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New multiresidue analytical method dedicated to trace level measurement of brominated flame retardants in human biological matrices

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Abstract

A new method has been developed for the multi-residue measurement of the main brominated flame retardants (α- and γ-hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBP-A) and polybrominated diphenyl ethers including decabromodiphenyl ether) in human biological matrices (serum, adipose tissue and breast milk). The proposed sample preparation procedure focused on reduced solvent and consumable consumption and associated procedural contamination, as well as reduced sample size. This protocol was fully validated and was proved to be suitable for identification of brominated flame retardant residues at ultra-trace level, as attested by preliminary results on real samples.

Keywords: Hexabromocyclododecane; Tetrabromobisphenol A; Polybrominated diphenylether; Sample preparation; HRMS; Serum adipose tissue; Milk

1. Introduction

The impact of brominated flame retardants on the environment and their potential risk for animal and human health is a present time concern for the scientific community [1,2]. Numerous studies related to the detection of hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBP-A) and polybrominated diphenylethers (PBDEs) (Fig. 1) have been developed over the last few years. The measurement techniques commonly used are mainly based on LC–MS/MS for HBCD stereoisomers [3,4], LC–MS/MS [5] or GC-NCI–MS with diazomethane derivatization [6] for TBBP-A, and GC techniques coupled with ECD [7], NCI-MS [8], EI-HRMS [9] and recently EI-MS/MS [10,11] for PBDEs. The sample treatment procedures used for PBDE are usually derived from the analytical methods dedicated to dioxins [12], generally including packed multi-layer columns and needing relatively high solvents volumes. Recently, some authors proposed the utilization of solid phase extraction (SPE) cartridges [13,14].

In this study, a new analytical strategy is presented for the multi-residue analysis of HBCD, TBBP-A and tri- to decaBDEs from human biological matrices. Our main objective was to develop a sample preparation procedure permitting the final collection of three fractions corresponding to the three classes of brominated flame retardants, and suitable for various biological matrices pending very minor adaptations. Special attention was paid to reduce the scale of all materials, solvents and consumables mainly to minimize the analytical contaminations. The final purpose of this analytical development is its further application to an exposure assessment study in French population groups for which, to our knowledge, no data exist regarding brominated flame retardants. A second objective was to provide an efficient analytical tool to study the transfer of these contaminants through the environment to living organisms.

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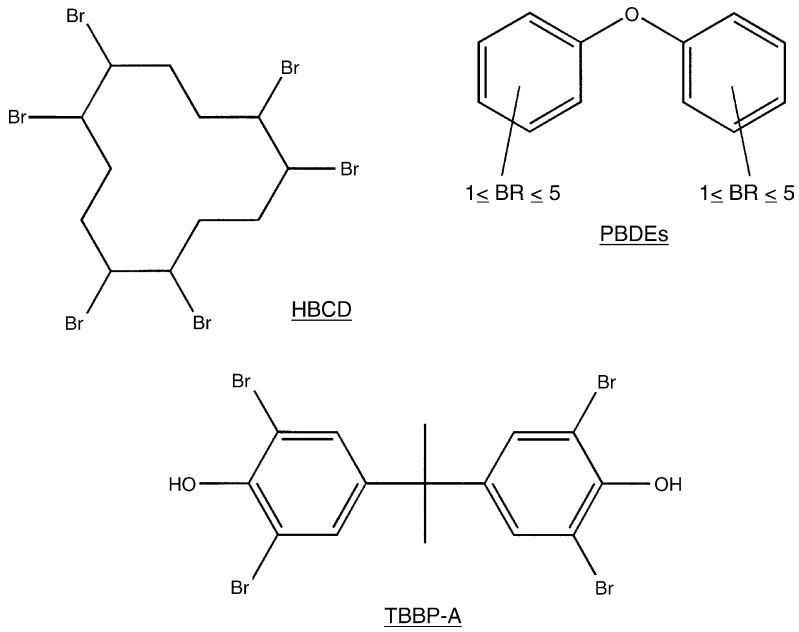

Fig. 1. Structures of hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBP-A) and polybrominated diphenylethers (PBDEs).

2. Experimental

2.1. Reagents and chemicals

Ethyl acetate, acetonitrile, *n*-hexane, dichloromethane and sulfuric acid were Picograde quality and provided by LGC Promochem (Wesel, Germany). *n*-Nonane (GC grade) was purchased from Sigma (Steinheim, Germany), and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) from Aldrich (Steinheim, Germany). Acetic acid 1 M, HPLC grade acetonitrile and methanol were provided by Solvents Documentation Synthesis (Peypin, France) and sodium acetate by Fluka (Buchs, Switzerland). Class I nitrogen was purchased from Air Liquide (Paris la Défense, France). β-Glucuronidase from *Helix pomatia* (Type H-5) was provided by Sigma. Enzymatic kit from Randox Laboratories Ltd. (Crumlin, UK) was used for total lipids determination in blood serums. Oasis HLB SPE cartridges (500 mg, 6 mL) were provided by Waters (Milford, MA, USA), and SiOH SPE cartridges (1 g, 6 mL) were purchased from United Chemical Technologies (Bristol, UK) or Interchim (Montluçon, France). Silica gel (G60) was provided by Fluka and sodium sulfate was from Merck (Darmstadt, Germany). All reference native and ^{13}C-labelled standard solutions were purchased from Cambridge Isotope Laboratories (Andover, USA) or Wellington Laboratories (Guelph, Canada), excepted Fluorometholone (Sigma). Solution used as the "LowBDE Mixture", referenced EO-5113 (CIL), contained 29 tri- to heptaBDE congeners, with relative concentrations compared to triBDE homologues of 1, 1.5, 2 and 2.5, respectively, for tetra-, penta-, hexa- and heptaBDE homologues. ^{13}C-labelled compounds as internal standards for quantification included: γ-HBCD, TBBP-A, BDE-28, 47, 99, 154, 153, 139 (external standard), 183 and 209.

2.2. Samples

Blood serum sample used for the validation of the developed analytical method was from bovine origin and was obtained at the National Veterinary School of Nantes (France). All other samples were human biological matrices collected by the Centre Hospitalier Universitaire de Toulouse during a research project supported by the Agence Française de Sécurité Sanitaire Environnementale (AFSSE). All these samples were obtained from volunteer women during caesarean deliveries. Two sample pools were constituted for validation experiments. The first one consisted in six under-skinned adipose tissue samples collected in the abdominal region. The second one consisted in milk samples from sixteen women, which were freeze-dried before pooled and homogenized. The study protocol was approved by a local ethical committee.

2.3. Equipment

Separation of the α-, β- and γ-HBCD stereoisomers was achieved using an Alliance 2690 HPLC pump with quaternary gradient system and automatic injector (Waters, Milford, MA, USA). Reversed phase liquid chromatography separation was realized on octadecyl grafted silica stationary phase Symmetry C_{18} (150 mm × 2.1 mm, 3.5 μm + guard column 10 × 2.1 mm) from Waters (Milford, USA). Elution solvents were methanol (A), acetonitrile (B) and water containing 0.5% (v/v) acetic acid (C). Mobile phase composition (A:B:C, v/v/v) was 30:10:60 from 0 to 1 min and 50:50:0 at 4.5 min. Flow rate was set at 0.25 mL/min and injected volume at 20 μL. Mass spectrometric data were acquired after negative electrospray ionization and on the basis of multiple reaction monitoring acquisition mode, using a Quattro LC triple quadrupole instrument (Micromass,