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Development of an analytical method for the determination of polybrominated diphenyl ethers in mussels and fish by gas chromatography—Inductively coupled plasma mass spectrometry

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ABSTRACT

Six congeners of polybrominated diphenyl ethers (PBDEs): BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154, were determined by a reliable and sensitive analytical method based on gas chromatography coupled to inductively coupled plasma mass spectrometry (GC-ICP-MS) in mussel and fish tissue samples. For their extraction, 30 min of ultrasound-assisted extraction with a 25% aqueous solution of tetramethylammonium hydroxide (TMAH) and an additional 2 h of mechanical shaking with tris(hydroxymethyl)aminomethane (Tris)-citrate buffer and iso-octane were applied. An effective cleaning, with minor solvent consumption, was achieved by passing the extract through a column filled with Florisil. PBDEs in the organic phase were quantified by GC-ICP-MS. Accuracy checks were performed by analyzing reference materials NIST SRM 2974a (freeze-dried mussel tissue) and SRM 1946 (fresh fish tissue homogenate) samples with a standard addition calibration method and by comparative analysis with species-specific isotope-dilution GC-ICP-MS. Good agreement of results between the determined and certified values were obtained (recoveries lied between 94 and 105%). Limits of detection (LODs) expressed on wet weight (ww) basis were 0.003 ng g^{-1} for BDE 28, 0.006 ng g^{-1} for BDE 47, 0.008 ng g^{-1} for BDE 99, 0.004 ng g^{-1} for BDE100, 0.005 ng g^{-1} for BDE 153 and 0.009 ng g^{-1} for BDE 154. The analytical method was applied for the determination of PBDEs in marine mussels and fish samples from the northern Adriatic Sea and fish samples from the Sava River. Among the six PBDEs congeners determined, BDE 47, BDE 100 and BDE 99 were commonly detected in the samples analysed.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of chemicals that have been on the market since the 1960s. They have been widely used as flame retardants in a variety of commercial products, such as polyurethane foams, electronic equipment, plastics, building materials and textiles [1]. PBDEs do not form chemical bonds to the matrix of the flame-retarded product and can be easily leached into the environment during their manufacturing and during the use or after the disposal of products that contain them [2,3]. Hence, current major sources of PBDE release into the environment are from waste processing and separation, sewage sludge disposal and landfill leachate outflows. Their occurrence has been demonstrated worldwide in sediments, soils, surface waters, sewage sludge, outdoor or indoor air, house dust and biota [4–7]. They can even be detected in samples taken from the deep ocean and remote sites in Arctic [8] and Antarctic environments [9].

PBDEs belong to a class of hydrophobic, poorly degradable persistent organic pollutants that tend to adsorb onto particulate matter, bio-accumulate in fatty tissues and bio-magnify through the food web [10]. They are frequently present in different aquatic organisms and mammals [10], including humans, where they have been detected in blood, adipose tissue and breast milk. Studies have demonstrated that exposure to PBDEs can cause adverse health effects in experimental animals and humans, including the disruption of growth and the immune and endocrine (thyroid hormone, for example) systems, as well as neurodevelopmental delays [11,12]. Accordingly, the production and use of technical penta-, octa- and deca-BDE mixtures has been restricted by law in the EU [13,14] and partially banned in the USA. Despite the bans, PBDEs will continue to be released into the environment from existing

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large reservoirs of PBDE-containing products for many years to come and remain a pollution problem in the foreseeable future.

The human population is mainly exposed to PBDEs through the diet and dust ingestion [10,15]. The diet is the predominant exposure pathway for Europeans [16,17]. In a study carried out by Voospoels et al. [18] a positive correlation between fish consumption and concentrations of PBDEs in blood serum was established. Fish consumption contributed approximately 40%–50% of the total dietary intake of PBDEs. The European Union Water Framework Directive (WFD) included six PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) in the group of priority hazardous substances [19]. Their regular monitoring is recommended by the WFD in order to prevent pollution of the aquatic environment. The Environmental Quality Standard (EQS) limit concentration for the sum of the six PBDE congeners in the biota (relating to fish), is set to 8.5 ng/kg wet weight (ww) [19]. The analysis of mussels, which are known bioindicator organisms used for monitoring organic and inorganic pollutants in aquatic environments, and fish samples provides valuable information about the risks associated with human exposure to PBDEs through the diet [20]. Thus, reliable and very sensitive analytical methods for the determination of PBDEs in fish and mussel tissues are needed in order to evaluate PBDE pollution of the aquatic environment and the consequent risk to human health.

Extraction of PBDEs from fish and mussel tissue sample matrices has been performed in recent years by using Soxhlet extraction [7,21,22], accelerated solvent extraction [23–25], ultrasound-assisted extraction [26] or microwave-assisted extraction [23,27]. After extraction, the content of PPDEs is determined with high sensitivity by using advanced instrumental techniques. Those based on the separation of PBDEs by gas chromatography (GC) [28] coupled with different highly developed detector systems, such as high resolution mass spectrometry [27,29], mass spectrometry operating in the negative chemical ionization mode [7,16,26] or electron capture detectors [22–24] are almost exclusively applied. Alternatively, inductively coupled plasma mass spectrometry (ICP-MS), which possesses good sensitivity and excellent selectivity for bromine determination, even in the presence of compounds containing sulfur or chlorine that can interfere with the detector systems listed above, can be used for the detection of the GC-separated PBDEs [30,31].

The aim of this work was to develop a simple analytical procedure that requires minimal sample preparation for a rapid, sensitive and reliable determination of the six PBDEs listed in the WFD in mussel and fish samples by GC-ICP-MS. For this purpose, the influence of different extracting agents (25% aqueous solution of tetramethylammonium hydroxide (TMAH), 0.5 mol L^{-1} acetic acid in methanol (MeOH) and 0.1 mol L^{-1} hydrochloric acid (HCl) in MeOH) and the subsequent simultaneous addition of tris(hydroxymethyl)aminomethane (Tris)-citrate buffer (co-extracting agent) and iso-octane on the extraction efficiency was studied when applying different modes of extraction (mechanical shaking, microwave- and ultrasound-assisted extraction). The extraction step was optimized by analyses of National Institute of Standards and Technology standard reference material, NIST SRM 2974a freeze-dried mussel tissue. A rapid and low solvent-consuming clean-up step, which included passing the extract through a column filled with Florisil, was also tested. The accuracy of the analytical method developed was checked by analyzing NIST SRM 2974a and NIST SRM 1946 (Lake Superior fish tissue) samples by the standard addition calibration method and performing a comparative analysis with the species-specific isotope-dilution (ID) GC-ICP-MS method. To demonstrate the applicability of the GC-ICP-MS analytical procedure developed for the determination of PBDEs in real samples, mussels and marine fish samples from the northern Adriatic Sea and fish samples from the Sava River were analyzed.

2. Experimental

2.1. Instrumentation

The analysis of 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.

For the separation of PBDEs on a 15-m column, the following GC temperature program was applied: 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 the transfer line were held at 280 °C. Helium at a flow rate of 1.5 mL min^{-1} was used as the carrier gas. The injection mode was splitless and the injection volume 2 μL. The operating parameters of the GC-ICP-MS instrumental set-up are presented in Supplementary Table S1. Their optimisation is described in our previous work [32].

Mechanical shaking of the samples during the extraction procedure was performed on a Vibromix 40 orbital shaker (elliptical table shaker) Tehtnica (Železniki, Slovenia), the ultrasound-assisted extraction of the samples on a 550D VWR International ultrasonic bath (West Chester, PA, USA), and the microwave-assisted extraction on a CEM MARS 5 microwave acceleration reaction system CEM (Matthews, NC, USA). Centrifugation of the sample extracts was carried out on Hettich Universal 320 Centrifuge Hettich GmbH & Co., KG (Tuttlingen, Germany). Tissue samples were homogenised in a OmniBlend V – 1.5 l BPA FREE blender OmniBlend Australia Pty Ltd (Centennial Byron Bay, Australia). Samples were stored at −20 °C in a freeze drier FN6192PW Gorenje (Velenje, Slovenia).

2.2. Reagents and materials

All the reagents used were of analytical reagent grade. MilliQ water (18.2 MΩ cm) Milipore (Bedford, MA, USA) was used for the 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 of the PBDEs were prepared in iso-octane at concentration levels of 5 μg mL^{-1} and stored in the dark at 4 °C. Working standard solutions were prepared daily in acetone. ^{81}Br isotopically enriched BDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154) were obtained from ISC Science (Oviedo, Spain) and were used for the calculation of BDE concentrations by isotope dilution (ID) GC-ICP-MS. The compositions of the ^{81}Br enriched BDE 28, BDE 47, BDE 99 and BDE 153 determined for isotopes 81 and 79 were 99.53% and 0.47%, respectively. The compositions of the ^{81}Br enriched BDE 100 for isotopes 81 and 79 were 68.5% and 31.5%, and of ^{81}Br-enriched BDE 154, 76.3% and 24.7%, respectively.

The standard reference material 2974a (freeze-dried mussel tissue) and the standard reference material 1946 (Lake Superior fish tissue) were purchased from the National Institute of Standards & Technology (NIST) (Gaitersburg, MD, USA). Tetramethylammonium hydroxide (TMAH), hydrochloric acid (HCl), acetone, acetic acid, citric acid monohydrate, 25% potassium hydroxide (KOH) and tris(hydroxymethyl)aminomethane (Tris) were obtained from Merck (Darmstadt, Germany).

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