



## Review

## Development and application of immunoaffinity chromatography for coplanar PCBs in soil and sediment

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## HIGHLIGHTS

- We examine a tandem bioanalytical method for coplanar PCB determination.
- Pressurized liquid extraction/immunoaffinity cleanup and ELISA detection for PCBs.
- Tandem bioanalytical method (PLE/IAC/ELISA) for PCB detection in soil and sediment.
- Bioanalytical methods provide advantages for environmental monitoring.

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## ABSTRACT

An immunoaffinity chromatography (IAC) column was developed as a simple cleanup procedure for preparing environmental samples for analysis of polychlorinated biphenyls (PCBs). Soil and sediment samples were prepared using pressurized liquid extraction (PLE), followed by the IAC cleanup, with detection by an enzyme-linked immunosorbent assay (ELISA). Quantitative recoveries (84–130%) of PCB-126 were obtained in fortified sediment and soil samples using the PLE/IAC/ELISA method. These results demonstrated that the IAC procedure effectively removed interferences from the soil and sediment matrices. The IAC column could be reused more than 20 times with no change in performance with 99.9% methanol/0.1% Triton X-100 as the elution solvent. Results of 17 soil and sediment samples prepared by PLE/IAC/ELISA correlated well with those obtained from a conventional multi-step cleanup with gas chromatography/mass spectrometry detection.

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## 1. Introduction

Polychlorinated biphenyls (PCBs) are synthetic chemicals that were commonly used as plasticizers, and in capacitors, transformers, and other electrical equipment for insulation. PCBs are a group of 209 different chemicals considered as pollutants of environmental and human health concern. They have been linked to adverse health effects in adults and children (Johnson et al., 1999; ATSDR, 2000; Aoki, 2001; Schantz et al., 2003) and are classified as probable human carcinogens by the U.S. Environmental Protection Agency (EPA) (IRIS, 2002). The manufacture of PCBs was banned in the US in 1977 and other countries followed with the Stockholm Convention on Persistent Organic Pollutants in 2001; however, they are still being detected in various environmental components (i.e., air, soil, dust, sediment and food) (Chuang et al., 1998; ATSDR, 2000; Kohler et al., 2002; Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004; Hopf et al., 2009; Chovancova et al., 2011; Fitzgerald et al., 2011). Elevated levels of PCBs in building caulking materials from around windows and in expansion joints in masonry buildings have also been reported (Herrick et al., 2004, 2007; Van Emon, 2009).

The three non-ortho coplanar PCBs (PCB-77, PCB-126, and PCB-169) are most structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and are considered the most toxic (van den Berg et al., 1998, 2006). Analytical determination of the coplanar PCBs with conventional methods usually involves an acid wash frequently coupled with either gel permeation chromatography (GPC), or silica/Florisil column chromatography, with gas chromatography/mass spectrometry (GC/MS) or electron capture GC detection (Kohler et al., 2002; Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004). Simpler, cost-effective, high-sample throughput cleanup and detection methods may assist in environmental site monitoring and human exposure assessment studies for the PCBs.

IAC combines the advantages of solid phase extraction (SPE) with the specificity of the antibody-antigen (Ab–Ag) interaction. IAC columns have been developed but not employed in large scale for small molecule environmental contaminants (Van Emon et al., 1998; Carrasco et al., 2001; Concejero et al., 2001; Wu et al., 2001; Shelper et al., 2002; Kaware et al., 2006; Altstein and Bronshtein, 2007; Chuang et al., 2007). Immunoassay methods have been developed for detecting PCBs at submicrogram levels depending on the congener and the sample processing procedure (Johnson and Van Emon, 1996; Van Emon and Lopez-Avila, 1992; Van Emon, 2001; Glass et al., 2005; Van Emon et al., 2007; Lin et al., 2008; Tsutsumi et al., 2008; Altstein et al., 2010).

Described here are: (1) the development of an IAC column with polyclonal rabbit anti-PCB antibodies (Abs) and HiTrap NHS activated Sepharose resin, (2) the development of a PLE method in tandem with an IAC column cleanup and ELISA detection (PLE/IAC/ELISA) and (3) the comparative results generated from different sample preparations (multi-step cleanup, acid wash, and IAC) and detection techniques (GC/MS and ELISA) for coplanar PCB analysis in soil and sediment samples.

## 2. Experimental section

### 2.1. Samples

Seventeen sediment and soil samples from various sampling locations in a field study conducted under the U.S. EPA Superfund Innovative Technology Evaluation Monitoring and Measurement Technology program were used for method validation (U.S. EPA, 2004; Dindal et al., 2007).

### 2.2. Chemicals

Distilled-in-glass grade dichloromethane (DCM), hexane, dimethyl sulfoxide (DMSO), ethyl ether (EE), methanol, toluene, and polypropylene glycol (PPG) were from VWR (West Chester, PA). PCB standards were obtained from Cambridge Isotope Laboratories (Andover, MA). Polyclonal anti-PCB Ab (which bound primarily with PCBs 126 and 169) and ELISA testing kits were from Abraxis (Warminster, PA). Glass fiber filters were from Dionex (Sunnyvale, CA). Polymeric Poros resin and silica gel (3-aminopropyl) were purchased from Fisher Scientific (Fair Lawn, NJ). Protein-Pak resin was from Waters (Milford, MA) and Affi-gel 102 was from Bio-Rad Laboratories (Richmond, CA). HiTrap NHS-activated Sepharose (referred hereafter as Sepharose) columns were purchased from Amersham Biosciences (Piscataway, NJ). Non-specific rabbit IgG Ab, bovine serum albumin (BSA), phosphate buffered saline (PBS), PBS containing 0.1% Triton X-100 (PBST), PBS containing 0.1% Tween 20, sulfuric acid, and anhydrous sodium sulfate were obtained from Sigma (St. Louis, MO). Hydromatrix (diatomaceous earth) was purchased from Varian (Walnut Creek, CA).

### 2.3. IAC development

Five types of control columns were prepared with non-specific rabbit IgG Ab or BSA using: (1) Polymeric Poros resin, (2) Protein-Pak resin, (3) Affi-gel 102 (aminoalkyl agarose), (4) silica gel (3-aminopropyl functionalized) and (5) Sepharose resin. Two types of IAC columns were prepared with polyclonal anti-PCB Abs with (1) Affi-gel and (2) Sepharose. Different combinations of loading solvents (10–25% methanol in water or in PBS) and elution solvents (50–75% methanol in PBS and 100% methanol) were employed. Sepharose resin yielded the best performance results among the five materials tested and was selected for the final development of the IAC procedure. Additional loading solvents evaluated for the Sepharose IAC column were: 1%, 10%, and 25% DMSO in PBST; 1% PPG/20% methanol in PBST; 10% and 20% methanol in PBST; and 10% methanol in PBS with 0.1% Tween 20. In each experiment, the control or IAC column was conditioned with 5 mL of PBS, followed by 3 mL of the loading solvent. After application of a known amount of PCB-126 to the conditioned column, the column was incubated at room temperature for 5 min; washed with 3 or 5 mL of the loading solvent; and eluted with 5 or 10 mL of elution solvent. The elution solvent used for the control column experi-

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