HIGH PERFORMANCE IMMUNOAFFINITY CHROMATOGRAPHY OF PCBs IN DRINKING WATER AND SERUM MATRICES

Concejero, M.A.; González, M.J.; de Frutos, M.

Department of Instrumental Analysis and Environmental Chemistry. Inst. of Organic Chemistry (CSIC). Juan de la Cierva 3. E-28006 Madrid (Spain)

Introduction

Polychlorinated biphenyls (PCBs) have been synthesized and used extensively in the past due to their characteristic physicochemical properties. Although most industrialized countries have stopped their production, they are still very widespread in the environment. These organochlorinated pollutants, mainly the so-called coplanar PCBs, are health aggressive compounds, having been assigned TEF values (1). Their chemical and physical stability makes easy their distribution around the planet and bioaccumulation in living organisms.

The analytical determination of these toxic compounds in environmental matrices using conventional methodologies involves tedious sample preparation, multistep cleanup procedures, and/or very specific detectors, what make these analyses expensive. Recently we developed a new antibody-based chromatography methodology for the analysis of these compounds, obtaining good recovery and R.S.D. values in a wide range of concentrations for these compounds and also for PCDD/Fs and related compounds in buffer solutions (2,3).

In this work the applicability of this immunoaffinity chromatography (IAC) methodology to analyze PCBs in drinking water and serum matrices is studied.

Material and methods

Procedure for antibody and IAC column generation

The synthesis of the PCB immunogen following a modified version of the procedure from Chiu *et al* (4), as well as the obtention of the polyclonal anti-PCB antibodies and their immobilization onto silica beads and column packing were previously described (2,3).

Samples

Due to the low levels of coplanar PCBs in the samples of drinking water and certified serum (1589a) assayed, both matrices, water and serum, were spiked with PCBs #77, 105, 118, 126, 156 & 169.

Filtered drinking water samples were prepared at two different supplementation levels:

W1: Drinking water supplemented to a final concentration of 1 pg/mL of each mentioned PCB congener.

W2: Drinking water supplemented to a final concentration of 0.4 pg/mL of each PCB mentioned congener.

Filtered human serum was firstly mixed 1:2 with PBS:MeOH (9:1) and then supplemented to a final concentration of 0.5 ng/mL of each mentioned PCB congener.

IAC procedure

Two mL of the sample, water or serum, were injected into the preequilibrated IAC column. Afterwards, the column was rinsed for 15 minutes with the mobile phase (PBS pH 7.2). After this step

the specifically bound compounds were eluted with 2 mL of the desorption solution (PBS:MeCN 60:40 (v/v)). All of the IAC eluted fractions corresponding to the injection, washing and desorption steps, were collected for their analysis by HRGC-LRMS.

The whole IAC method was performed at a constant mobile phase flow rate of 0.2 mL/min.

Quantitation

The fractions collected from the IAC column were extracted into methylene chloride, passed through anhydrous sodium sulphate, and concentrated with a keeper solvent (nonane, 10 mL). The quantitation of the PCBs was performed by analysis of the nonane solution by HRGC-LRMS, using a Varian 2000 mass spectrometer in mode MS/MS coupled to a Varian CP-3800 gas chromatograph. A fused silica capillary DB-5 column (J&W, 60 m, 0.25 mm. i.d., 0.25 mm film thickness) was used, being helium the carrier gas.

Results and Discussion

Figure 1 shows the UV chromatograms of the IAC analysis of drinking water and serum matrices spiked with PCBs. In this figure the different steps of the IAC protocol, injection, washing and desorption, are indicated. The differences in the UV absorption of the injection and washing steps between both samples can be observed. Whereas for water samples the absorption values in both steps are low, the absorption values of the same steps for the serum samples are very high, specially in the first step. These high absorption values correspond to the components of serum that are not recognized by the antibodies in the IAC column.

In figures 2 a-c the CG-MS chromatograms obtained from the different fractions of serum collected from the IAC column are shown. From the GC-MS chromatogram corresponding to the injection fraction (Fig 2 a) it can be seen that there are a lot of different substances which increase the

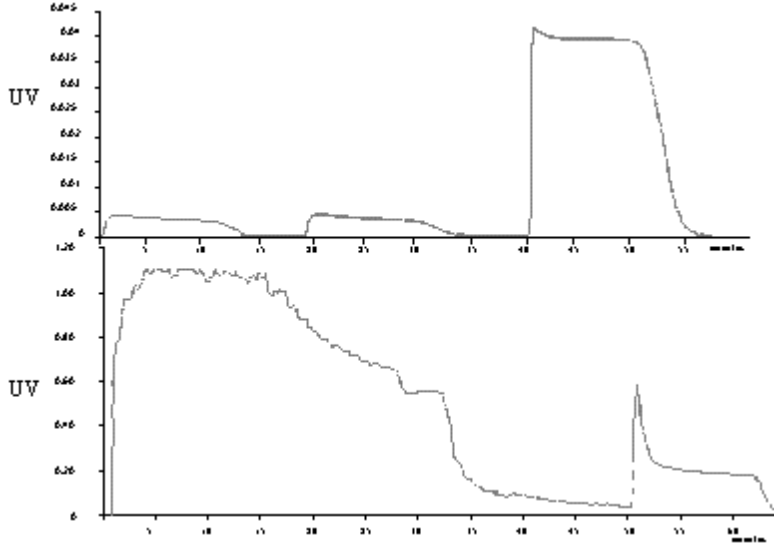

Figure 1. UV chromatograms of the IAC analysis of water (a) and serum (b) samples

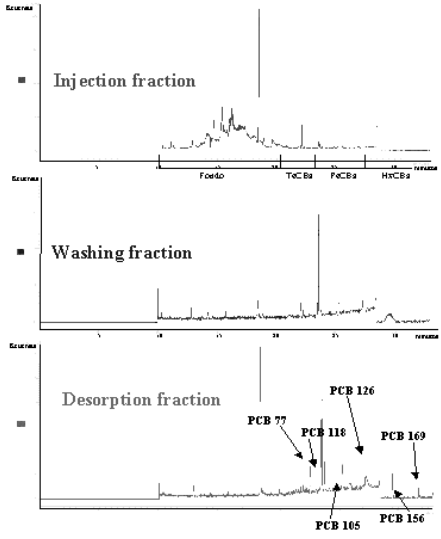

Figure 2. GC-MS chromatograms corresponding to the different fractions collected from the IAC column. a) injection fraction, b) washing fraction, c) desorption fraction from the analysis of the serum sample.

background signal of the GC-MS chromatogram. This high background could interfere in the determination and quantitation of PCBs if these serum components were not eliminated prior to the GC-MS analysis. The absence of signals from the PCB congeners in this non-retained fraction can also be observed. The GC-MS chromatogram corresponding to the washing fraction of the serum sample also shows the absence of signals from the PCB congeners spiked.

The signals of the PCBs supplemented to the samples were found in the GC-MS chromatogram corresponding to the retained and further desorbed IAC fraction (Fig 2 c). This result together with the lack of interferences observed in this fraction show the selectivity of antigen-antibody interaction.

Table 1 shows the recoveries and R.S.D. values obtained by the IAC analysis of the spiked drinking water and serum samples. It can be observed that all the recoveries are over 75% with low R.S.D. values. These results are in the range recommended by the U.S.E.P.A. method 1668 for the analysis of PCBs, which allows recoveries between 60 and 140 % and a maximum R.S.D. value of 40 %.

Congener	Water sample W1 (1pg/mL) (n=3)		Water sample W2 (0.4 pg/mL) (n=3)		Serum sample S1 (0.5 ng/mL) (n=3)	
	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.
PCB 77	87 %	5.7 %	75 %	6.4 %	83 %	6.4 %
PCB 105	114 %	10.0 %	80 %	3.0 %	96 %	7.4 %
PCB 118	108 %	8.0 %	85 %	4.2 %	110 %	7.2 %
PCB 126	110 %	9.1 %	79 %	9.3 %	101 %	5.4 %
PCB 156	113 %	7.4 %	81 %	10.2 %	113 %	16.4 %
PCB 169	116 %	8.2 %	83 %	11.6 %	92 %	10.5 %

ANALYSIS II

In this work the feasibility of the IAC column and methodology developed for the extraction of the PCB congeners assayed from water and serum matrices, and the capability of this immunocolumn to perform in a single step the extraction and cleanup of the sample are shown.

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