IMMUNOAFFINITY CHROMATOGRAPHY AS AN ISOLATION METHOD FOR PCBs AND OTHER DIOXIN-LIKE COMPOUNDS

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are a widespread group of environmental pollutants. They have a high lipophilicity and chemical resistance, what means a high capacity of accumulation in the environment.

There are only 12 out of the 209 possible PCB congeners showing toxicity similar to PCDD/Fs, and having been assigned Toxic Equivalent Factors (TEFs). These 12 congeners are the non- and mono-*ortho* chlorine substituted, with two chlorines in *para* position and at least one chlorine in *metha* position. These congeners are the so-called coplanar PCB congeners. Due to their toxicity and capability of accumulation, this group of pollutants has been widely studied during the past 10 years.

The analytical determination of these toxic compounds in environmental matrices using conventional methodologies involves sophisticated and tedious sample preparation, multistep cleanup procedures, and/or very specific detectors[1]. To overcome these disadvantages new analytical methods, such as Immunocromatography, are under development. The high specificity of the antigen-antibody reaction can be used to simplify the isolation methods. Antibodies raised against PCBs can be bound to a chromatographic support. The Immunoaffinity Chromatography (IAC) column obtained can be used to isolate and concentrate this family of pollutants in a single step instead of the various steps needed for the cleanup of a sample using conventional methodologies. In fact, low pressure immunoaffinity chromatography has been used to isolate PCDDs and PCDFs[2-4].

The goal of this work was to develop a high performance IAC method to improve the analytical methodology for PCB analysis. The IAC column generated against the coplanar toxic PCB congeners should reduce the cleanup steps, time of analysis, costs, and the amount of hazardous solvents used in the analysis of PCBs. The feasibility of the anti-PCB column to isolate PCBs and other related compounds, such us PCDD/Fs, is shown.

MATERIAL AND METHODS

Antiserum and IAC column generation: The synthesis of the hapten with structures shown in figure 1 was done following the method of Ya-Wen Chiu[5] with minor modifications. The hapten bound to Keyhole Limpet Hemocyanin (KLH) through a spacer arm was used as immunogen to raise

antibodies against PCBs. The antiserum was purified using a protein-G affinity column. Following purification, the antibodies were immobilized onto Epoxy-Silica beads according to manufacture's instruction. The IAC column was made by packing the antiPCB beads into an HPLC empty column.

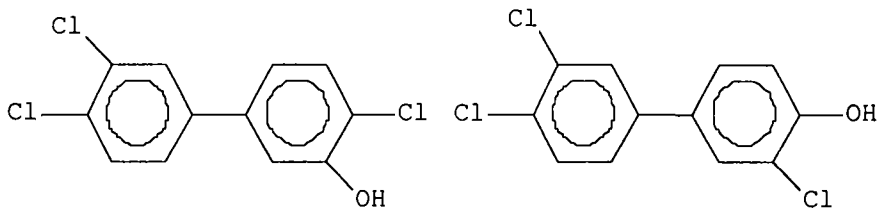

Fig 1. Hapten structures

Procedure for IAC purification:
In order to determine the recoveries of the most toxic PCBs and other related compounds from the IAC column three different working solutions in Methanol/PBS (10:90) were prepared. Two of the solutions were prepared from a standard mixture containing the PCB congeners IUPAC No. 77, 105, 118, 126, 156 and 169. Solutions M1 and M2 had final concentrations of each congener of 1 ng/ul and 0.001 ng/ul respectively. The third solution, M3, was prepared from a standard mixture of the 17 most toxic PCDD/F congeners, to final concentrations of 3.3 pg/g for TCDD/F, 6.6 pg/g for PeCDD/Fs, HxCDD/Fs and HpCDD/Fs and 13.3 pg/g for OCDD/F. All the PCB and PCDD/Fs congeners used for the working stock solutions were purchased from Ehrenstorfer (Germany).

20 ul of solution M1 were injected into the preequilibrated IAC column. Afterwards, the column was washed with 2 ml of Methanol/PBS (10:90) to remove the interferences and the unspecifically bound compounds. After this washing step the specifically bound compounds were eluted with 2 ml of Acetonitrile/PBS (40:60). The whole method was performed at a constant flow of 0.2 ml/min.

In order to simulate real concentrations and to evaluate the capability of the column to manage high volumes of diluted samples as a concentration step, 2 ml of the M2 solution were injected into the IAC column.

To study the performance of the IAC column to retain structurally related compounds, 20 ul of solution M3 were injected into the IAC column. The washing and elution steps were the same as in experiments with M1 and M2.

Quantification:
The 2 ml eluates from the IAC column were extracted into methylene chloride, passed through dry sodium sulphate, and concentrated with a keeper solvent (isooctane, 10 ul). The quantification of the PCBs was performed by analysis of the isooctane solution by HRGC-LRMS, using a Varian 2000 mass spectrometer coupled to a Varian CP-3800 gas chromatograph. The quantification of PCDDs and PCDFs was performed by analysis of the isooctane solution by HRGC-HRMS using an AutoSpec Ultima coupled to a Fisons Series 8000 (8060) gas chromatograph. For both analysis a fused silica capillary DB-5 column (J&W, 60m, 0.25 mm i.d., 0.25 μm film thickness) was used, being helium the carrier gas.

RESULTS AND DISCUSSION:

Table 1 presents the recoveries of the extraction of PCBs from the IAC column for the M1 and M2 solutions. The IAC column shows a high affinity for the coplanar PCB mixture used in the study, obtaining good recoveries for all these congeners, except for PCB 156 in the diluted solution.

Table 1. Recoveries of extraction of PCBs from the IAC when working with the solutions M1 and M2

IUPAC No.	M1 Average (n=3)	M1 R.S.D.	M2 Average (n=3)	M2 R.S.D.
PCB #77	95.9%	9.4%	90.2%	5.1%
PCB #105	97.9%	14.6%	117.4%	12.0%
PCB #118	87.6%	14.5%	106.4%	15.9%
PCB #126	96.6%	17.7%	108.2%	8.0%
PCB #156	102.2%	22.4%	56.8%	1.9%
PCB #169	88.1%	25.3%	118.4%	8.2%

The R.S.D. obtained are in the range of the values accepted by the U.S. EPA methods.
Table 2 presents the recoveries for the extraction of PCDD/Fs using the IAC column. Recoveries higher than 60% for all PCDD/Fs congeners except for 1,2,3,4,7,8,9-HpCDF, OCDF and OCDD were obtained. These congeners with low recoveries were not detected in the non-retained fraction, most probably indicating that they could remain retained into the IAC column. A different elution solvent should be necessary to elute them.

Coplanar PCBs have a structure very similar to PCDD/Fs. Due to this similarity a good affinity of the polyclonal IAC column for PCDD/Fs was expected. This crossreactivity would allow to perform in a single step the fractionation of the dioxin-like compounds from other possible interferences.

Table 2. Recoveries of extraction of PCDD/Fs from the IAC when working with the solution M3.

Congener	Recovery	Congener	Recovery
2,3,7,8-TCDF	74.0%	2,3,7,8-TCDD	80.6%
1,2,3,7,8-PeCDF	82.9%	1,2,3,7,8-PeCDD	101.3%
2,3,4,7,8-PeCDF	77.7%		
1,2,3,4,7,8-HxCDF	94.6%	1,2,3,4,7,8-HxCDD	120.9%
1,2,3,6,7,8-HxCDF	107.0%	1,2,3,6,7,8-HxCDD	94.8%
2,3,4,6,7,8-HxCDF	102.0%	1,2,3,7,8,9-HxCDD	112.7%
1,2,3,7,8,9-HxCDF	67.8%		
1,2,3,4,6,7,8-HpCDF	116.3%	1,2,3,4,6,7,8-HpCDD	79.2%
1,2,3,4,7,8,9-HpCDF	44.1%		
OCDF	36.9%	OCDD	51.8%

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