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A method for analysis of marker persistent organic pollutants in low-volume plasma and serum samples using 96-well plate solid phase extraction

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ABSTRACT

The objective of this study was to develop and validate a 96-well plate solid phase extraction method for analysis of 23 lipophilic persistent organic pollutants (POPs) in low-volume plasma and serum samples which is applicable for biomonitoring and epidemiological studies. The analysis of selected markers for internal exposure: 16 polychlorinated biphenyls (PCBs), 5 organochlorine pesticides (OCPs), octachlorinated dibenzo-*p*-dioxin (OCDD), and polybrominated diphenylether 47 (BDE 47) was evaluated by comparing two SPE sorbents and GC-HRMS or GC–MS/MS detection. The final method extracted 23 POPs from 150 μL of serum and plasma using a 96-well extraction plate containing 60 mg Oasis HLB sorbent per well prior to GC-HRMS magnetic sector analysis. The extraction method was applied to 40 plasma samples collected for an epidemiological study. The recovery of selected POPs ranged from 31% to 63% (n = 48), and detection limits ranged from 2.2 to 45 pg/mL for PCBs, 4.2 to 167 pg/mL for OCPs, 7.8 pg/mL for OCDD and 6.1 pg/mL for BDE 47. This method showed good precision with relative standard deviations of selected POP concentrations in quality control samples (n = 48) ranging from 11% to 25%. The trueness was determined with standard reference material serum (n = 48) and the deviation from certified values ranged from 1 to 27%. Of the 23 POPs analyzed, 18 were detected in 43% to 100% of plasma samples collected for the epidemiological study. The method showed good robustness with low inter-well plate variation (11–31%) determined by twelve 96-well plate extractions, and can extract 96 samples, including quality controls and procedural blanks in 2–3 days. Comparison with GC–MS/MS analysis showed that similar concentrations (within 0.5% to 30%) of most POPs could be obtained with GC-APCI-MS/MS. Larger deviations were observed for PCB 194 (60%) and *trans*-nonachlor (43%). The developed method produces accurate concentrations of low-level marker POPs in plasma and serum, providing a suitable high-throughput sample preparation procedure for biomonitoring and epidemiological studies involving large sample size and limited sample volume. GC-HRMS was chosen over GC–MS/MS, however the latter showed promising results, and could be used as an alternative to GC-HRMS analysis for most POPs.

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1. Introduction

Persistent organic pollutants (POPs) encompass a wide range of lipophilic substances that are known to be persistent in the environment and toxic to humans [1]. Four major groups of POPs include polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenylethers (PBDEs) [1]. The PCBs, PBDEs and OCPs are man-made chemicals. PCBs were used in electrical transformers or added as plasticizers to paint. PBDEs are used as flame retardants, added into plastics or used for fabric coating [1]. PCDDs are not intentionally produced, but are formed as a result of incomplete combustion during incineration and as

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manufacturing byproducts in pesticide and other chlorinated substance production [1]. Today, diet and indoor dust are two of the main sources of POP exposure for humans [2,3].

Although these chemical's production and usage were restricted by the Stockholm Convention in 2004 and 2009, and by other national and international regulatory parties, their stable chemistry enables them to persist in the environment and in humans [1]. Human internal exposure has been linked to various negative health effects such as hormone related cancers and endocrine disruption [4–6], and has been associated with diabetes and risk factors for cardiovascular disease [7,8]. Biomonitoring and epidemiological studies are important elements in risk assessment and are frequently used to study the temporal trends of POP concentrations in humans and to evaluate the effects low-level POPs have on human health [9–15]. Epidemiological studies require large sample sizes in order to achieve statistical power when evaluating small changes or differences in the effects of interest [16]. In addition, population-based biomonitoring studies often have a large number of biological samples, but low sample volumes. As a result, there is an increased demand on sensitive high-throughput sample preparation and analysis methods. In order to determine the concentration of POPs in humans, they are usually extracted from plasma or serum using liquid-liquid extraction (LLE) or solid-phase extraction (SPE) [17].

Typically, most SPE methods require 0.5 mL to 2 mL of plasma or serum and usually process between 10 to 30 samples at one time [17]. LLE–based methods that require less sample volume (200 μL) and increase sample throughput to 40 processed samples in one analysis have been developed [18,19]. By developing a SPE method with increased throughput the sample preparation procedure becomes even more cost-effective. The challenges associated with high-throughput miniaturized sample preparation methods include maintaining sensitivity, robustness, and low detection limits. This is a time consuming process, which requires reevaluating and potentially modifying conventional methods. However, these challenges need to be overcome in order to efficiently process the many samples required for important biomonitoring and epidemiological research.

The objective of this study was to develop and validate a high-throughput SPE sample preparation method for the extraction of 23 chlorinated and brominated (Cl/Br) POPs from maximum 150 μL of serum or plasma. These 23 POPs are believed to be representative indicators of POP exposure since they are relevant in terms of human levels and toxic effects, are commonly tested for in epidemiological studies, and are listed in the Stockholm Convention. To compensate for the low levels of POPs present in 150 μL samples, the inherently sensitive gas chromatography high resolution mass spectrometry (GC-HRMS) analysis technique was employed. To determine if the analysis of low-level POPs using gas chromatography atmospheric pressure chemical ionization tandem mass spectrometry (GC-APCI-MS/MS) was comparable to GC-HRMS analysis, the resulting POP concentrations in quality control samples measured with both techniques were evaluated. The validated method was applied to 40 samples collected for an epidemiological study.

2. Materials and methods

2.1. Sampling and storage

The National Institute for Standards and Technology (NIST) Standard Reference Material (SRM) 1957, pooled reference plasma obtained from Örebro University Hospital, Sweden, newborn bovine serum (NBS), and HPLC-grade water were used for method development and quality control (QC). The NIST SRM was reconstituted in HPLC-grade water and all QC materials were stored at −20 °C until analysis. After the current method was developed and validated, the QC samples continued to be included when the new extraction method was applied to plasma samples collected for an epidemiological study.

2.2. Chemicals

Methanol, dichloromethane, and toluene were obtained from Honeywell Riedel-de Haën (Steinheim, Germany). Sulfuric acid (H_2SO_4) and *n*-hexane were purchased from Merck (Darmstadt, Germany). Newborn bovine serum (sourced in New Zealand), tetradecane, silica gel (60 Å, 70–230 mesh), and anhydrous sodium sulfate (Na_2SO_4) used for water removal and lipid degradation were from Sigma Aldrich/Supelco (Steinheim, Germany). HPLC-grade water, acetonitrile and 2-propanol were obtained from Fisher Scientific (Leicestershire, UK). A native calibration standard solution consisting of 16 polychlorinated biphenyl (PCB) congeners: #74, #99, #105, #118, #138, #153, #156, #157, #170, #180, #189, #194, #206, #209, octachlorodibenzo-*p*-dioxin (OCDD) and brominated diphenyl ether (BDE) 47, were purchased from Cambridge Isotope Laboratories (Andover, MA). The native pesticides, hexachlorobenzene (HCB), *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and 2,2-bis (4-chlorophenyl)-1,1-dichloroethene (*p,p*'-DDE) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The ^{13}C-labeled PCBs: #70, #101, #105, #118, #138, #153, #156, #170, #180, #194, #206, and #209, ^{13}C-labeled pesticides (HCB and *p,p*'-DDE), ^{13}C-labeled OCDD, and ^{13}C-labeled BDE #47 standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada). The internal standard stock solution used for isotope dilution contained 30 pg/μL ^{13}C-labeled PCBs and ^{13}C-labeled pesticides, 5 pg/μL ^{13}C-labeled OCDD, and 3 pg/μL ^{13}C-labeled BDE #47 in 2-propanol. The recovery standard stock solution added before injection and used for determining the recovery of the internal standards included three ^{13}C-labeled PCBs: #81, #114, and #178, obtained from Wellington Laboratories (Guelph, Ontario, Canada), and was prepared at a concentration of 30 pg/μL in toluene. All standard solutions were kept in a refrigerator at 8 °C.

2.3. Extraction and clean-up

Two SPE sorbents, the Prime HLB and Oasis HLB (both from Waters Corporation, Milford, USA), and a range of sulfuric acid concentrations (0.30 mM to 30 mM in water) and acetonitrile protein precipitation solutions (0%-50% in water) were tested during the method development. In the final method aliquots of 150 μL plasma or serum were diluted in 9 mM sulfuric acid and 20% acetonitrile solutions and applied to a 96-well plate containing 60 mg Oasis HLB media per well. The developed extraction method is based on a previous method which used 500 μL plasma or serum and 150 mg Oasis HLB cartridges [20]. All samples were allowed to thaw at laboratory temperature, and then homogenized by vortex mixing prior to sub-sampling. An aliquot of 150 μL was added to 1 mL glass collection vials. Plasma and serum proteins were precipitated by adding 300 μL 9 mM sulfuric acid in HPLC-grade water followed by 500 μL 20% acetonitrile in water. The samples were sonicated for 15 min after addition of the sulfuric acid solution, then ^{13}C-labeled internal standards (^{13}C-PCBs and ^{13}C-OCPs: 300 pg, ^{13}C BDE 47: 30 pg, and ^{13}C-OCDD: 50 pg) in 2-propanol were added and allowed to equilibrate for 15 min. The 20% acetonitrile solution (500 μL) was then added to each sample and the samples were sonicated again for 30 min. The Oasis-HLB 96-well plate was preconditioned with 1 mL methanol followed by 1 mL HPLC-grade water. The samples were added to the wells. After loading the samples, the transfer pipet tips and sample vials were rinsed with 500uL 20% acetonitrile and added to the respective wells. The sample solution was

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