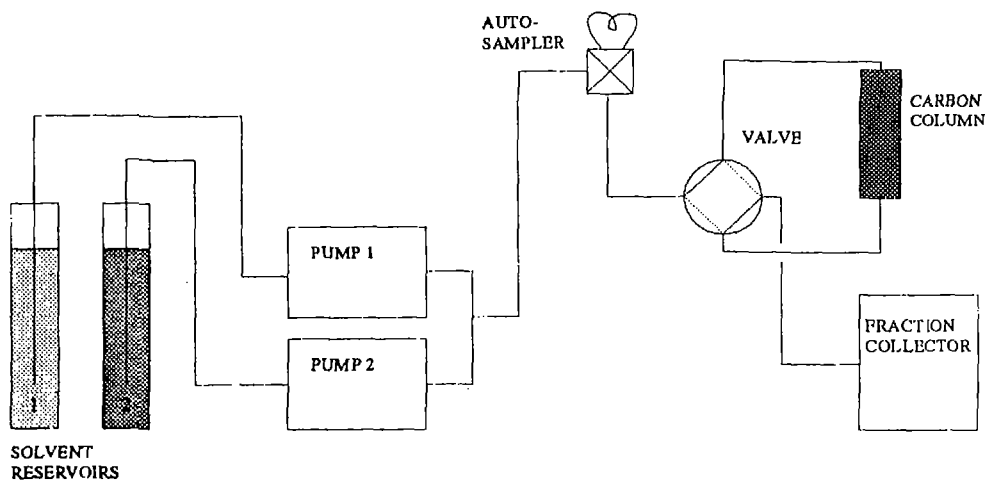


Figure 1. HPLC equipment. Solvent 1 is a mixture of 10 % methylene chloride in hexane. Solvent 2 is toluene. The solvents are mixed after the HPLC pumps providing the gradient elution shown in Figure 2. The valve controls the direction of the mobile phase through the column.

MATERIALS AND METHODS

The HPLC equipment used for the separation of the polychlorinated polycyclic aromatic compounds is shown in Figure 1. The equipment consist of two HPLC-pumps LKB 2150 (Bromma, Sweden), a HPLC-controller LKB 2152 (Bromma, Sweden), an auto sampler AS-4000 (Hitachi, Japan) equipped with a 100 μ L sample loop, a HPLC carbon column, and a Gilson FC 204 fraction collector (Gilson Medical Electronics Inc., Middleton, WI, US). The 4,5 x 250 mm HPLC carbon column was packed with 100 mg Amoco PX-21 (2-10 μ m) activated carbon dispersed on LiChrospher RP-18 (15-25 μ m). The solvent reservoirs contain 10 % methylene chloride in hexane, solvent 1, and toluene, solvent 2. The flow rate was 4 mL/min. Following sample injection (400 ng/congener) solvent 1 is blended with a continuously increasing portion of solvent 2 from 5 min. to 40 min. resulting in a final solvent composition of 10 % solvent 2 and 90 % solvent 1. At 40 min. the valve changed the direction of the flow and pure toluene was used to backflush the column. The gradient program used for the separation is shown in Figure 2. The polarity of the hexane/methylene chloride solvent, the use of toluene and the adsorption on the carbon separate the PCB congeners. Seventeen fractions were collected with the fraction collector. The first sixteen fractions each contained 10 mL of the mobile phase. The volume of the last fraction was 80 mL toluene.

The analyses of the fractions were carried out with HRGC/LRMS in EI mode. The gas chromatograph used for the analysis of PCBs is a Hewlett Packard 5890 in splitless mode. The column is a RTx-5 (Restek corp., Bellefonte, PA, USA), 60 m x 0.32 mm ID,

fused silica column. The capillary column is directly coupled to the ion source of a VG 12-250 mass spectrometer. After a 3 μ L injection of an aliquot of the fractions the oven is

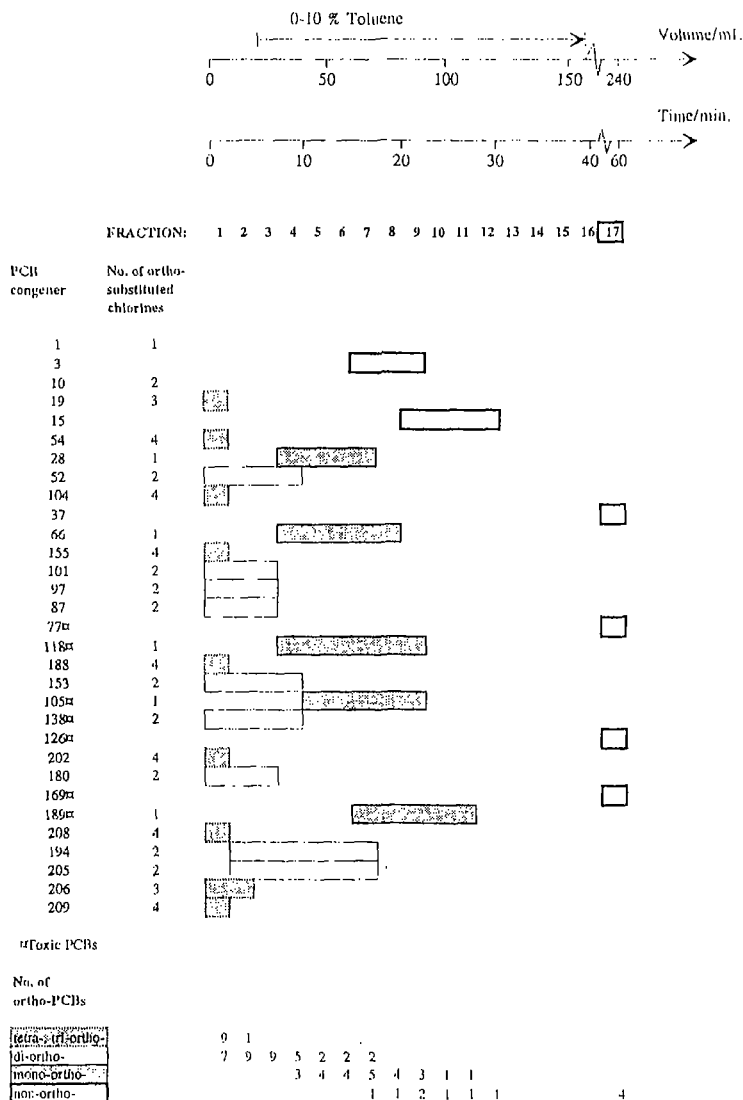


Figure 2. Elution of a PCB mixture through the HPLC carbon column. The mixture contained monochloro- to decachloro-CB. All homologues are represented in the mixture. Differing numbers of chlorine atoms in the PCB *ortho* positions are also well represented. Analysis time, 60 minutes, as well as total elution volume, 240 mL, are shown at the top of the figure.

temperature programmed as follows: 80°C (2 min), 15°C/min, 200°C, 4°C/min, 300°C (10 min). The PCB responses are recorded with full scan, $m/z = 100-500$.

RESULTS AND DISCUSSION

The result of the separation of the PCB mixture is shown in Figure 2. The tetra- and tri-*ortho* PCBs, di-*ortho* PCBs, mono-*ortho* PCBs, and non-*ortho* PCBs are mainly collected in the first, second and third, fifth to ninth, and seventeenth fraction, respectively. The retention of the individual PCB congeners on the column depend on the number of chlorine atoms and the number of chlorine substituents in the *ortho* positions. Many chlorine atoms and/or few *ortho* chlorine atoms in the PCB molecule increases the retention. The non-*ortho* PCBs (PCB 37, PCB 77, PCB 126, and PCB 169) are collected in the last fraction. Recent experiments with 1 % methylene chloride in hexane instead of 10 % methylene chloride in hexane makes the separation between the PCB congeners even better. This work is a prestudy for a more complete validation using biological samples.

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