Solid-Phase Disk Extraction: An Improved Sample Preparation for Organohalogen Pollutants from Human Serum

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Introduction

Organochlorine pesticides (OCPs) are pest control agents with a worldwide use in agriculture. Polychlorinated biphenyls (PCBs) represent another group of chemicals with widespread applications in products such as heat-transfer fluids, hydraulic fluids, capacitors and transformers. Due to their high chemical stability and lipid solubility, these two groups of compounds are ubiquitous environmental pollutants, routinely detected in fish, wildlife, human adipose tissue, blood and breast milk.

A fast, simple, sensitive and efficient extraction method prior to analytical detection for a range of these organochlorine compounds (OCCs) in serum is important for assessment studies that document health issues related to human exposure. This paper describes as first the use of (SPE) 'disk technology" to provide a simplified and improved sample preparation procedure for the extraction of a wide range of both OCPs and PCBs from human serum, prior to GC-MS analysis. The method is much more elegant and efficient than any extraction method (solid phase or liquid-liquid) hitherto reported in the literature (1,2).

Materials and Methods

Blood collection of patients was conducted in collaboration with the University Hospital of Antwerp (UZA, Edegem, Belgium). Blood samples were centrifuged (15 minutes at 3000 rpm) within 24 hr after collection. The serum was pooled and kept frozen (-20°C) until analyzed. Serum protein-binding was disrupted in formic acid (extra pure, Merck, Darmstadt, FRG), after which analytes were extracted and concentrated using C_{18} solid-phase extraction disk cartridges (Empore disks (C_{18}, 7 mm/3mL and 10mm/6mL) by 3M Company, St. Paul, MN USA). The following OCCs were selected for this study: pesticides: β-HCH, γ-HCH (lindane), DDT and its metabolites, heptachlor epoxide, and dieldrin. As PCBs we selected the 7 PCB congeners many European government and regulatory bodies propose as marker compounds to monitor occurrence and distribution

(numbering according to Ballschmiter and Zell (3): IUPAC Nos. 28, 52, 101, 118, 138, 153, 180). All analytes under investigation were purchased from J.T. Baker (Deventer, the Netherlands). The $^{13}C_{12}$-labelled chlorinated biphenyls used as surrogate analyte and internal standard (PCB 110 and 149, respectively) were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA).

The extraction scheme using SPE cartridges is shown in Table 1. The eluates were twice washed with concentrated sulphuric acid (extra pure, Merck, Darmstadt, FRG). After separation of the phases by centrifugation (5 min at 3500 rpm), the organic layer was removed. After washing with water and evaporating the eluate, the internal standard $^{13}C_{12}$ PCB 149 was added to the final concentrate, prior to GC-MS analysis.

Table 1: Solid-phase extraction scheme for OCCs using particle-loaded membranes.

	1 mL serum	8 mL serum	Remarks
•SPE C_{18} cartridge	7 mm / 3 mL	10 mm / 6 mL	
•Sample Preparation (uL)			
Formic acid	1000	8000	
Acetonitrile	50	400	
TEA	10	80	sonification 30 min
•Column Conditioning (uL)			
DCM	250	500	dry thoroughly
Methanol	250	500	no drying allowed
Water	500	1000	
•Sample Loading	1 column	2 columns	2 columns in parallel
•Column Wash			
Water	500	2 x 500	dry thoroughly
•Elution Step (uL)			
EtAc : Hex : TEA (8:2:0.2)	2 x 200	2 x 300	
EtAc : Hex : TEA (2:8:0.2)	2 x 200	2 x 300	
Hexane		300	
Methanol		300	
•Surrogate analyte recovery	85 (6,6 %)	97,5 (4,9 %)	

Quantification is achieved using capillary gas chromatography with highly selective mass spectrometry in SIM (selected ion monitoring) mode. For this we employed a Hewlett Packard 5890 series II *Plus* gas chromatograph (HP, Palo Alto, USA), equipped with a HP 5972A quadrupole mass spectrometric detector and a fused silica DB-XLB capillary column (J&W Scientific, Folsom, USA). The mass spectrometer was operated in the electron impact (EI) ionization mode at 70 eV. Multi-level calibration curves were created for the quantification using standard solutions of the analytes in hexane.

Results and Discussion

Protein binding

Dale and Miles (4) used hexane for the extraction of chlorinated insecticides from blood but found that the recoveries were not quantitative, probably owing to binding of the analytes to serum proteins. Pretreatment of the serum by denaturation was found to liberate the compounds from protein binding sites (5). Experiments with acid denaturation methods (formic acid, trichloroacetic acid, perchloric acid), organic solvents (methanol, acetonitrile) and zinc salt in alkaline solution were performed. Formic acid deproteination technique yielded the highest recoveries for the individual compounds studied.

SPE

The Empore technology entraps sorbent particles within an inert matrix of polytetrafluoroethylene (PTFE). The resulting membrane (90% sorbent, 10% PTFE, w/w) yields a denser, more uniform extraction bed than can be achieved in a traditional SPE cartridge made from loosely packed particles. The diffusion distance between particles is minimized, adsorption is more efficient, and extraction can be accomplished using less sorbent mass. Higher throughput can be realized, as solvent and elution volumes are reduced to microliters (instead of milliliters) using a small sorbent bed thickness of about 0.75 mm. The potential for automation of the extraction procedure further improves the attractiveness of the disk approach to SPE.

It was suspected that protein binding prevented the analyte from attaching to the adsorption sites on the C_{18} solid phase during the load step, either by strong hydrophobic sites or polar sites from exposed silanol groups. Addition of detergents or triethylamine (TEA) during sample application have been shown to mask some of these sites, resulting in higher recoveries (6).

Elution solvents such as dichloromethane, ethylacetate and n-hexane, as pure solvents and solvent mixtures, were evaluated. The elution pattern consisting of 2 times 200 µL of a mixture of ethylacetate :hexane :TEA (80 :20 :0.2), followed by 2 times 200 µL of a mixture of ethylacetate :hexane :TEA (20 :80 :0.2) yielded higher recoveries for these analytes than any pure solvent.

Sulphuric acid clean-up

Removal of lipids from nonpolar extracts can be achieved by washing with concentrated sulphuric acid or by passing the solvent through adsorption columns of Florisil, aluminium oxide or silica gel. The latter method does not provide complete removal of

lipids, because the polarity of fats is similar to those of pesticides (7). The clean-up of extracts with concentrated sulphuric acid removed the ubiquitous phthalate esters, as well as lipids, thus eliminating the disturbance in the GC chromatogram and giving a truer determination of the analytes under investigation (7). Note that heptachlor epoxide and dieldrin are degraded during clean-up of extracts with concentrated sulphuric acid.

Analyte recovery

The extraction method provided consistent recoveries at three fortification levels; recoveries ranged from 48 - 143 % for OCPs (6.25, 12.5 and 25 ng/mL) and 71 - 126 % for PCBs (0.625, 1.25 and 2.5 ng/mL). A mean recovery of 85 ± 6.6 % of $^{13}C_{12}$ PCB 110, the compound added to all samples as a surrogate analyte, was considered satisfactory in our protocol.

The method of extraction has been further evaluated by analyzing 8 mL aliquots of human pooled blank serum using a larger 10mm/6mL disk cartridge size (the 1.0 mL serum aliquots were extracted using a 7mm/3mL disk cartridge). For these experiments 8 mL serum were required to reach sufficient GC-MS sensitivity for measuring background levels. To avoid overloading of the 10mm/6mL cartridges, two columns in parallel, containing each 4 mL of sample were used. The modifications made to extract this larger amount of serum are summarized in Table 1. The background levels obtained for four PCBs and one pesticide are in agreement with literature values of serum concentrations in the Netherlands and Sweden (8,9). This method has been succesfully used in our laboratory for routine analyses of pesticides and PCBs in human serum.

References

1. Brock JW, Burse VW, Ashley DL, Najam AR, Green VE, Korver,MP, Powell MK, Hodge CC, Needham LL; *J. Anal. Toxicol.* 1996, 20, 528-536.
2. Pauwels A, Schepens PJC; *Int. J. Environ. Anal. Chem.* 1998 (in press).
3. Ballschmiter K, Zell M; *Fresenius Z. Anal. Chem.* 1980, 302, 20-31.
4. Dale WE, Miles W, Gaines TB; *J. Assoc. Off. Anal. Chem.* 1970, 53(6), 1287-1292.
5. Franken JJ, Luyten BJM; *J. Assoc. Off. Anal. Chem.* 1976, 59(6), 1279-1285.
6. Hsu CL, Walters RR; *J Chromatogr.* 1993, 629, 61-65.
7. Waliszewski SM, Szymczynski GA; *Bull. Environ. Contam. Toxicol.* 1991, 46, 803-809.
8. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Paauw, Tuinstra LGMT, Boersma ER, Sauer PJJ; *Chemosphere* 1994, 28(9), 1721-1732.
9. Asplund L, Svensson B, Eriksson U, Jansson B, Jensen S, Wideqvist U, Skerfving S; *Arch. Environ. Health* 1994, 49(6), 477-486.