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Determination of polychlorinated biphenyls and organochlorine pesticides in small volumes of human blood by high-throughput on-line SPE-LVI-GC-HRMS

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ABSTRACT

A fully automated and robust method featuring on-line solid-phase extraction (SPE) and large volume injection (LVI) gas chromatographic (GC) high resolution mass spectrometry (HRMS) is used to determine polychlorinated biphenyls (PCBs) and organochlorine pesticides, such as penta- and hexachlorobenzene (PeCBz, HxCBz), hexachlorocyclohexane isomers (HCH) and 4,4′-dichlorodiphenyldichloroethene (a metabolite of dichlorodiphenyltrichloroethane (DDT)), with only 200 μl of human blood, serum or plasma. After spiking the sample with ^{13}C-labeled internal standards and precipitating the proteins, the sample is passed through a 10 mm × 2.0 mm ID SPE cartridge filled with C18 material that adsorbs the analytes. After washing and drying, the cartridge is extracted with hexane/dodecane (99/1, v/v); the extract is directly injected into a LVI where GC/HRMS analysis follows. The fully automated system utilizes a robotic autosampler and a modular SPE system including two high-pressure syringe pumps, an automatic SPE cartridge exchanger unit and 6 switchable valves. All sample preparation steps are performed within 20 min during the GC run of a previous sample, limiting the throughput with only the GC runtime. The contents are quantified using the isotope dilution method. Due to laboratory air contamination problems, we achieved LOQs of 0.017 (PeCBz), 0.009 (HxCBz), 0.007 (HCH), 0.016 (DDE), while for the six indicator PCBs, we achieved values of 0.030 (PCB-28), 0.044 (PCB-52), 0.024 (PCB-101), 0.009 (PCB-138), 0.015 (PCB-153) and 0.008 (PCB-180) μg/l serum. Under clean laboratory air conditions, these values may be improved. This method is recommended when high throughput is desirable and/or only small amounts of material are available, such as during studies involving children.

1. Introduction

Polychlorinated biphenyls (PCB, 209 congeners) as well as organochlorine pesticides, such as 4,4′-dichlorodiphenyltrichloroethane (DDT) and isomers of hexachlorocyclohexane (α-, β-, γ-HCH), as well as both penta- and hexachlorobenzene (PCBz, HCBz), are persistent organic pollutants (POPs). These compounds persist in the environment, bio-accumulate within the food-chain and can be identified in humans worldwide. Because of their physicochemical and toxicological profiles, these compounds were included in a list of the 21 most dangerous POPs by the Stockholm Convention [1]. Cost-effective measurement of these compounds in small amounts of human blood or serum is desirable, particularly in large epidemiological studies, such as the German Environmental Survey (GerES) or birth cohort studies requiring examinations of children [2].

In Germany, the suitable methods used to determine these compounds in human blood, plasma or serum samples via solvent extraction, classical column clean-up and capillary gas chromatography (GC) were developed in the 1980s and subsequently standardized, optimized and applied to occupational and environmental studies until recently [3–7]. The methods use a few ml of blood, plasma or serum and have a limit of detection of approximately 0.1–0.01 μg/l, depending on the detector. However, when determining dioxin-like PCBs, such as the non-ortho-PCB 77, 81, 126 or 169, at background levels in blood samples collected from the general population, larger sample volumes are still necessary. Methods combining the preparation of complex samples with selective and sensitive detection methods, such as GC/high resolution mass spectrometry (HRMS), are needed to counteract the low concentrations of these PCB congeners (in the pg/l range) and to avoid false positive results [6].

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Other techniques, such as solid phase extraction (SPE) [8–13] or gel permeation chromatography (GPC) [14], coupled with GC and electron capture (ECD) or mass spectrometric detection have been established. Methods prioritizing miniaturization and blood volume reduction use extracts from dried 50 μl blood spots with GC/HRMS. The LODs of the PCBs ranges from 0.002 to 0.005 μg/l [15].

Sample preparation steps, such as SPE and column-based clean-up methods, have been automated [16–20], and instruments adapted for analyzing human blood containing POPs are commercially available from different manufacturers, such as Zymark (Hopkinton, MA, USA), Fluid Management Systems (Watertown, MA, USA) and others.

From a technical point of view, the off-line systems suffer from severe drawbacks: incomplete automation, limited sample throughput, high sample and solvent volumes and the inevitable evaporation of the organic solvent after the SPE extraction and/or clean-up.

The existing on-line combination strategies exhibit their own drawbacks. These drawbacks can be divided in two categories. Several systems can be mounted on top of syringe-based autosamplers. In this case, the transfer of every solvent and sample is realized using the autosampler syringe. An SPE cartridge has replaced the needle or been mounted between the syringe barrel and needle. Depending on the system, these cartridges may be automatically exchanged. One major advantage of this concept is the ease of on-line coupling with an analytical system, such as GC/MS. After the extraction step, the eluates can be injected like any other liquid sample. However, when utilizing large sample volumes, these strategies are inefficient because the extraction time is directly correlated with the size of the syringe. The syringe size is usually limited to 10–500 μl because the same syringe is used for the injection; therefore, using milliliter scale sample volumes is very time consuming. More problems arise when samples with high viscosities or numerous particles are handled.

During our previous experiments using C18 barrel insert and needle assemblies (BINs) and syringes from SGE Analytical Science (Ringwood, Victoria, Australia), we attempted to adapt a micro extraction technique utilizing a packed sorbent (MEPS) [21–23] to determine the halogenated compounds in human blood or serum. After adapting the sample handling and protein precipitation (serum) to our needs, the method was susceptible to faults, becoming unusable for routine analysis. Either the SPE cartridges became blocked or the syringe could not handle the backpressure generated by the sample. Therefore, the syringe-based SPE strategies were abandoned.

Consequently, SPE systems were developed that utilize their own pumps to deliver all solvents and samples. Due to the high pressure delivery requirements mentioned above, these systems contain pressure proof valves, making them more complex than the syringe-based systems. Moreover, the on-line coupling to the analytical system is more demanding; it requires fixed tubing connected to the injector system, prohibiting its use for any other standard analytical procedure. In addition to the instrumental difficulties, there are often shortcomings related to the software control over these systems.

In recent years, on-line SPE has been combined with capillary electrophoresis or liquid chromatography (LC) with MS; these systems have become popular for different applications. Due to the general SPE problems, restrictions set by the physicochemical characteristics of the analytes and the necessary elimination of the solvent only a few applications, particularly in the field of drinking water analysis for environmental contaminants, are suitable for on-line SPE with GC [24,25].

To solve the problems mentioned above during the determination of POPs in human blood or serum, we combined a rapid, high-pressure-proof solid phase extraction unit equipped with an automated SPE cartridge changer, a flexible autosampler system able to handle various sample volumes, and a robust, large volume GC injector with pressure control. This apparatus is attached to a GC/HRMS system. When using this technique, the complete adsorbed amount of the analytes can be transferred without loss to the highly selective and sensitive detection system. This is the first adaption of on-line-SPE for the determination of organochlorine POPs in human blood samples.

2. Materials and methods

2.1. Sample preparation system

A basic schematic of the sample preparation system is shown in Fig. 1. The system was composed of a CTC CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland), a modular SPE system with two syringe pumps (HPD) and an automatic SPE cartridge exchanger unit (ACE) (Spark Holland B.V., Emmen, The Netherlands, distributed as an SPE Exchange Module (SEM) by Axel Semrau GmbH & Co. KG, Sprockhövel, Germany). The autosampler was equipped with a side-port syringe (250 μl, ILS, Stützerbach, Germany) for aspirating the sample and injecting the extraction eluate. The system was also equipped with a 2-way 6-port-injection valve (A) with a 500 μl sample loop between port 5 and 6. The exchanger unit contained three 2-way 6-port valves (B–D). Both syringe pumps (HPD 1 and HPD 2) were equipped with multi-position 6-port valves to pump up to eight different solvents. The syringe pumps were connected to valve D at positions 2 and 6. The exchanger unit could be equipped with two trays containing 96 cartridges each. The SPE cartridges had a size of 10 mm × 2.0 mm ID and were filled with HySphere C18 HD (HD = high density, code Y09x) material as 7 μm spherical particles (Spark Holland B.V., Emmen, The Netherlands). HySphere C18 HD is an end-capped, silica-based phase with a high loading of octadecyl chains. The exchangeable SPE cartridges were placed between positions 3 and 6 at valve B. Nitrogen (5.0, Linde, Munich, Germany) was used as purge gas and connected to port 5 at valve C. The exit at port 2 of valve C was connected to the side-port of the syringe, while port 2 of valve A, port 4 of valve B and port 6 of valve C were connected to a waste vessel. All connections were made of stainless steel or peek material. The system was controlled using the Chronos master software 3.5 advanced (Axel Semrau GmbH & Co.KG, Sprockhövel, Germany).

2.2. Reagents and standards

Picograde acetonitrile, n-hexane, methanol, 2-propanol and toluene, as well as optigrade water, were supplied from LGC Standards GmbH (Wesel, Germany); pro-analysis grade n-dodecane was purchased from Merck (Darmstadt, Germany).

Standard solutions of the following were obtained from Cambridge Isotope Laboratories (Andover, MA, USA): $^{13}C_{12}$-labeled indicator PCBs (28, 52, 101, 138, 153, 180; 40 μg/ml), $^{13}C_{12}$-labeled dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189; 1 μg/ml), $^{13}C_6$-labeled penta-(PeCB) and hexachlorobenzene (HxCB; 100 μg/ml), $^{13}C_6$-labeled α- (100 μg/ml), β- (50 μg/ml) and γ-hexachlorocyclohexane (HCH; 100 μg/ml), and $^{13}C_{12}$-labeled 4,4′-dichlorodiphenyldichloroethene (DDE). The stock solutions were purchased in nonane and initially diluted (1:4 or 1:10) with toluene. All further dilutions utilized methanol to reach final concentrations of 0.5 (PCB 28, 52, 101), 2.5 (PCB 138, 153), 1.25 (PCB 180), 1.0 (dioxin-like PCB), 10 (PeCB, HxCB), 1.0 (α-, β- and γ-HCH) and 8.0 ng/ml (DDE). Standard solutions of the unlabeled compounds were obtained from Cambridge Isotope Laboratories and diluted with toluene.