SIMPLE AND FAST METHOD FOR THE MEASUREMENT OF PLOYBROMINATED DIPHENYL ETHERS AND SOME NOVEL BROMINATED FLAME RETARDANTS IN HUMAN SERUM

Pirard C., Charlier C.

Laboratory of Clinical, Forensic and Environmental Toxicology, CHU of Liege, Liege, 4000, Belgium (c.pirard@chu.ulg.ac.be)

Introduction

Brominated flame retardants (BFRs) were widely used these past decades in textiles, plastics, electric or electronic equipments, etc, to prevent or reduce the ignition and rate of combustion. Due to their persistence, bioaccumulation and health concerns, several BFRs including polybromodiphenylethers (PBDEs) were added to the list of the Persistent Organic Pollutants (POPs) inducing the ban of their production and uses in several countries. Nevertheless, they are still currently contaminated our environment and thus the exposure needs still to be monitored. Moreover in recent years, several emerging BFRs have been developed as alternatives to these banned or restricted chemicals, and data on their human exposure are still scarce. In order to perform reliable biomonitoring studies, the determination of biomarkers should be accurate and robust, but also fast and user-friendly to be implemented in routine analyses. All novel and historical BFRs used to be detected in the serum of the general population at the ppb level resulting in a challenge for the analytical chemists. The determination of such lipophilic compounds usually required multistep Liquid-Liquid Extraction (LLE) or Solid Phase Extraction (SPE), and additional purification steps to remove lipid interferences (acidic silicagel, gel permeation chromatography, alumina, etc). This necessary cleanup used to be laborious, reagent and time consuming. The aim of this study was develop a fast and simple analytical procedure to measure 15 historical and novel BFRs in human serum, and allowing high throughput analyses for large scale epidemiological studies. The BFRs targeted were 8 PBDEs (PBDE-28, -47, -99, -100, -153, -154, -183, and -209), 1 hexabromobiphenyl (PBB-153), and 6 novel BFRs namely pentabromotoluene (PBT), hexabromobenzene (HxBBz), pentabromoethylbenzene (PBEB), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), and decabromodiphenylethane (DBDPE).

Materials and Methods

The extraction was carried out on 1 ml of serum previously equilibrated for 1 hour with 10 µl of internal standard and 0.5 ml of acetic acid glacial. Then a LLE was performed twice with 2x3ml of a hexane/acetone mixture (95/5). The combined organic fractions were concentrated till 0.5ml using SuperVap® 12 positions concentration system from Fluid Management System (Watertown, MA, USA), and loaded on 1ml PHREE cartridge (Phenomenex, Torrance, CA, USA) to remove lipid traces. The eluate obtained by centrifugation was transferred to silanized GC vial with nonane as keeper, and let evaporate at room temperature. From the 50 µl obtained, 2µl were injected on a gas chromatograph (GC) coupled to a mass spectrometer (7890A GC/7000A Triple Quad MS, Agilent Technologies, California, USA), and equipped with a RTX-1614(15mx0.25mm IDx0.10µm df, Restek, Bellefonte, PA, USA). Helium was used as carrier gas a constant flow of 2ml/min. The injection was performed in pulsed splitless mode with an additional pressure of 65psi, and at a temperature of 140°C held for 0.1min, then increased to 275°C at 700°C/min. The initial oven temperature was set at 140°C held for 1 min, then successively increased to 180°C at 10°C/min, to 195°C at 3°C/min, to 240°C at 10°C/min, to 250°C at 5°C/min and finally increased at 100°C/min to 325°C held for 6 min. The MS operated in Negative Chemical ionization mode using methane as reagent gas. The transfer line, the source and the quadrupoles were set respectively at 300°C, 200°C and 150°C. Both 79 and 81 masses were selected in Single Ion Monitoring for all target BFRs except for native PBDE-209 and PBDE-209 $^{13}C_{12}$ for which 486, 488 (for native), 496 and 498 (for labelled) were monitored. Besides decabrominated BFRs (PBDE-209 and DBDE) quantified using PBDE-209 $^{13}C_{12}$, PBDE-51, -156 and -181 were used for the quantification of all

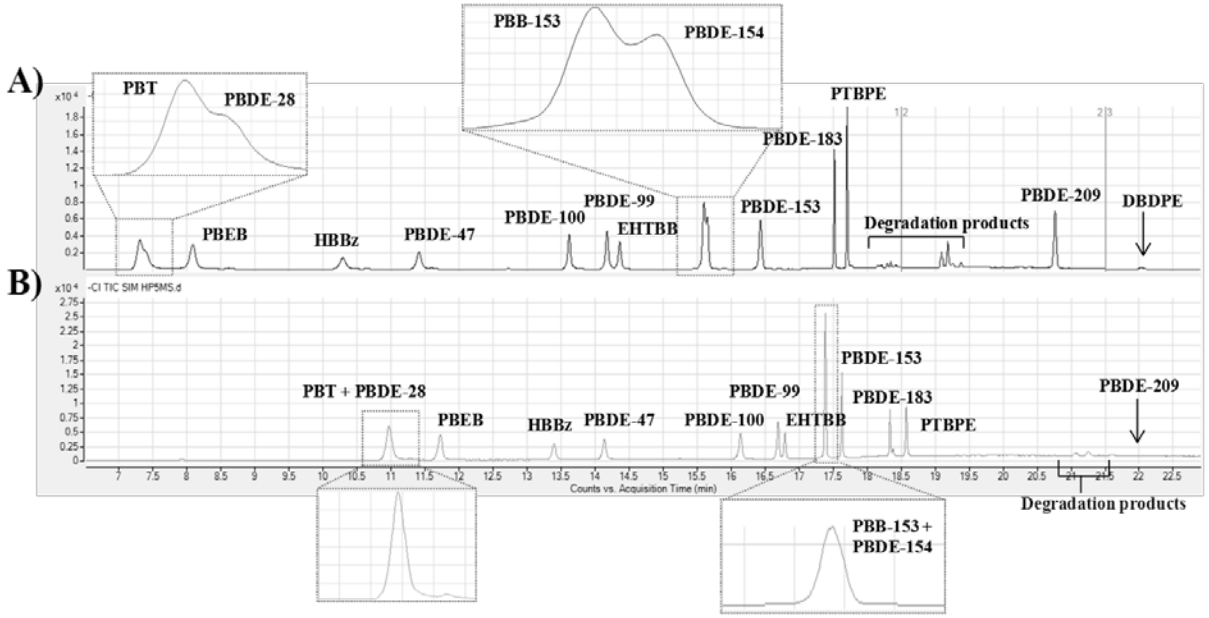
other BFRs depending on their brominated degree. The 9 point calibration curve was built by spiking serum (from anonymous donors and previously checked for low contamination) with native standard solutions to obtain concentrations ranging from 1pg/ml to 1500pg/ml serum (except for PBDE-209 ranging from 10 to 15000pg/ml) and was extracted and analysed simultaneously with unknown samples.

Results and Discussion

Whereas PBDEs and novel BFRs were usually analysed using a 30m column to be separated, both decabrominated PBDE-209 and DPEB used to require a separate injection on a 15m column to shorten the GC run and thus reduce their thermal degradation. Figure 1 shows the chromatograms obtained with the RTX-1614 and with a traditional HP-5MS, both 15m length columns. When using the RTX-1614, the separation of all targets was achieved in 23 minutes. Nevertheless, partial separations were obtained for PTB and PBDE-28, and PBB-153 and PBDE-154, but still allowing a proper quantification, while perfect coelution still occurred on the HP-5MS.

A small degradation of PBDE-209 and DPDE could not be avoided but was demonstrated to occur in the inlet during the sample vaporisation and therefore was independent of the GC run duration.

Figure 1: RTX-1614(up) versus HP-5MS (bottom) on standard solution (2pg injected).



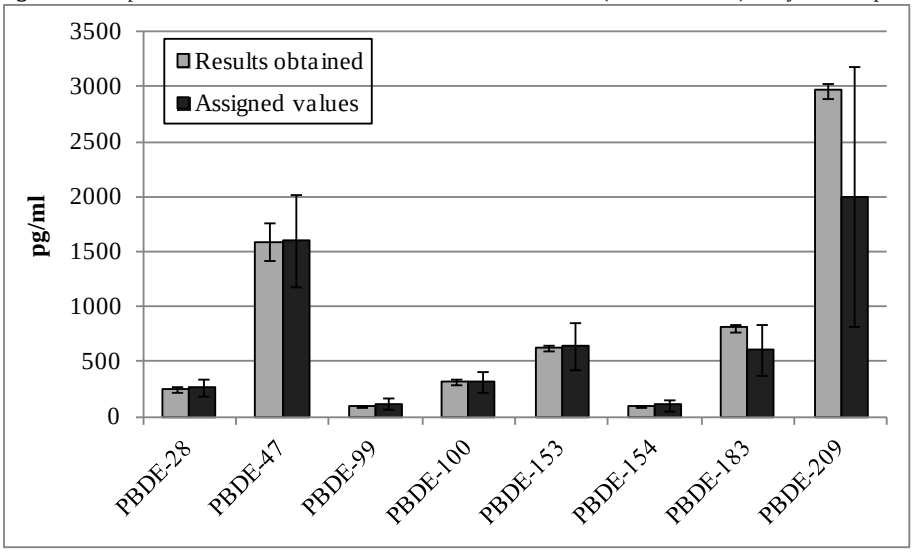
The use of PHREE cartridges after LLE provided clean extracts removing efficiently the lipid interferences, and limited the sample handling resulting in a reduced solvent consumption and extraction time. Due to the sensitivity of the whole technique, small sample volumes (1ml) were required to obtain fair limits of detection (see Table 1) comparable to other method consuming larger serum volumes. The accuracy and precision of the developed method were assessed by analysing in triplicate a home-made quality control (QC) consisting in serum sample spiked at 100pg/ml. Table 1 also shows the means and standard deviations (SD) of the accuracy and recovery. Recoveries were evaluated by comparing serum samples (N = 3) spiked (at 100ng/ml) before and after the extraction procedure using PCB-198 as surrogate standard.

Table 1: Accuracy and recovery (mean and standard deviation) evaluated on serum samples spiked at 100ng/ml (N = 3). LOD were determined as the concentration in the serum giving a S/N ratio greater than 3.

	Accuracy (%)		Recovery (%)		LOD
	Mean	SD	Mean	SD	(pg/ml serum)
PBDE-28	85	4	80	7	1
PBDE-47	83	3	81	7	<1
PBDE-99	93	2	70	9	<1
PBDE-100	94	3	69	8	1
PBDE153	87	4	74	11	10
PBDE-154	102	12	65	12	1
PBDE-183	96	7	80	14	5
PBDE-209	78	3	56	19	100
PTB	80	8	82	1	1
PBEB	88	3	70	6	5
HxBBz	85	10	77	5	1
EHTBB	98	18	55	13	<1
PBB-153	101	14	62	13	1
BTBPE	94	13	76	17	5
DBDPE	125	21	82	18	500

The analytical technique was also tested in triplicate on materials from the AMAP interlaboratory ring test for POPs in human serum. Results obtained were shown on Figure 2. Excellent correlations were observed between results obtained and assigned values except for PBDE-209 for which unexplained higher levels were measured.

Figure 2: Comparisons between results obtained for AMAP materials (AM-S-W-1402) analyzed in triplicate and assigned values.



Conclusions

The fast extraction procedure provided clean extracts and good recovery rates from small serum volume (1ml) and did not need further labor and time consuming purification steps. The 15m length RTX-1614 allowed the simultaneous measurement of the 15 BFRs including PBDE-209 in a single injection. This user-friendly method was demonstrated to be accurate, and could be easily implemented in routine laboratories.