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Determination of organochlorine pesticides and polychlorinated biphenyls in human serum using headspace solid-phase microextraction and gas chromatography-electron capture detection

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Abstract

A simple procedure for the determination of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human serum using headspace solid-phase microextraction (HS-SPME) was developed. The analysis was carried out by gas chromatography (GC) equipped with electron capture detector (ECD). A 2^{7-4} Plackett–Burman reduced factorial design for screening and a central composite design for optimizing the significant variables were applied. A 100 μm PDMS fiber, 3/5 headspace ratio (3 ml in 5 ml vial), 85 °C extraction temperature, 50 min extraction time, and 1 ml of acidic solution (pH 3) added to 1 ml of diluted serum (1:1) were chosen for the best response in HS extraction mode. The detection limits found were from 1 pg/ml (PCB 167) to 52 pg/ml (β-HCH), the relative standard deviation for the procedure varied from 3% (PCB 52) to 12% (PCB 189) and the accuracy was checked by using validated solid-phase extraction (SPE) procedure. The method that avoids the use of clean-up steps and the hazardous solvents enabled reliable determinations of the OCPs and the PCBs except β-HCH. The method was applied to the analysis of 33 human serum samples. The most abundant target compound was *p-p′*-DDE (range, 0.3–8.0 ng/ml; median value, 2.1 ng/ml). Among the PCBs the prevalent congeners were 138, 153 and 180.

Keywords: Organochlorine pesticides (OCPs); Polychlorinated biphenyls (PCBs); Headspace solid-phase microextraction; GC-ECD; Plackett–Burman design; Central composite design; Human serum

1. Introduction

Most organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) in environment. Nine of the OCPs, as well as PCBs, were the subjects of the Stockholm convention on POPs. The proposed treaty called for urgent global actions to reduce and eliminate releases of these compounds [1]. However, these chemicals already occur in the environment and in food chains, and because of their resistance to degradation and high solubility in organic solvents and lipids these compounds bioaccumulate in human tissues and fluids, and pose a risk of causing adverse effects to human health. The PCBs have been shown to cause cancer in animals and other non-cancer effects including effects on the immune, reproductive, nervous and endocrine systems [2]. Studies in humans provide supportive evidence for potential carcinogenic and non-carcinogenic effects [2].

Measurements of the OCPs and the PCBs or their metabolites in body tissues and fluids (often called biological monitoring) have been done as useful approach for assessing the exposure risk in the epidemiological studies. Human serum is one of the biological materials that can be conveniently obtained and used for these types of studies [3–6].

Determination of PCBs and OCPs in serum is carried out using a liquid–liquid extraction (LLE) [7,8] or solid-phase extraction (SPE) by columns [9], C_{18} cartridges or disks [10,11]. Most of the reported procedures require posterior clean steps to remove interferences from the coextracted bulk fatty matrix material. Laborious operations such as conditioning, washing, elution, and solvent evaporation are included in the steps of

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sample preparation. Also, clotting, channeling and percolation are inconveniences of the SPE procedures. Those laborious and time-consuming clean-up steps lead to cleaner extracts and, consequently, to lower detection limits. However, the risk of analytical error increases because of the incorporation of more steps in the sample preparation. The cleaned extract is later analyzed using capillary gas chromatography (GC) with electron capture detector (ECD) [7–9,11], mass spectrometry (MS) detection [10,11], high-resolution mass spectrometry with isotope-dilution quantification (IDHRMS) [12,13] or isotope dilution time-of-flight mass spectrometry (IDTOFMS) [13].

Nowadays, solid phase microextraction (SPME) is considered an useful alternative to LLE or SPE. SPME does not require solvents and it can be carried out directly from the liquid phase or from headspace over the liquid samples (HS). Also, SPME involves fewer steps and less sample handling. The HS sampling is more advisable when the matrix could affect the determination of a target analyte. In addition, HS *versus* immersion extraction often shows an important reduction of extraction time [14]. The application of SPME technique, including advantages and disadvantages, to biological fluids has been reviewed [15,16]. These works agreed that an HS method should be applied whenever possible in body fluids analysis. Among other advantageous characteristics, the burden of the fiber with proteins is considerably decreased and the fiber lifetime greatly increased [15]. With respect to the target analytes of this study, an SPME technique has been reported for the determination of OCPs and PCBs in different environmental matrices including water and biological tissues [17–20]. However, only few works have been devoted to OCP determination in human serum using SPME or HS-SPME [21,22]. Nevertheless, in the revised literature there are no references about the potential of HS-SPME technique for simultaneous PCBs and OCPs determination in serum.

There are several experimental variables affecting the HS-SPME procedure such as type of fiber, stirring rate, temperature, extraction time, volume of the headspace, and salt addition. The study considering the variables one by one to get the best possible conditions for HS-SPME has been showed in several studies working with volatile compounds in biological fluids [23,24], PCBs in water [17] and OCPs in water [20] and in human serum [22]. However, this procedure requires a high number of runs and is also time consuming. A structured experiment design that could simultaneously take into account several variables, seems a more convenient approach searching for the optimal operational conditions in a reasonable number runs [25]. As an example in aqueous medium, a response surface methodology (Doehlert design) has been used for optimization of SPME extraction conditions to determine organochlorine pesticides [26]. The response surface methodology uses a multifactor space model where a set of experiments is carried out in a systematic way in order to predict the optimum and the interaction effects. Also, a design with the screening and optimization steps has been applied to find the best experimental conditions using HS-SPME for the determination of alkyl ethers as well as benzene, toluene, ethylbenzene, and xylene isomers (BTEX) in water [27]. Hence, the use of this type of structured methodology should be a short and useful approach to find the best experimental conditions for HS-SPME working with human serum.

This work mainly focused on obtaining a convenient method for the simultaneous determination of the OCPs and PCBs in serum using HS-SPME-GC-ECD. In the method developing the advantageous characteristics of the HS-SPME technique that avoid the use of clean-up steps and the hazardous solvents was considered. A Plackett–Burman reduced factorial design was planned in order to know the significant experimental variables, and a central composite design had run out trying to get the best experimental conditions for the HS-SPME extraction of analytes from serum. Finally, the procedure was applied to the analysis of 33 human serum samples.

2. Experimental

2.1. Reagents and materials

Two mixture standard solutions PCB "Key" (28, 52, 101, 118, 138, 153 and 180) and PCB "dioxin-like" (77, 81, 105, 114, 123, 126, 156, 157, 169 and 189) each at 10 ng/μl in isooctane, and PCB 46 and 143 solutions (internal standards) each at 100 ng/μl, were purchased from LGC Promochem (Middlesex, UK). Organochlorine pesticides (HCB, β-HCH, Heptachlor epoxide, *p*,*p*'-DDE and *p*,*p*′-DDT) were purchased from Dr. Ehrenstorfer (LGC Promochem). The criteria for the standard solution selection were based on reported abundance (PCB "Key" and OCPs) and toxicity (PCB "dioxin-like"). The PCB 118 included in PCB "Key" is one of the twelve "dioxin-like" congeners. These "dioxin-like" congeners display all the following characteristics: (a) there are "co-planar" with non-ortho chlorine substitution or only one mono-ortho of the 2, 2′, 6 or 6′ positions, (b) have a total of four or more chlorine substituents, (c) have both para positions (4 and 4′) chlorinated, and d) have two or more of the meta positions (3, 3′, 5, 5′) chlorinated. H_3PO_4 acid and Triton X-100 were from Fluka (Buchs, Switzerland). NaH_2PO_4 (99% purity) was supplied by Carlo Erba (Milan, Italy). Acetonitrile and *n*-hexane of HPLC grade, 37% HCl acid, sodium hydroxide and Na_2SO_4 (99% purity) were obtained from Panreac (Panreac Química S.A., Barcelona, Spain).

Two stock mixture solutions containing 2.5 mg/l of each PCB and 0.1 mg/l of OCPs respectively were prepared in cyclohexane. These solutions were stored at −20 °C. From these mixtures a 20 μg/l solution of each PCB and OCP was prepared in acetonitrile. Two standard solutions 0.1 M H_3PO_4 and 0.074 M NaH_2PO_4 were prepared and stored at 4 °C. From these solutions a 3 pH buffer was prepared.

SPME holders and fibers [100 μm thickness poly(dimethylsiloxane) (PDMS) and 65 μm poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB)], 5 ml sample vials and PTFE-silicone septa were obtained from Supelco (Bellefonte, PA, USA). Before using, vials were heated at 300 °C for 10 h.

A thermo bath Lauda RE 104 (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany) was used to maintain the temperature and a Heidolph MR 3003 (Heidolph Elektro GmbH & Co KG, Kelheim, Germany) was used to