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Determination of polybrominated diphenyl ethers in human serum by gas chromatography – inductively coupled plasma mass spectrometry

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are flame retardants that are added to a wide range of consumer products. Due to their extensive use in the past, their presence has been documented in multiple environmental compartments and living organisms, including humans. To assess the exposure of humans to PBDEs, a new simple, reliable, and sensitive method was developed for the determination of six PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154) in human serum by gas chromatography–inductively coupled plasma mass spectrometry (GC-ICP-MS). The PBDEs were extracted from 1 mL ofserum by 30 min of mechanical shaking with formic acid. Subsequently, 2 mL of iso-octane was added and 30 min of mechanical shaking was applied. For clean-up of the extract Florisil column was applied. The analytical method was validated by analysis of human serum standard reference materials SRM 1957 (Non-Fortified Human Serum) and SRM 1958 (Fortified Human Serum). Good agreement of the determined concentrations with those certified was found. The repeatability and reproducibility of the analytical method was within 5.9% and 6.1%, respectively, whereas the limits of detection (LODs) for the PBDEs analysed were between 0.0016 and 0.0039 ng mL^{-1} wet weight (ww). The feasibility of the method was tested by analysing human serum samples. In this study, the determined concentrations in sera were in a range similar to that of as those reported for certain other European countries.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of persistent organic pollutants that are added to –numerous commonly-used products such as textiles, polyurethane foam, plastics, mobile phones, television sets, computers and construction materials for reducing their inherent flammability [1,2]. Becouse PBDEs are not covalently bound to the matrix of the consumer products, they are susceptible to leaching into terrestrial and aquatic environment, where they exhibit a tendency to bind to organic fraction of particulate matter, soils and sediments. Their hydrophobicity and resistance to degradation enables them to bioaccumulate and biomagnify in living organisms [3–5]. Numerous studies have demonstrated that PBDEs could exhibit toxic effects on the endocrine, nervous, and reproductive systems [6–9]. Consequently, regulations that restrict the use of PBDEs have been enacted [10,11]. Nevertheless, PBDEs will continue to enter the environment from available products and will remain there and in living organisms for numerous years in future.

Humans are exposed to PBDEs mainly through the ingestion of food and dust and inhalation of indoor air. In Europe, the ingestion of food is the predominant pathway of exposure. Among food items, seafood and fish in particular are considered to be the main source of exposure [12,13]. In North America, the inhalation of contaminated indoor air and ingestion of house dust contribute the most to the human body burden due to the higher flammability standards in this part of the world [14]. The presence of PBDEs in human serum, milk and adipose tissue has been reported worldwide. In North America, PBDEs in the serum are in the concentration range of 30-100 ng g^{-1} lipid weight (lw), whereas the concentrations in Europe and China are approximately 10 times lower [15–18]. In terms of specific congeners, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154 are reported to account for 90% of the total body burden [19]. Children below the age of 4 years have higher concentrations of PBDEs compared to older children and adults due to the ingestion of mother's milk and higher exposure to house dust [20]. This is of a major concern because early childhood represents the developmental period in which physiological detoxification pathways

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and numerous other different growth and developmental processes that occur throughout this time are still underdeveloped, rendering them particularly vulnerable to endocrine disruption. Over the last decades, persistent contaminants such as PBDEs were recognised as a threat to children's health; there is a need for monitoring their presence in the environment and in living organisms to safeguard humans, particularly children's health. For human biomonitoring, simple and accurate analytical methods for determining PBDEs in low volumes of serum are required.

For extracting PBDEs from the human serum to the organic phase, ultrasound-assisted extraction combined with solid phase extraction is used almost exclusively [21–25]. A few authors also report the employment of liquid–liquid extraction [26,27]. The clean-up step, which is required for removing lipids and potentially-interfering species from the extract, is usually performed on columns consisting of multiple layers of silica, acid silica and sodium sulphate [21–23]. This step, together with SPE extraction, is quite laborious and also requires large amounts of organic solvents. For the separation of PBDEs gas chromatography is predominately used due to the PBDEs' physicochemical properties (vapour pressure and polarity) and the method's higher inherent separation efficiency when compared to liquid chromatography. Detection of separated PBDE congeners is commonly detected by electron capture negative ionization mass spectrometry (ECNI-MS) due to its high sensitivity and relatively low cost. Electron impact mass spectrometry (EI-MS) and electron impact high resolution mass spectrometry (EI-HRMS) are also often employed notwithstanding a few drawbacks such as low selectivity (EI-MS) and high price (EI-HRMS). On the other hand, inductively coupled plasma mass spectrometry (ICP-MS) is rarely employed for the determination of the PBDEs, although it is a highly sensitive and versatile detector for bromine, which is easily coupled to GC [28,29].

The aim of our work was to develop simple, reliable, and sensitive analytical method for the determination of the six PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154) by GC-ICP-MS in small amounts of human serum. For this purpose, the effect of different extracting agents (formic acid, formic acid in 2-propanol (4:1, v/v), 0.1 M HCl in MeOH, and 25% TMAH) and subsequent addition of iso-octane on the extraction efficiency when applying different modes of extraction (mechanical shaking, microwave- and ultrasound-assisted extraction) was systematically studied. The efficacy of a rapid and low solvent- consuming clean-up step using Florisil adsorbent for removal of lipids from the extract was tested. The optimised analytical method was validated by determining PBDEs in two certified human serum reference materials, SRM 1957 (Non-Fortified Human Serum) and SRM 1958 (Fortified Human Serum). The applicability of the method was demonstrated by the determination of the PBDEs in the human serum samples.

2. Experimental

2.1. Instrumentation

The analysis of the PBDEs was carried out on an Agilent 6890 GC Agilent Technologies (Santa Clara, CA, USA) equipped with an Agilent 6890 Series Autosampler Injector. The GC was coupled to an Agilent 7700x ICP-MS via a heated transfer line and fitted with a 15 m × 0.25 mm DB-5MS capillary column (film thickness 0.25 μm) coated with 5% phenylmethylpolysiloxane (Agilent J&W Scientific, Palo Alto, CA, USA). Hyphenated instrumental set-up was controlled by Agilent MassHunter software. The following GC temperature program was applied for separation of the PBDEs: the temperature was raised from 120 °C to 300 °C at a heating rate of 30 °C min^{-1} and held there for 5 min. The inlet temperature and transfer line were held at 280 °C. Helium was used as the carrier gas and set at a flow rate of 1.5 mL min^{-1}. The injection mode was splitless and the injection volume 2 μL. The operating parameters of the GC-ICP-MS are presented in Supplementary Table S1. Their optimisation is described in our previous work [30].

The mechanical shaking of the samples was performed on a Vibromix 40 orbital shaker (elliptical table shaker) Tehtnica (Zelezniki, Slovenia), ultrasound-assisted extraction on a 550D VWR International ultrasonic bath (West Chester, PA, USA), and microwave-assisted extraction on a CEM MARS 6 microwave accelerated reaction system CEM (Matthews, NC, USA). The sample extracts were centrifuged on Hettich Universal 320 Centrifuge Hettich GmbH & Co., KG (Tuttlingen, Germany).

2.2. Reagents and materials

All the reagents used were of analytical reagent grade. MilliQ water (18.2 MΩ cm) Millipore (Bedford, MA, USA) was used for preparation of all the aqueous solutions. Individual standards of seven BDE congeners (28, 47, 77, 99, 100, 153 and 154) at a concentration of 50 μg mL^{-1} were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Standard stock solutions (5 μg mL^{-1}) of the PBDEs were prepared in iso-octane and stored in the dark at 4 °C. The working standard solutions were prepared daily in acetone. The standard reference materials SRM 1957 (freeze-dried Non-Fortified Human Serum) and SRM 1958 (freeze-dried Fortified Human Serum) were purchased from the National Institute of Standards & Technology (NIST) (Gaitersburg, MD, USA). Formic acid, tetramethylammonium hydroxide (TMAH), hydrochloric acid (HCl), acetone, citric acid monohydrate, 25% potassium hydroxide (KOH) and tris(hydroxymethyl)aminomethane (Tris) were obtained from Merck (Darmstadt, Germany).

Hexane, methanol (MeOH), 2-propanol and iso-octane were purchased from J. T. Baker (Deventer, Holland). Tris-citrate buffer (pH 6.0) was prepared daily from a 0.2 mol L^{-1} solution of Tris with appropriate addition of citric acid. The absorbent columns (500 mg, 3 mL) for cleaning the extracts were Strata FL-PR Florisil (170 μm, 80 A), purchased from Phenomenex, Inc. (Torrance, CA, USA).

2.3. Cleaning procedure

To prevent contamination, all the glassware were rinsed three times with tap water, soaked in 10% nitric acid for 48 h, rinsed three times with tap water and three times with MilliQ water and heated at 400 °C for 4 h. Prior to use, all the glassware were rinsed with hexane and acetone and dried at room temperature.

2.4. Sample preparation

Venous blood (venous puncture) from a transplanted renal patient was taken during clinical examination after informed consent was obtained, and collected into Pyrex glass bottle without additives. After collection, 10 mL aliquots of blood were transferred into glass centrifuge tubes and centrifuged for 10 min at 855 *g*. The serum aliquots were collected and stored at -20 °C. Prior to analysis, serum was thawed and equilibrated to room temperature.

2.5. Total lipid content

The total lipid content (TL_S) was obtained on the basis of enzymatic assays by summing the individual lipid species measured: total cholesterol (TC) and triglycerides (TG), using formula proposed by Bernet et al. [31].

$$TL_S = (2.27 \times TC) + TG + 62.3 \text{ mgdL}^{-1} \quad (1)$$

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