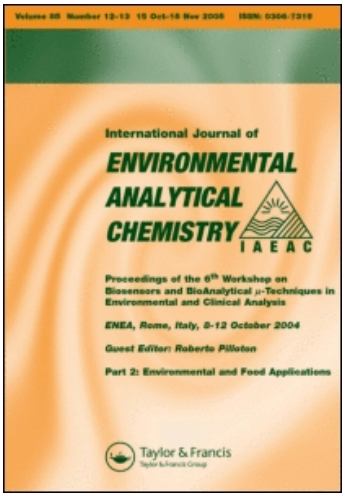

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AUTOMATED GEL PERMEATION CHROMATOGRAPHIC CLEAN-UP OF HUMAN ADIPOSE TISSUE FOR MULTIRESIDUE ANALYSIS OF ORGANOCHLORINE COMPOUNDS

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Human adipose tissue samples have been analysed for organochlorine pesticides and polychlorinated biphenyls (PCBs). Following a micro liquid-solid extraction, clean-up is performed by automated gel permeation chromatography. The GPC separation provides clean extracts, allowing subsequential analysis by capillary gas chromatography-electron capture detection without additional clean-up.

Keywords: Adipose tissue; electron capture detector; gel permeation chromatography; organochlorine pesticides; polychlorinated biphenyls

INTRODUCTION

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are two classes of organochlorine compounds (OCCs) that are structurally and toxicologically related. These two groups of chemicals have shown to be ubiquitous environmental pollutants due to their chemical stability and lipid solubility. They are routinely detected in fish, wildlife, human adipose tissue, blood and breast milk [1,2]. The concentrations determined in adipose tissue of human populations are the best indices in estimating the extent of exposure and risk assessment [3].

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The following OCCs were selected for this study based on their occurrence, persistence, toxicity, and prevalence in human matrices [4,5]. The group of pesticides consisted of β-HCH, γ-HCH (lindane), DDT and its metabolites DDD and DDE, heptachlor epoxide, and dieldrin. Polychlorinated biphenyls form a group of 209 possible congeners. Specific PCB congener analysis has become the preferred method of monitoring PCBs in the environment [6,7]. Many European government and regulatory bodies have selected seven PCB congeners (numbering according to Ballschmiter *et al.* [8]: IUPAC Nos. 28, 52, 101, 118, 138, 153, 180) as marker compounds to monitor occurrence and distribution [9–11]. These congeners were also analyzed in this study.

Although since the 70's the use of OCPs and PCBs has been banned in developed countries, recent data on total pesticide and PCB concentrations in human adipose tissue in European countries, such as Germany, Denmark, Slovakia or Poland indicate a steady-state and/or a relatively high contamination, implying continuous leakage of these compounds into the environment and unchanged exposure rates [12]. The biological contamination resulting from the use of OCPs and PCBs has created the need for multi-residue methods for fast quantitative determination of these chlorinated compounds.

The challenging task in the determination of OCPs and PCBs in fat matrices is the removal of lipids. An overview of sample treatment techniques used in the analysis of organochlorine micropollutants in fatty samples in given by Liem *et al.* [13]. Different techniques have been used including hydrolysis followed by liquid-liquid extraction, column chromatography (Florisil, silica, alumina), solid-phase extraction, gel permeation chromatography (GPC), etc... A GPC clean-up technique (also called size exclustion chromatography) for pesticide residue determination was introduced by Stalling *et al.* in 1972 [14]. This technique is recommended by the AOAC for the clean-up of OCPs in animal fat and is the subject of several recent publications [15–18].

In this paper, automated gel permeation chromatography was used for the fractionation of OCCs from human adipose tissue extracts. The fractions obtained were analysed by gas chromatography with electron capture detection (GC-ECD) without further clean-up, offering excellent sensitivity.

EXPERIMENTAL

Sample collection

Sampling of patients was conducted in collaboration with the Fertility Unit from the University Hospital of Antwerp (UZA, Edegem, Belgium). Because the inva-

sive nature of the process required to collect adipose tissue, sampling population was limited to surgical patients. This study has been accepted by the UZA Ethical Committee (UZA 96/44/107) and all patients accepted their participation by signing an Informed Consent. One gram of abdominal subcutaneous adipose tissue per patient was surgically resected during laparoscopy and was stored at –20°C until analyzed.

Chemicals

n-Hexane, *iso*-octane and methylene chloride were purchased pesticide grade from E. Merck (Darmstadt, FRG). Reference pesticide standards were purchased in crystalline form from J.T.Baker (Deventer, the Netherlands). The seven PCB congeners (as a standard mixture) and PCB 149 (internal standard) were purchased from J.T.Baker at a concentration of 10 ng/μL in *iso*-octane. A standard mixture solution containing all analytes under investigation was prepared in *n*-hexane.

Sample preparation

From each human adipose tissue sample, a 200 mg aliquot was thawed and weighed. 2 mL methylene chloride and PCB 149, as internal standard, were added. Extraction was performed under ultrasonic agitation for 2 h. The samples were centrifuged and the methylene chloride fraction was filtered through a 0.45 μm PTFE filter before injection in the GPC system. The maximum fat concentration was thus 100 mg/mL. To measure the lipid percentage of each sample, a separate subaliquot of the tissue was homogenized and extracted four times with a mixture of *n*-hexane:metylene chloride (2:1).

Gel permeation chromatography

A GPC system consisting of an isocratic HP 1050 HPLC pump (Hewlett-Packard, Waldbronn, Germany), a Gilson 233 XL on-line column switching system (Gilson, Villiers-le-Bel, France) and a Kontron variable wavelength UV detector were used. The Gilson switching system is equipped with a dispenser, sample and collection vial racks and two high pressure six-port valves. This system was set-up to allow both automated injection and automated sample collection [19]. After rinsing the system, a 100 μL sample loop installed on the first switching valve, was filled with the sample extract and injection was performed. The effluent of the column passes the detector and the second switching valve to waste. At

the time of elution of the pesticide/PCB fraction, the second valve is rotated and the fraction is collected in a collection vial. Separation was done on two 30 cm × 7.5 mm id × 5 μm particle size and 50 Å pore diameter PL Gel columns (Hewlett-Packard, Waldbronn, Germany) coupled in series. Methylene chloride was used as mobile phase at a flow rate of 1 mL/min. UV detection was performed at 220 nm. The collection window was 3 min, corresponding to 3 mL methylene chloride. 100 μL *iso*-octane was added to the collected fraction, which was concentrated (to 100 μL) under a gentle stream of nitrogen at room temperature.

Chromatographic equipment and analytical conditions

A HP 6890 Series gas chromatograph (Hewlett-Packard, Wilmington, DE, USA) equipped with split/splitless injection and a micro-ECD was used. Injection was done using a HP 7673 autosampler. For the separation, a 60 m × 0.25 mm id × 0.25 μm film thickness DB-XLB capillary column (J&W Scientific, Folsom, USA) was used. The carrier gas was hydrogen at a constant pressure of 135 kPa. A volume of 1 μL was injected splitless at an injector temperature of 260°C. The GC temperature program was as follows: initial 65°C held for 2 min, then to 220°C at a rate of 50°C min^{-1}, 1.7 °C min^{-1} to 255°C, and then 30°C min^{-1} to 290°C, held for 5 min. The micro-ECD temperature was kept at 320°C, using argon-methane (95+5) make-up gas at a flow rate of 20 mL/min.

Quantitation was carried out using PCB 149 as internal standard. Calibration curves for all solutes had correlation coefficients $r^2 \geq 0.996$ for concentrations ranging from 1–25 ng/g for all analytes under investigation.

RESULTS AND DISCUSSION

In contrast to other chromatographic techniques, gel permeation chromatography also called size-exclusion chromatography, offers a unique separation based on differences in molecular size. Therefore it is an excellent technique for the fractionation of compounds with molecular masses of 200–400 Dalton (e.g. organochlorine compounds) from higher molecular weight compounds, such as lipids (600–1000 Dalton) [20–22]. Generally, large columns slurry packed with polystyrene-divinylbenzene gels (Bio-Beads) are used, resulting in large solvent consumption and long analysis times. Patterson *et al.* [23] and Rhijn *et al.* [24] miniaturized this technique for sample clean-up by using high pressure LC columns packed with small particles of PS-DVB crosslinked polymers. In particu-

lar, 30 cm × 7.5 mm id columns packed with 5 μm particle size and 50 Å porosity are efficient for low molecular weight separations (100 < MW < 1000 Dalton). These columns can thus be used to fractionate lipids (mainly triglycerides) from environmental pollutants such as pesticides, PCBs and PAHs.

A typical GPC chromatogram obtained for the analysis of a human adipose tissue extract is shown in Figure 1. The triglycerides and sterol esters elute first (17–20 min), followed by di- and monoglycerides and sterols. The elution window of the organochlorine pesticides and PCBs was determined by analyzing a highly concentrated reference sample and using sequential fraction collection. OCPs and PCBs eluted in a time window from 21 to 24 min. These start and stop times for fraction collection were introduced in the controller of the Gilson 233 XL switching system, allowing fully automated injection and fraction collection.

The combination of two 30 cm × 7.5 mm id GPC columns offers a good compromise between loadability, resolution, flow rate and analysis time. From a 2 mL extract of 200 mg fat, 100 μL was injected. This corresponds to a maximum of 10 mg fat load on the column combination. This fat quantity does not overload the GPC column combination used here. Moreover, by using a 1 mL/min flow rate, the total analysis time was less than 30 min. Solvent consumption is hereby drastically reduced in comparison to column chromatography on BioBeads (30 mL versus 400 mL methylene chloride per analysis) [25]. Another advantage of the GPC clean-up is the robustness of the method. Since the high molecular weight solutes elute first, no residues remain on the column and after the permeation limit, the next sample can be injected. The same column combination was used in this study for more than 80 samples without deterioration of the GPC separation or shift in retention time of the compounds on the UV detector.

The analysis of the extract obtained by GPC fractionation was done by capillary gas chromatography with ECD detection. The most important PCB congeners and different organochlorine pesticides can easily be detected. For the optimisation of this method, 30 samples were analysed. The average pesticide concentrations (on a lipid weight basis) can be summarized as follows: dieldrin 12 ng/g adipose tissue (range 8–17 ng/g), *p,p'*-DDD 10 ng/g (range 7–17 ng/g), and *p,p'*-DDE 319 ng/g (range 132–613 ng/g). The average PCB concentrations were 112 ng/g (range 58–228 ng/g), 142 ng/g (range 91–194 ng/g) and 83 ng/g (range 49–138 ng/g), respectively for PCB 118, PCB 138, and PCB 180. Due to co-elution of *p,p'*-DDT and PCB 153, these compounds could not be quantified on the GC-ECD. The other compounds were not found quantitatively in these samples. The GC-ECD chromatogram obtained for a human adipose tissue extract is shown in Figure 1.

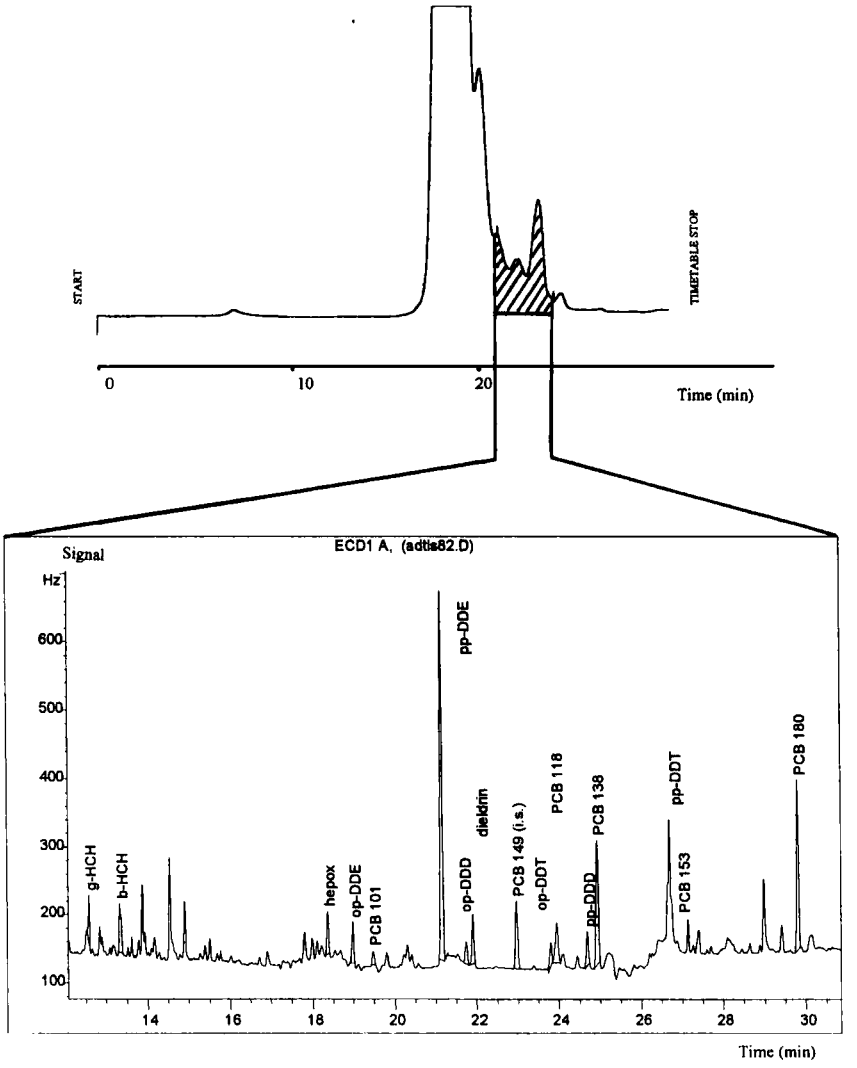

FIGURE 1 GPC clean-up on PL Gel columns of a human adipose tissue sample and GC-ECD analysis of the fraction containing the chlorinated compounds. Upper trace: UV-chromatogram of GPC separation. Lower trace: ECD-chromatogram of OCC fraction

The most abundant PCBs detected in the samples were PCBs 138 and 180. These compounds are known to be preferentially bioaccumulated in living organisms, are widely found to be the major constituents of the PCB residual mixtures in human adipose tissue [12,26]. PCB 153 is also known to be bioaccu-

mulated in organisms, but the capillary column used do not allow its separation from *p,p'*-DDT, even after optimisation of all the chromatographic parameters. Anyway, this type of capillary column is believed to offer the best overall PCB separations [27].

For the pesticides, p,p'-DDE was the most abundant isomer. This is in accordance with previous observations [28], showing that DDT and its metabolites constitute the principal OCPs found in the human body. Detailed quantitative results in correlation with toxicological data will be published later.

The combination of micro-extraction, GPC and GC-ECD offers enough sensitivity for the study of OCP and PCB contamination. The injection of an equivalent of 10 mg of fat in combination with a final volume of 100 µL extract (2 mL methylene chloride fraction exchanged into 100 µL *iso*-octane) allowed a detection limit better than 1 ng/g for the OCPs and PCBs monitored in this study.

CONCLUSIONS

Sample clean-up by automated gel permeation chromatography using high performance GPC columns offer an efficient and robust method to fractionate organochlorine compounds (OCPs and PCBs) from lipids, extracted from human adipose tissue. The organochlorine pollutants in the collected fraction can be quantified by GC-ECD without further additional clean-up. The sensitivity of the method was 1 ng/g.

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