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Simple and fast method for the measurement of legacy and novel brominated flame retardants in human serum

Catherine Pirard [a, b, *], Corinne Charlier [a, b]

[a] Laboratory of Clinical, Forensic and Environmental Toxicology, CHU of Liege, B35, 4000 Liege, Belgium
[b] Center for Interdisciplinary Research on Medicines (CIRM), University of Liege (ULg), CHU (B35), 4000 Liege, Belgium

HIGHLIGHTS

- Simultaneous measurement of 15 BFRs including BDE-209 within a single injection.
- Results obtained from interlaboratory test material were close to the assigned values.
- The analytical technique successfully passed the validation process.
- The user-friendly method could be easily implemented for high throughput analyses.
- Provides clean extracts avoiding usual solvent and time consuming purification steps.

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ABSTRACT

Recent and reliable human biomonitoring data on brominated flame retardants (BFRs), either legacy or new BFRs, are still needed to assess human exposure. The aim of this work was therefore to develop and validate an accurate, fast and user-friendly analytical strategy for the determination of 15 legacy and novel BFRs in human serum namely 8 polybrominated diphenylethers (BDE-28, -47, −99, −100, −153, −154, −183, and −209), 1 hexabromobiphenyl (PBB-153), and 6 novel BFRs (pentabromotoluene, hexabromobenzene, pentabromoethylbenzene, 2-ethylhexy-2,3,4,5-tetrabromobenzoate, 1,2-bis(2,4,6-tribromophenoxy) ethane, and decabromodiphenylethane). This analytical procedure consisted in a simple liquid-liquid extraction followed by elution on a PHREE cartridge avoiding further laborious purification steps. The final determination was performed by gas chromatography coupled to mass spectrometry in electron capture negative ionization mode (GC-ECNI-MS). The 15 m long RTX-1614 allowed the simultaneous measurement of the 15 BFRs including low and high brominated species within a single injection on a single column. Except for 2-ethylhexy-2,3,4,5-tetrabromobenzoate (EHTBB) which showed very high response variations resulting in poor linearity, trueness and precision, and decabromodiphenylethane for which very low sensitivity was achieved, the 13 other BFRs passed the validation process with recoveries varying between 56 and 82%, and limits of quantification (LOQs) ranging from 2.5 to 6.0 pg/ml (34.5 pg/ml for BDE-209). Within the validated range of concentrations, the relative bias from the introduced levels were below 20% while the intra and inter precisions were maintained below 15%. The reliability of the technique was confirmed by successfully analyzing interlaboratory test materials (AMAP ring test for POPs in human serum).

1. Introduction

Brominated flame retardants (BFRs) are used in a wide range of consumer products such like textiles, plastics, electric or electronic equipments, insulation foams, building materials, to prevent or reduce the ignition and rate of combustion (de Wit, 2002; Sjodjin et al., 2003). Among them, polybrominated diphenylethers (PBDEs) manufactured as mixtures, mainly the penta-, octa- and deca-formulations, represented an important class extensively used as additive flame retardants since the 1970s. Because not chemically bound to the polymer, they can migrate from the final

* Corresponding author. Laboratory of Clinical, Forensic and Environmental Toxicology, CHU of Liege, B35, 4000 Liege, Belgium.
E-mail addresses: c.pirard@chuliege.be (C. Pirard), c.charlier@chuliege.be (C. Charlier).

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product during lifetime, disposal or recycling. Due to their persistence and lipophilicity, they were able to bioaccumulate in the environment and reach the human organism through the food chain. Indoor environment has also been suggested as an important source of exposure for humans through the inhalation, ingestion and/or dermal contact of domestic dust however all exposure pathways are still not fully determined. Their widespread occurrence and health concerns led to the ban of use and production in Europe and North America of the penta- and octa-formulations in 2003 and 2004, whereas phase out and restrictions on the deca-brominated formulation occurred 5–10 years later (Covaci et al., 2011; de Wit et al., 2010; Gramatica et al., 2016). Moreover, tetra-, penta-, hexa-, hepta- and deca-PBDEs were progressively added to the list of the Persistent Organic Pollutants (POPs) from 2009 to 2017 (UNEP, 2007). Nevertheless, their ubiquity in all compartments of environment and subsequent human exposure still are occurring.

On the other hand, other brominated compounds have been gradually used as substitutes to these legacy BFRs. This is the case for instance for decabromodiphenylethane (DBDPE) or 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), used now as replacement of deca-BDE and octa-BDE respectively in various plastics and textiles (Covaci et al., 2011), or 2-ethylhexy-2,3,4,5-tetrabromobenzoate (EHTBB) contained in Firemaster 550 which is added to polyurethane foam instead of penta-BDE (Stapleton et al., 2012). These “novel BFRs” (NBFRs) although defined as new to the market or newly or recently observed in the environment (Covaci et al., 2011), have been produced most of the time since several decades, but their increasing production led to increasing interest and consequently to increasing recent data in the environment (Arp et al., 2011; Covaci et al., 2011). The reason for the apparent re-emergence in recent studies of other NBFRs such like hexabromobenzene (HBBz), pentabromotoluene (PBT), or pentabromoethylbenzene (PBEB) which is not longer manufactured to date, remains unclear but definitively reflects a re-emergence in concern for non-PBDE BFRs (Arp et al., 2011). However, reliable human exposure or body burden data are still scarce likely due to the analytical difficulties encountered for instance for high brominated compounds such like DBDPE, the few availability of commercial reference materials or standards, or the very few analytical methods specifically dedicated to NBFRs (Covaci et al., 2011). Indeed although several times reviewed (Covaci et al., 2011; Kierkegaard et al., 2009; Papachlimitzou et al., 2012), their determinations were usually based on retrospective analyses from extracts initially intended to PBDE measurements, or in the best case included in legacy BFR analyses but without specific optimization and validation (Covaci et al., 2011; Papachlimitzou et al., 2012).

Both novel and legacy BFRs used to be detected in the serum of the general population at the ppb or ppt level resulting in a challenge for the analytical chemists. Historically the PBDE's analytical methods were mainly derived from the complex dioxin analyses, requiring huge volumes of serum sample (up to 10 ml), involving laborious extraction and multistage purification steps and the use of high resolution mass spec (HRMS) for final determination (Guvenius et al., 2003; Papke et al., 2004; Pirard et al., 2003; Sjodin et al., 2004). Nevertheless efforts were expended to move from HRMS to low-cost and user-friendly low resolution mass spec (LRMS) using preferentially electron capture negative ionization mode (ECNI) because of its high sensitivity toward brominated compounds despite its lower selectivity (Eljarrat et al., 2003; Gomara et al., 2007; Thomsen et al., 2002; Vizcaino et al., 2009), and to develop the sample preparation in order to reduce solvent and matrix consumption. Nowadays, 3–5 ml of serum are typically needed to achieve sufficient sensitivity, and extraction and cleanup procedures are simplified or partially automated in well-equipped labs (reviewed in Covaci et al., 2007; Gao et al., 2016; Lu et al., 2017; Ramos et al., 2007; Thomsen et al., 2007). Nevertheless, due to the high lipophilicity of the analytes, the sample preparation usually still requires multistep purification for lipid and other interference removals. These latter include subsequently to solid phase (SPE) or liquid-liquid extraction (LLE), additional steps such like gel permeation chromatography (GCP), acidic treatment, elution through multilayer silicagel, alumina, or Florisil, etc (Covaci et al., 2007). All these fastidious steps could be solvent and time consuming, and increase the risk of external contamination of the sample (Gao et al., 2016; Kierkegaard et al., 2009; Papke et al., 2004). Moreover, the determination of high brominated compounds (i.e. BDE-209, DBDPE) used to be touchy due to their thermal instability and low volatility, inducing frequently separate GC injections on shorter columns (Kierkegaard et al., 2009), increasing the analysis duration.

Therefore due to the need to still monitor historical BFRs including BDE-209 for which human data remain scarce, as well as to collect reliable recent data on NBFRs, the aim of this work was to develop and validate a robust, accurate, fast and user-friendly analytical strategy for the measurement of 15 legacy and novel BFRs in human serum allowing high throughput analyses for large scale epidemiological studies. The BFRs targeted were 8 PBDEs (BDE-28, -47, −99, −100, −153, −154, −183, and −209), 1 hexabromobiphenyl (PBB-153), and 6 novel BFRs namely PBT, HBBz, PBEB, EHTBB, BTBPE, and DBDPE.

2. Material and methods

2.1. Chemicals and materials

The individual standard solutions of PBEB, BTBPE, DBDPE, HBBz, PBT, EHTBB, BDE-51, BDE-156 and BDE-181 (50 μg/ml) as well as the $^{13}C_{12}$-labeled internal standard MBDE-209 (25 μg/ml) were purchased from Wellington Laboratories (Ontario, Canada), while the BDE-CM containing BDE-28, -47, −99, −100, −153, −154, −183, and −209 (at 2.5 μg/ml for all congeners except for BDE-209 which was at 25 μg/ml) was come from AccuStandard Inc (New Haven, CT, USA). Water was obtained from a Milli-Q® UltrapureWater Purification Systems (Millipore, Brussels, Belgium). Glacial acetic acid (99.5%) and acetone (AR®) were bought from Macron Fine Chemicals (Gliwice, Poland), hexane (ultra resi-analyzed grade) and 2-propanol (LC-MS reagent grade) from J.T. Baker (Pennsylvania, USA), and nonane anhydrous (≥99%), sodium sulfate anhydrous (≥99%) and fetal bovine serum (research grade) from Sigma Aldrich (St Louis, USA). PHREE phospholipid removal cartridges (1 ml) were purchased from Phenomenex (Torrance, CA, USA).

2.2. Stock standard solutions

The native stock solution was prepared in isopropanol to obtain 50 ng/ml for all compounds except for BDE-209 and DBDPE which were set at 500 ng/ml. The BDE-51, -156, −181 and the $^{13}C_{12}$-labeled BDE-209 were used as internal standard and were diluted in isopropanol to obtain respectively 10 ng/ml and 100 ng/ml. All standard solutions were stored in amber vials at 4 °C.

2.3. Sample preparation

Internal standard (20 μl) was added to 1 ml of serum, 0.3 ml of glacial acetic acid and 0.7 ml of water and let equilibrated using multi-tube vortex at 2500 rpm for 1 h, before being extracted twice with 4 ml of a hexane/acetone mixture (95/5) by vortexing at 2500 rpm for 10 min. The combined organic fractions were