Immunoaffinity chromatography: Feasibility for the fractionation of dioxin-like compounds

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Introduction:

Polychlorinated byphenils (PCBs) as well as most organochlorinated pesticides (DDT, lindane, aldrin, heptachlor, etc.) have been synthesized, and used extensively in the past. Although most industrialized countries have stopped their production, they still are very widespread in the environment. These compounds, PCBs, organochlorinated pesticides and their metabolites, are health aggressive compounds. Their chemical and physical stability makes easy their distribution around the planet, being possible to find trace levels of them even in remote places, such as in polar areas. (1,2)

The analytical determination of these toxic compounds in environmental matrices using conventional methodologies involves sophisticated tedious sample preparation, multistep cleanup procedures, and/or very specific detectors, what make these analyses expensive. New analytical methods, such as immunochromatography, are under development in an effort to overcome these disadvantages. Because of the high specificity of the antigen-antibody reaction this methodology can be used to simplify the isolation methods.

Recently we developed an anti-PCB immunoaffinity column for the extraction and cleanup of PCBs, obtaining good recoveries not only for PCBs, but also for the structure related PCDD/Fs (3,4). Most organochlorinated pesticides also have a chemical structure similar to PCBs, suggesting a possible cross-reaction as it was observed for PCDD/Fs.

Traditionally IAC methodology has been used mainly as an isolation and concentration methodology. Some authors have described the possibility of this methodology to be used not only for these purpoises, but also as a chromatographic separation methodology prior to other detection techniques (6). Few studies, mainly regarding weak affinity systems, have been focused in this way.

The objective of this work was to determine the feasibility of the immunocolumns developed to recognize these insecticides and PCBs mixed and to optimize the working conditions of this immunoaffinity system to allow the fractionation of the different compounds retained by the IAC column. The feasibility of the anti-PCB column to retain some pesticides and the ability to reach their fractionation are shown.

Material and methods

Procedure for antibody and IAC column generation:
The PCB antigen synthesis, the obtention of the polyclonal anti-PCB antibodies, as well as their immobilization and column paking into an empty stainless steel HPLC column were described previously (3,4).

Solvents and solutions:
A working solution containing a mixture of the most toxic coplanar PCBs together with some organochlorinated insecticides in PBS:MeOH (9:1) at a final concentration of 1 pg/μL of each congener was prepared.

The PCB congeners used were the IUPAC No. 77, 105, 118, 126, 156 & 169, whereas the insecticides employed were *pp'*-DDT, *pp'*-DDE, *pp'*-DDD, β & γ–lindane, dieldrin, DBP, heptachlor and heptachlor epoxide.

In order to check the effect of variations of the percentage of organic modifier on the desorption of the different compounds, four desorption solutions were prepared:
1) PBS:Acetonitrile(MeCN) (65:35)
2) PBS:MeCN (70:30)
3) PBS:MeCN (75:25)
4) PBS:MeCN (80:20)

The mobile phase for the whole method was PBS pH 7.2

IAC procedure:
Twenty μL of the working solution were injected into the preequilibrated IAC column. Afterwards, the column was rinsed for 15 minutes with mobile phase. After this rinsing step the specifically bound compounds were eluted by injecting 2 mL of desorption buffer. The non-retained and retained fractions were collected for their analysis by HRGC-LRMS. The elution fraction was collected in fractions of 1 minute in order to create the profile of elution of the different compounds studied from the IAC column. Fig 1 shows a scheme of the instrument used for this analysis.

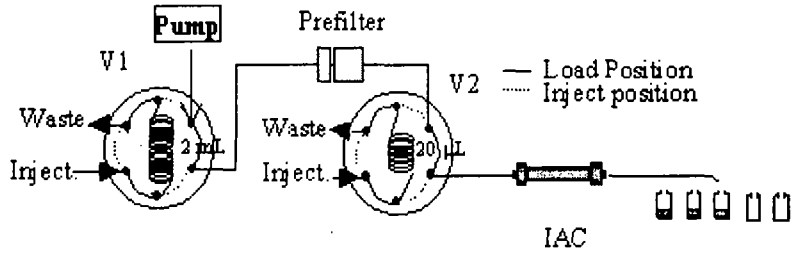

Fig 1: Analytical instrument.

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

Quantitation.
The fractions collected during the desorption step from the IAC column were extracted into methylene chloride, passed through anhydrous sodium sulphate, and concentrated with a keeper solvent (nonane 10 μL). The quantitation of the PCBs and insecticides were performed by analysis of the nonane solution by HRGC-LRMS, using a Varian 2000 mass spectrometer 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 μm film thickness) was used being helium the carrier gas.

RESULTS AND DISCUSSION

In a previous work the feasibility of the anti-PCB column to recognize PCBs and PCDD/Fs was shown (13, 14). PCDD/Fs , have a structure very similar to PCBs, and in particular to the so-called coplanar PCBs, so a good affinity of the anti-PCBs IAC column for PCDD/Fs should be expected. This fact suggests the possiblility of cross-reactivity of other PCB structure-related compounds.

Figure 2 shows the elution profiles of PCBs and some organochlorinated insecticides under different desorption conditions. Values in the Y axis are normalizes to maximum value of 100. In this figure it can be observed that variation of the affinity with the decrease in the percentage of the organic modifier in the desorption buffer is different for each compound. Although when working with a low percentage of the organic modifier (20 % MeCN) the bands for the PCBs, are very wide, this desorption buffer let us to fractionate most of the organochlorinated pesticides from PCBs, and also allows the fractionation between them.

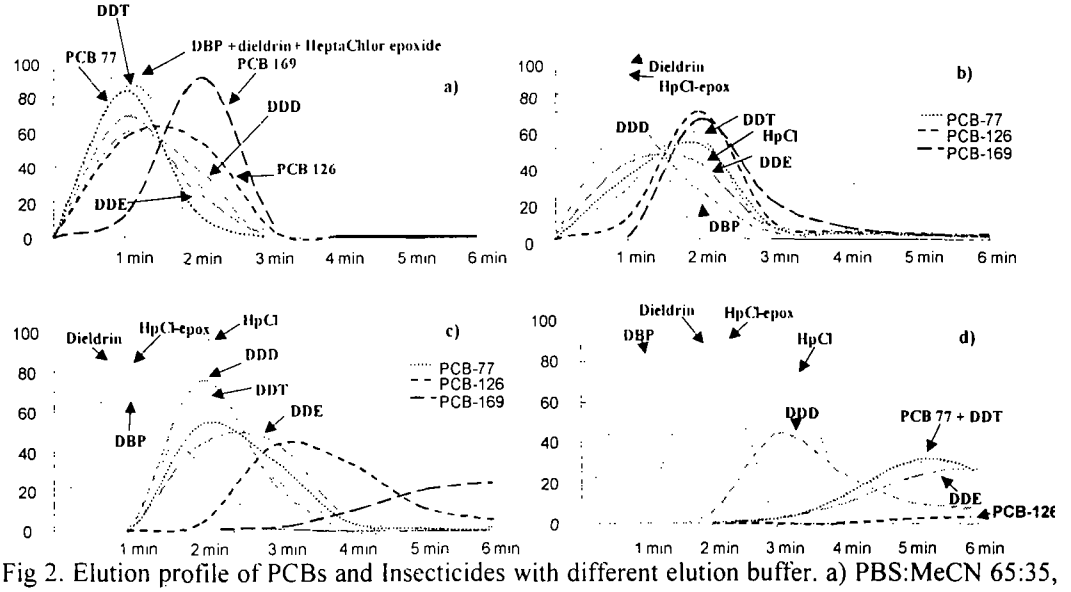

Fig 2. Elution profile of PCBs and Insecticides with different elution buffer. a) PBS:MeCN 65:35, b) PBS:MeCN 70:30, c) PBS:MeCN 75:25, d) PBS:MeCN 80:20.

Lindanes (α and γ), were the only organochlorinated pesticides studied that were not detected in the elution fraction. These compounds were fractionated from the rest as they were recovered in

the non-retained fraction. This result shows the specificity of the anti-PCB column, which does not recognize every chlorinated compound.

In this work the importance of the cross-reaction of polyclonal antibodies for the analysis of organochlorinated pollutants have been probed. Also the ability to use the IAC column as a fractionation instrument and not merely for the extraction and concentration has been shown.

ACKNOWLEDGEMENTS

The authors thanks I.N.I.A. for project CAL00-041
M.A. Concejero also thanks Mapfre-CSIC for a predoctoral grant.

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