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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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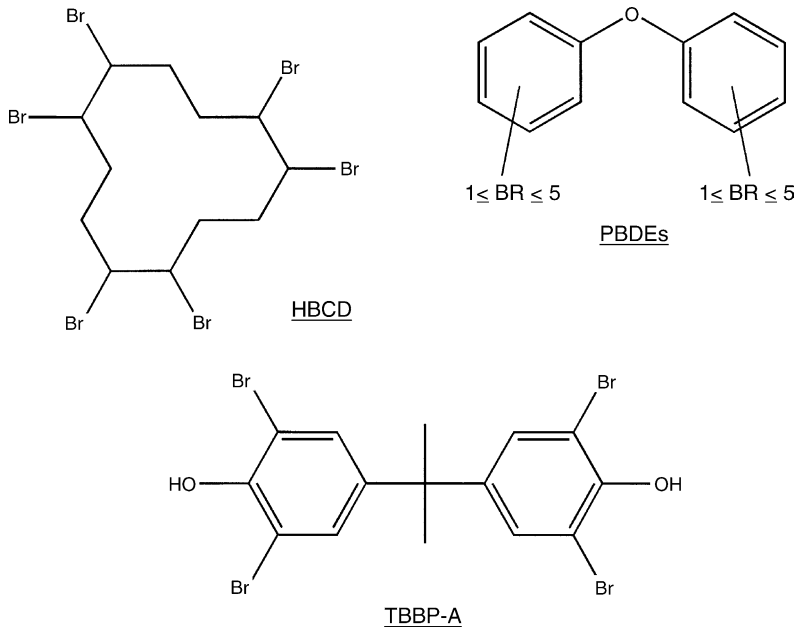


Fig. 1. Structures of hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBP-A) and polybrominated diphenylethers (PBDEs).

## 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,

Manchester, UK). Monitored fragment ions were the *m/z* 79 and *m/z* 81 bromine ions coming from the $[M–H]^- > [Br]^-$ transition.

Separation of TBBP-A and PBDE congeners was achieved using a Hewlett-Packard 5890 (Palo-Alto, CA, USA) gas chromatograph with capillary column (15 m × 0.25 mm × 0.25 μm) coated with low bleeding diphenyl (5%)-dimethylpolysiloxane (95%) copolymer (UB5-P, Interchim, Montluçon, France). The temperature gradient started from 120 °C (2 min), rose to 280 °C (10 °C/min) and then to 320 °C (20 °C/min, 8 min). Injected volume was 3 μL in the splitless mode. Helium (N55) was used as carrier gas at the constant flow of 1 mL/min. Only PBDE congeners 28/33 were not correctly resolved in these conditions. Detection was performed on a SX-102A (Jeol, Tokyo, Japan) double focusing electromagnetic instrument ($R = 10{,}000$), using perfluorokerosene (PFK) as calibrating reference. Electron ionization energy was set at 70 eV and source temperature was set at 230 °C. Monitored ions were the two most intense ones among the $[M–CH_3]^+$, $[M]^{\bullet+}$ or $[M–Br_2]^{\bullet+}$ ion clusters, respectively, for TBBP-A (silylated compound), tri- to pentaBDEs and hexa- to decaBDEs.

### 2.4. Sample treatment

A global overview of the developed sample preparation procedure is presented in Fig. 2. Blood serum (10 g), liquid fat (0.5 g) or freeze-dried milk (1 g) were spiked with $^{13}C$-labelled internal standards (γ-HBCD, TBBP-A, BDE-28, 47, 99, 154, 153, 183 and 209) and kept one night for equilibration. For serum samples, a first liquid/liquid extraction with ethyl acetate (12 + 8 mL) was performed. For freeze-dried milk samples, a first solid/liquid extraction with acetone/dichloromethane 1:1 (v/v) (12 + 6 mL) was realized. Thus, for all samples, a liquid/liquid extraction with simultaneous partitioning was applied on dried extracts (serum, milk) or directly on liquid fat samples. Three milliliters of acetonitrile were added after same volume of *n*-hexane, and in total *n*-hexane extraction was repeated three times (3 × 3 mL).

The acetonitrile phase containing HBCD and TBBP-A but also potential conjugated metabolites, was reduced to 200–500 μL, completed to 9 mL with an acetate buffer (0.2 M, pH 5.2) and subjected to an enzymatic hydrolysis (β-glucuronidase from *H. pomatia* purified juice, 10,000 Sigma units) during 4 h at 50 °C. After cooling and centrifugation, the incubated extract was thus loaded on an HLB cartridge used in reverse phase. Water/acetonitrile mixtures were applied for the washing steps (5 mL of 1:9 (v/v), 5 mL of 6:4 (v/v)) followed by 1 mL of pure acetonitrile. Thus, the column was dried under a gentle stream of nitrogen, before eluting HBCD (7 mL *n*-hexane/dichloromethane 1:1, v/v) and then TBBP-A (8 mL dichloromethane). The two resulting fractions (HBCD or TBBP-A) were then purified onto an SiOH cartridge using an optimized *n*-hexane/dichloromethane solvent system.

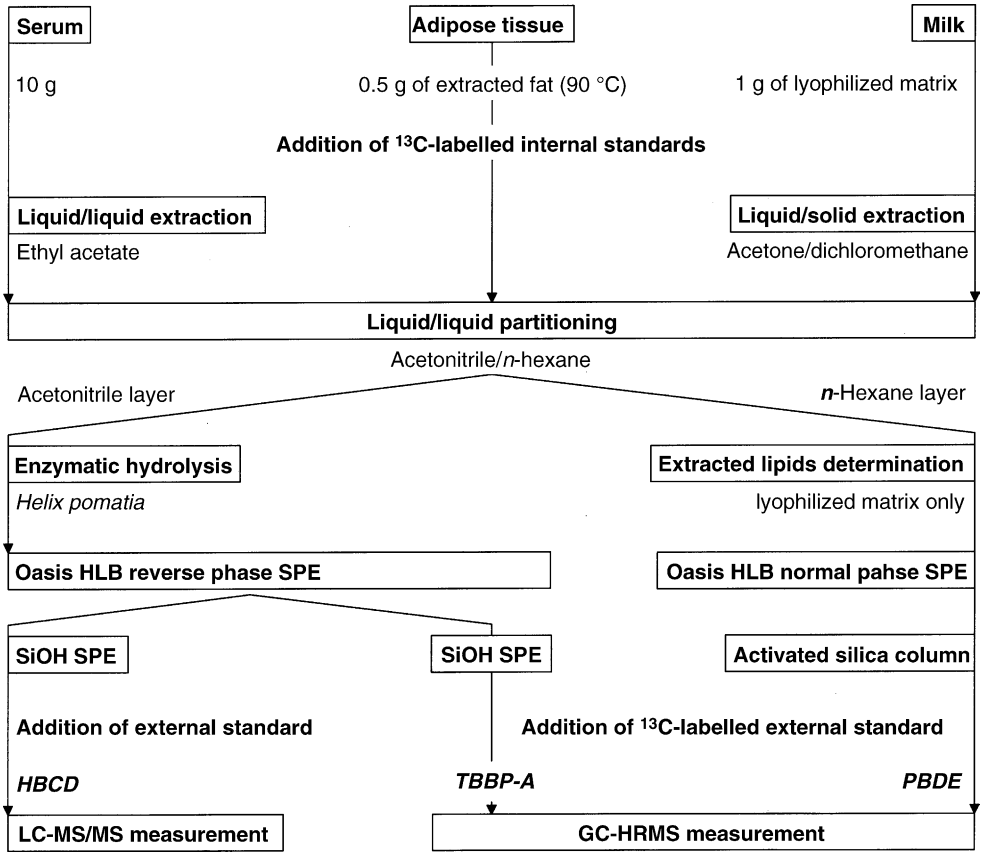


Fig. 2. Overview of the developed sample preparation procedure for the analysis of HBCD, TBBP-A and PBDEs in various animal matrices.

The *n*-hexane phase containing all PBDE congeners was submitted to two SPE purification steps. First, a HLB cartridge was used in normal phase with 5 mL *n*-hexane for the washing step and 5 mL *n*-hexane/dichloromethane 1:1 (v/v) for elution. Secondly, a multilayer column packed with 1 g, 3 g and 4 g of neutral, 22% and 44% of sulfuric acid activated G60 silica, respectively, between two layers of anhydrous sodium sulfate was used only with *n*-hexane as washing and eluting solvent. Analytes were eluted in a restricted "window" of 10 mL.

Finally, this procedure led to three fractions to be analyzed by mass spectrometry. The HBCD fraction was reconstituted in 50 μL of a methanol/acetonitrile 1:1 (v/v) solution containing fluorometholone as external standard for LC–MS/MS injection. The TBBP-A and PBDEs fractions were injected in GC-HRMS after reconstitution into 10 μL of a *n*-nonane/MSTFA (9:1, v/v) mixture containing the external standard ($^{13}$C-BDE-139). Total lipids content was determined using a colorimetric method for blood serums, or gravimetrically for freeze-dried milk samples (*n*-hexane extract after liquid/liquid partitioning).

### 2.5. Validation procedure

For each biological matrix, linearity was assessed on the basis of eight fortification levels in addition of an unfortified sample. These fortification levels were defined following an exponential increasing rule in a global concentration range adapted to each compound and covering at least three-order of magnitude in terms of concentration range. As an actual rule in the field of contaminant analysis, the obtained regression curve corresponded to the analyte signal relative intensity (analyte versus internal standard peak areas) plotted against the relative amount of the analyte (analyte versus internal standard concentrations). Therefore, the obtained slope for this calibration curve reflects the relative response factor of the analyte compared to its $^{13}$C-labelled internal standard. Basically, this slope should be equal to 1 assuming that their responses are equal. Repeatability was evaluated by the analysis of eight surrogate samples (intra-day variability). For PBDE congeners, a second set of eight replicates was analyzed separately, allowing the evaluation of the inter-day variability. All the used spiking levels are presented in Table 1. As significant contaminations in unfortified samples were observed for α-HBCD in adipose tissue and for TBBP-A in freeze-dried milk, no surrogate was realized for the repeatability assays in this case. The recovery yields were classically estimated on the basis of internal standard versus external standard ratio calculated for an extracted sample and for a standard at the same concentration.

Table 1
Fortification levels (pg) used for the validation experiments

| | Linearity range (pg) | Repeatability (pg) | | |
|---|---|---|---|---|
| | | Serum | Fat | Milk |
| α-HBCD | 100–100,000 | 1000 | – | 2000 |
| γ-HBCD | 100–100,000 | 1000 | 1000 | 2000 |
| TBBP-A | 20–20,000 | 100 | 100 | – |
| LowBDE Mixture[a] | 10–1280 | 50 | 50 | 100 |
| tetraBDE-47[b] | 0–98,720 | – | – | – |
| hexaBDE-153[b] | 0–17,440 | – | – | – |
| decaBDE-209 | 400–100,000 | 5000 | 2500 | 10,000 |

Linearity included eight fortification levels following an exponential increasing rule in the cited global concentration range. Repeatability included one or two sets of eight replicates.

[a] As described in chemicals paragraph. Cited quantities on the basis of tri- and tetra-BDE homologues.

[b] In addition of quantity contained in LowBDE Mixture.

## 3. Results and discussion

### 3.1. Validation results

The main validation results are shown in Table 2, respectively, for HBCD, TBBP-A, tri- to heptaBDE and decaBDE. Concerning the calibration curves, no more than three eventual aberrant points were eliminated for each compound (mostly the one corresponding to the maximal concentrations that reached the limit of the linearity domain). For bovine serum, total lipids content was found to be 1.79 ± 0.13 g/L, while the weighted extracted fat content for freeze-dried milk represented 145.8 g/kg of the dried weight (±2.4%).

#### 3.1.1. Hexabromocyclododecane

Recovery yields estimated for $^{13}$C-γ-HBCD were found to be around 40%, which was satisfying regarding the purification process. The coefficients of determination ($R^2$) obtained for the calibration curves were higher than 0.999, demonstrating a good linearity. As expected, the slope of the regression curve was close to 1 for γ-HBCD. A lower slope was observed for α-HBCD (around 0.6) with a noticeable variability between matrices, probably due to a slightly different extraction yield between α and γ stereoisomers. Regarding the repeatability, the intra-day variabilities were between 8 and 25%. The absence of the $^{13}$C-α-HBCD as internal standard probably contributes to the less satisfying results obtained for α-HBCD. Only liquid chromatography authorizes the separation of the HBCD three major stereoisomers, but the associated ionization and detection techniques are not always fully sufficient for trace analysis in complex biological matrices. Estimated limits of detection (LOD) were 30, 500 and 500 ng/kg for serum, fat and freeze-dried milk, respectively, as illustrated by Fig. 3. In conclusion, the presented analytical method appeared efficient in terms of specificity and quantification, but remained limited in terms of sensitivity (for trace analysis in biological matrices) due to the low ionization efficiency of ESI for this relatively apolar compound. Probably the sensitivity should be clearly improved on a last generation instrument. Another possible level of action should be the utilization of recently introduced ultra performance liquid chromatography to increase signal to noise ratio.

#### 3.1.2. Tetrabromobisphenol A

Recovery yields were about 40%, which can be considered as a satisfying value regarding the specificity of the method. $R^2$ observed for the regression curves were higher than 0.999

Table 2
Main parameters resulting from linearity (six to nine levels, $n = 1$) and repeatability (1 level, $n = 8$ or $n = 2 \times 8$) validation of developed protocol for α and γ-HBCD stereoisomers, TBBP-A, tri- to heptaBDE congeners and decaBDE

| | Bovine serum | Human fat | Breast milk |
|---|---|---|---|
| α and γ-HBCD | | | |
| $R^2$ | >0.999 | >0.999 | >0.999 |
| Slope | 0.74/1.02 (α/γ, resp.) | 0.63/1.02 (α/γ, resp.) | 0.56/1.08 (α/γ, resp.) |
| RSD intra-D | 20%/8% (α/γ, resp.) | 18%/25% (α/γ, resp.) | 12%/11% (α/γ, resp.) |
| LOD | 30 ng/kg | 500 ng/kg | 500 ng/kg |
| LOQ | 100 ng/kg | 1500 ng/kg | 1500 ng/kg |
| TBBP-A | | | |
| $R^2$ | >0.999 | >0.999 | >0.996 |
| Slope | 1.43 | 1.42 | 1.48 |
| RSD intra-D | 4.1% | 6.5% | 6.1% |
| LOQ | 0.2 ng/kg | 4 ng/kg | 3 ng/kg |
| tri- to heptaBDE | | | |
| $R^2$ | >0.995 | >0.995 | >0.995[a] |
| Slopes | 0.40–1.68 | 0.25–1.62 | 0.25–1.27 |
| RSD inter-D | 5–15% | 3–19% | 6–22% |
| LOQ | <0.5 ng/kg[b] | <10 ng/kg[b] | <10 ng/kg[b] |
| decaBDE-209 | | | |
| $R^2$ | >0.999 | >0.999 | >0.999 |
| Slope | 2.02 | 2.04 | 1.95 |
| RSD inter-D | 3.7% | 3.2% | 4.2% |
| LOQ | 30 ng/kg | 500 ng/kg | 200 ng/kg |

[a] Excepted BDE-100, 99, 116 and 139.
[b] Excepted BDE-166 and heptaBDEs.

for serum and fat, and higher than 0.996 for milk. For monitoring the $^{13}$C-labelled internal standard, the $m/z$ 682.8509 was preferred to the $m/z$ 684.8489 for quantification since the native compound slightly contributes to the second one ($[^{12}C_{19}{}^{13}CH_{25}O_2{}^{79}Br_4Si_2]^{\bullet+}$). Then, the slopes obtained for the regression curves were close to the expected value (1.43–1.48 depending on the matrix, while the theoretical relative response factor based on the diagnostic ions should be 1.53). As the repeatability is concerned, satisfying intra-day variability values were obtained (<6.5%). Maximum accuracy deviation was estimated to 9%, for serum. In conclusion, the developed extraction/purification and detection procedure appeared globally very impressive and particularly sensitive for TBBP-A, at the ng/kg range.

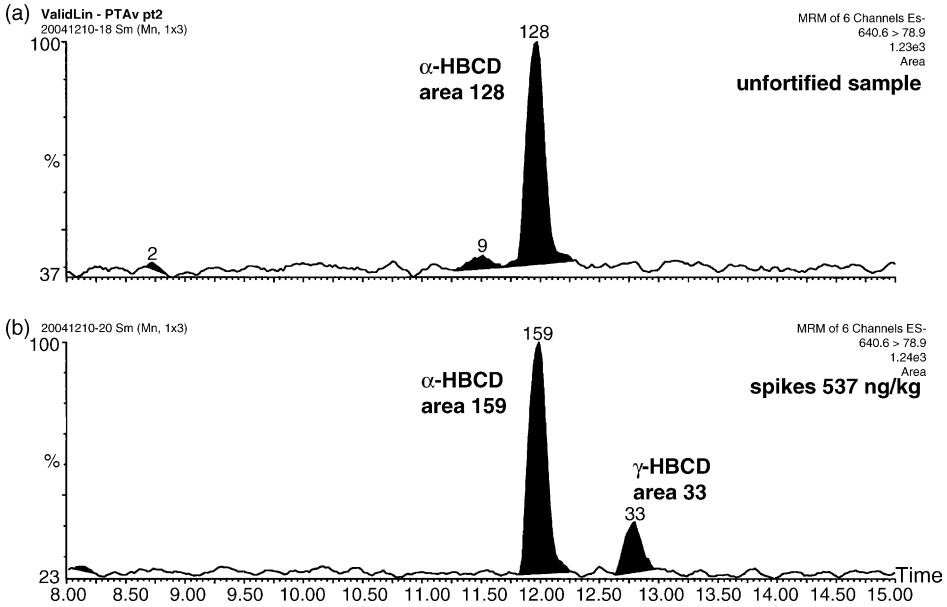


Fig. 3. LC-(ESI-)–MS/MS diagnostic ion chromatograms ($[M–H]^- > [Br]^-$) obtained for HBCD in the human adipose tissue fat pool used for validation, without (a) or with (b) fortification with α and γ stereoisomers (537 ng/kg each).

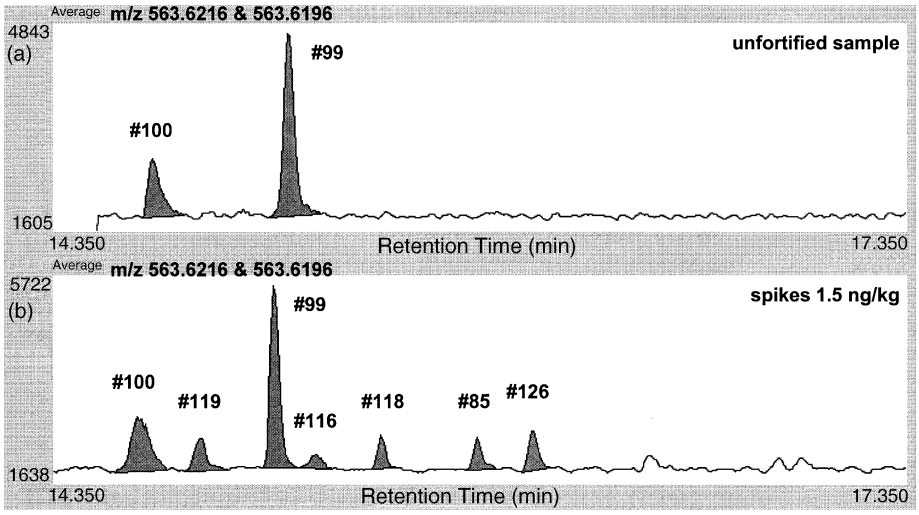


Fig. 4. GC-(EI+)-HRMS diagnostic single ion monitoring chromatograms ($[M]^{\bullet+}$) obtained for seven pentaBDE congeners in the bovine blood serum used for validation, without (a) or with (b) fortification at 1.5 ng/kg each.

### 3.1.3. Tri-to heptabrominated diphenylether congeners

Recovery yields obtained for tri- to heptaBDE congeners were in the 60–110% range which appeared satisfying. For the 29 studied congeners in the three matrices, all the observed $R^2$ were higher than 0.995, excepted for four congeners in milk. In this matrix, $R^2$ obtained for hexaBDE-138 and pentaBDE-116 were higher than 0.990 which remain acceptable. For pentaBDE-100 and 99, $R^2$ was, respectively, about 0.98 and 0.92. A slightly disturbing matrix effect observed in the elution area of pentaBDE added to a significant sample contamination probably explains these results. Regarding repeatability, the inter-day repeatability was globally in the 5–15%, 3–19% and 6–22% for serum, fat and milk, respectively. Accuracy deviations were lower than 10%, excepted for some congeners: BDE–47 and 183 in serum, BDE–119 in fat, BDE-25, 75 and 77 in milk. Fig. 4 shows ion chromatograms obtained for pentaBDEs in serum. Estimated limits of quantification (LOQ) were lower than 0.5 ng/kg fresh sample in serum and than 10 ng/kg in fat and milk (respectively on fat and dried weight) for tri- to hexaBDE. The observed LOQ for pentaBDE-116 and heptaBDE were two or three times higher than for other congeners, mainly due to a matrix effect more disturbing and noticeable on the blank ion chromatograms.

### 3.1.4. Decabrominated diphenylether

The very complex and specific behavior of decaBDE-209 during all the analytical steps and particularly the GC-HRMS measurement made hexaBDE-139 unsuitable as external standard for recovery yield estimations. Moreover, the difficulty to achieve easily an accurate calibration of the instrument in the expected mass range ($m/z$ 797–812) as well as the poor reproducibility linked to the thermal degradation of this PBDE in the splitless injector contributes to the difficulty of its analysis. Nevertheless, observed signals made us believe that real recovery yields were in the 80–110% range. Regarding linearity, the presence of suitable $^{13}$C-labelled decaBDE-209 as internal standard provided $R^2$ higher than 0.999. Concerning repeatability, the inter-day variability was not higher than 5%, which appeared very satisfying. A satisfying accuracy deviation close to 5% was observed for milk, but these values were near 50% for serum and fat. An analytical contamination during extraction steps of linearity sets was incriminated, what is the subject of the next paragraph. Estimated LOQ were 30, 500 and 200 ng/kg, respectively, for serum, fat and milk samples. It can be noticed that improved performances were obtained since these validation results, on one side using a reduced film thickness (0.10 μm instead of 0.25 μm) for GC capillary column, and on the other side by the suppression of MSTFA in the external standard solution initially used to maintain constant the silylation of GC column but aggravating thermal degradation of decaBDE-209 in splitless injector. With these two modifications, the LOQ was reduced to 50 ng/kg in fat sample for instance.

## 3.2. Analytical contaminations

One major issue highlighted by this study was the occurrence at multi-stage levels of analytical contaminations. This is probably the main challenge regarding brominated flame retardants measurement. Compounds concerned were TBBP-A, BDE-28, 47, 100, 99, 154, 153 and 209, and identified sources included nitrogen drying system, freeze-drying, solvents and glassware. Class I nitrogen with clean associated system have been preferred to a laboratory nitrogen generator. As already observed for similar compounds such as polychlorinated biphenyls, freeze-drying can be a high source of contamination [15], especially for decaBDE in our laboratory. The solvents quality has to be tested and controlled. At last, special attention must be paid to the glassware due to adsorption phenomenon [16] and washing-machine contamination. As illustration, Fig. 5 shows ion chromatograms obtained in a procedural blank submitted to the serum analytical protocol, leading to analytical contaminations in the ng/kg range. We regularly improved the analytical quality in terms of contamination of our procedural blank, and since this presented example, analytical contamination was decreased by a factor 10 for TBBP-A and by factors 3–4 for PBDE (decaBDE not

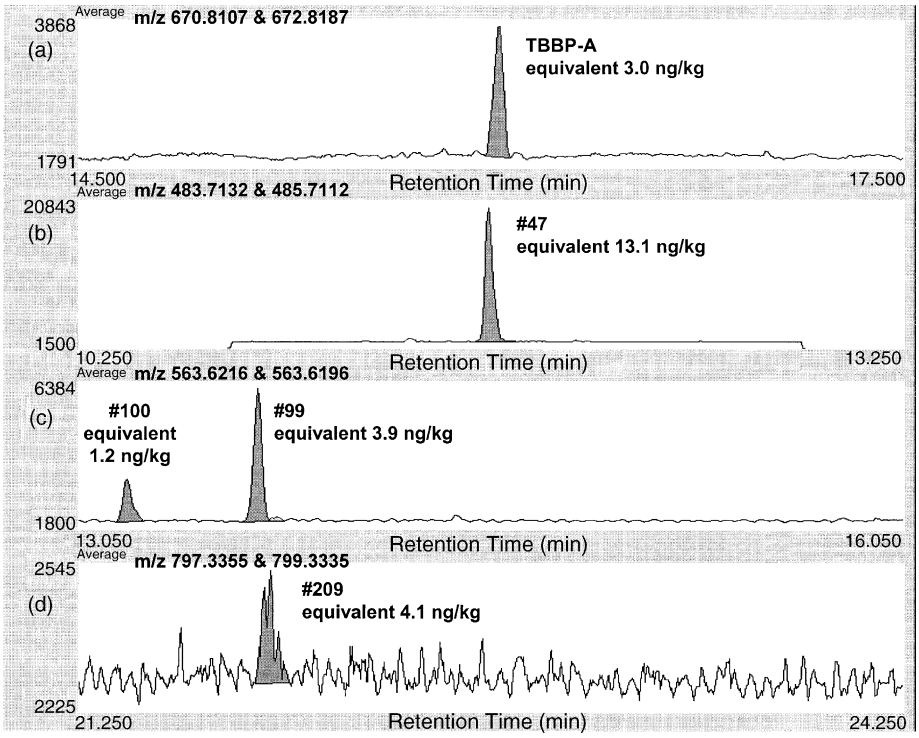


Fig. 5. GC-(EI+)-HRMS diagnostic single ion monitoring chromatograms ($[M–CH_3]^{\bullet+}$, $[M]^{\bullet+}$, $[M]^{\bullet+}$ and $[M–Br_2]^{\bullet+}$), respectively, obtained for TBBP-A (a); tetraBDE (b); pentaBDE (c) and decaBDE (d) in a procedural blank (no matrix) submitted to the serum analytical protocol, illustrating the analytical contamination.

included). In conclusion, it can be estimated that if this subject was not the purpose of the present work, it will undoubtedly merit more investigation. The need of extensive quality control/quality assurance (QA/QC) criteria with special attention on procedural blank concerning PBDEs in biological tissues was recently highlighted by Päpke et al. [17]. Consensual rules for quantification of such compounds with systematic blank contamination undoubtedly merit discussion by regulatory instances in charge of establishing validation guide and analytical criteria.

### 3.3. Applications on real samples

Analyzes of individual samples were realized using the validated protocol and improved precautions in order to minimize analytical contamination. Examples of ion chromatograms obtained are shown for TBBP-A, hexaBDE and decaBDE, respectively, in milk, serum and adipose tissue. Fig. 6 shows that TBBP-A was identified and quantified at 1400 ng/kg dry matter (equivalent 7000 ng/kg lipid weight) in the milk sample. Pro-

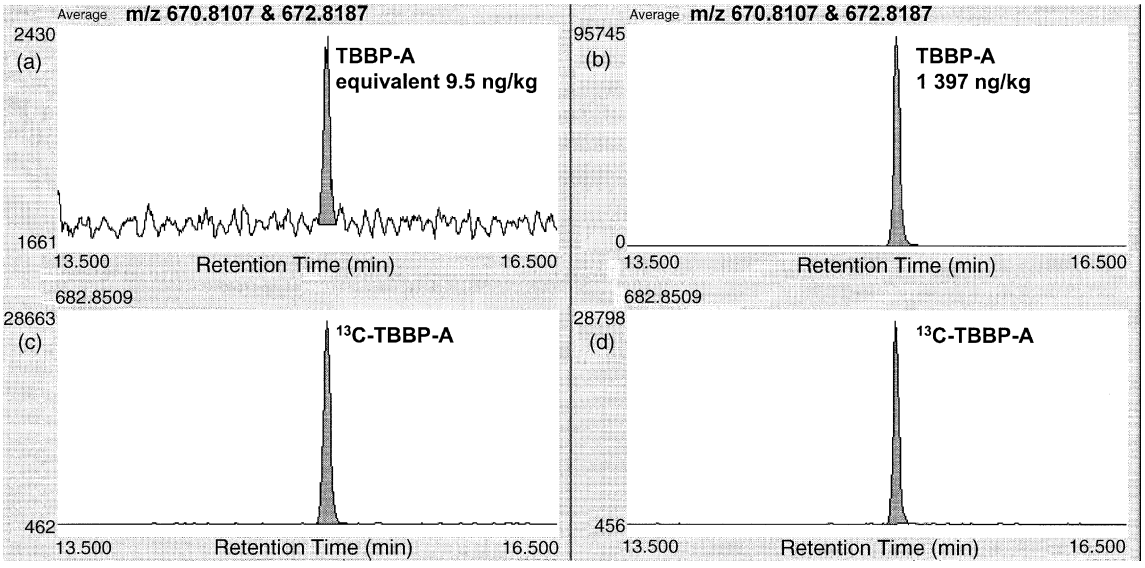


Fig. 6. GC-(EI+)-HRMS diagnostic single ion monitoring chromatograms ($[M–CH_3]^{\bullet+}$) obtained for native TBBP-A (a, b) and $^{13}C$-labelled internal standard (c, d) in a procedural blank (a, c) and a milk sample (b, d).

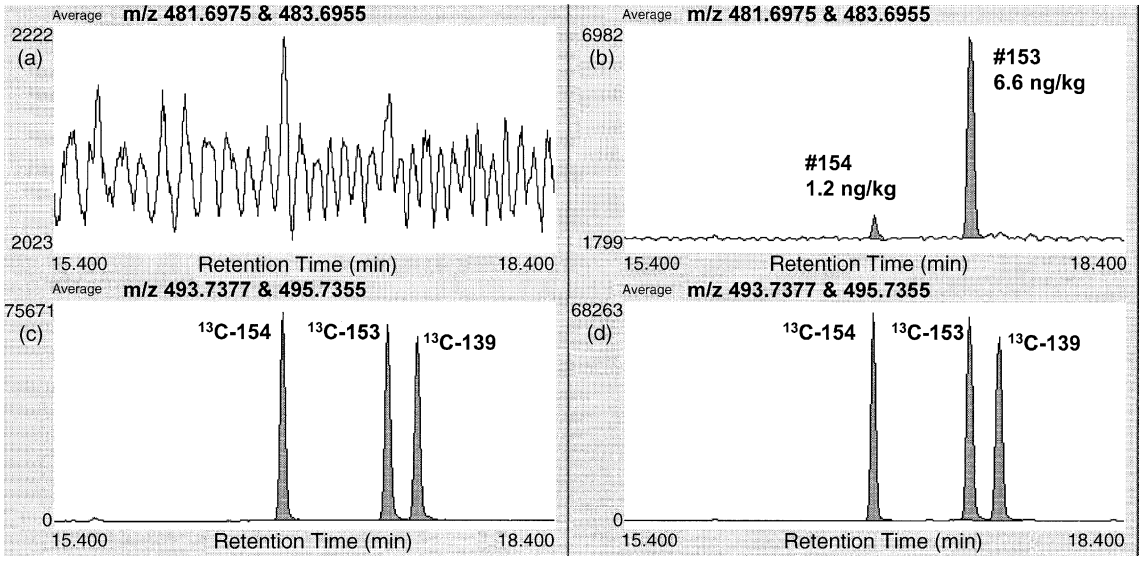


Fig. 7. GC-(EI+)-HRMS diagnostic single ion monitoring chromatograms ($[M–Br_2]^{\bullet+}$) obtained for native hexaBDE (a, b) and $^{13}C$-labelled internal/external standards (c, d) for a procedural blank (a, c) and for a human serum sample (b, d).

cedural contamination represented the equivalent of 9.5 ng/kg, which appeared in this case negligible compared to the sample contamination. The example of hexaBDE diagnostics ion chromatograms (Fig. 7) shows that no analytical contamination was observed for these compounds, allowing the identification and quantification of hexaBDE-154 and 153 at low ng/kg levels in a serum sample (respectively 1.2 and 6.6 ng/kg fresh matter). The last example (Fig. 8) concerns decaBDE in adipose tissue. The first observation was the significant analytical contamination appearing on the blank ion chromatogram (equivalent to 450 ng/kg). Several additional experiments permitted to demonstrate that this analytical contamination, essentially occurred during the first lipids extraction step. Nevertheless, decaBDE was identified and quantified in the presented sample at a very significant level (4400 ng/kg lipid weight).

## 4. Conclusions

A multi-residue analytical method was developed for the determination of the main representatives belonging to the three classes of brominated flame retardant (HBCD, TBBP-A, PBDEs) in various human biological matrices such as serum, adipose tissue and milk. This method was validated and the results demonstrated fit-for-purpose performances regarding the defined objectives. Application on real samples permitted to prove its suitability, and to collect first data regarding the occurrence of brominated flame retardants, especially for TBBP-A in serum and PBDEs in fat. One important source of difficulty was highlighted, which was the control of analytical contamination. Work is now in progress in order to characterize and minimize the different identified contamination sources. The further step

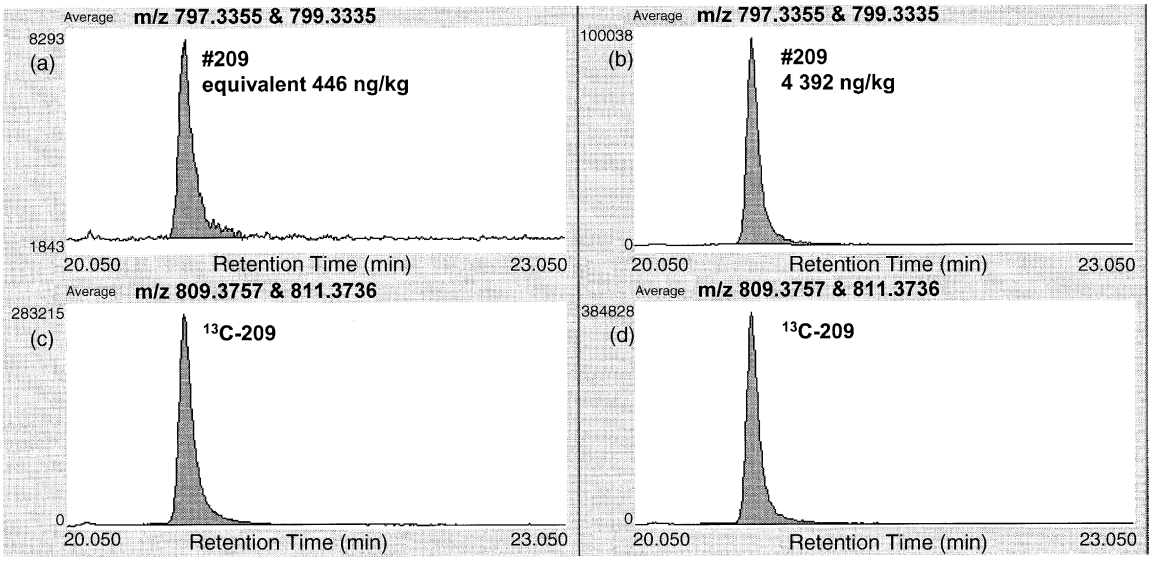


Fig. 8. GC-(EI+)-HRMS diagnostic single ion monitoring chromatograms ($[M–Br_2]^{\bullet+}$) obtained for native decaBDE (a, b) and $^{13}C$-labelled internal standard (c, d) for a procedural blank (a, c) and a human adipose tissue sample (b, d).

will be to use this methodology to assess the fetal and neonatal exposure to brominated flame retardants through the analysis of samples from women and newborn collected during caesarean deliveries.

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## References

[1] H. Hakk, R.J. Letcher, Environ. Int. 29 (2003) 801.
[2] L.S. Birnbaum, D.F. Staskal, Environ. Health Perspect. 112 (2004) 9.
[3] K. Janak, C. Thomsen, G. Becher, Proceedings of the Third International Workshop on Brominated Flame Retardants, Toronto, Ontario, 2004, p. 313.
[4] W. Budakowsky, G. Tomy, Rapid Commun. Mass Spectrom. 17 (2003) 1399.
[5] T. Hayama, H. Yoshida, S. Onimaru, S. Yonekura, H. Kuroki, K. Todoroki, H. Nohta, M. Yamaguchi, J. Chromatogr. B 809 (2004) 131.
[6] A. Sjödin, H. Carlsson, K. Thuresson, S. Sjölin, A. Bergman, C. Östman, Environ. Sci. Technol. 35 (2001) 448.
[7] L. Hovander, M. Athanasiadou, L. Asplund, S. Jensen, E. Klasson Wehler, J. Anal. Toxicol. 24 (2000) 696.
[8] K. Akutsu, H. Obana, M. Okihashi, M. Kitagawa, H. Nakazawa, Y. Matsuki, T. Makino, H. Oda, S. Hori, Chemosphere 44 (2001) 1325.
[9] M. Alaee, D.B. Sergeant, M.G. Ikonomou, J.M. Luross, Chemosphere 44 (2001) 1489.
[10] S. Crhova, M. Cerna, R. Grabic, T. Tomsej, T. Ocelka, Organohalogen Compd. 58 (2002) 241.
[11] R. Cariou, J.P. Antignac, L. Debrauwer, D. Zalko, B. Le Bizec, F. André, Organohalogen Compd. 61 (2003) 53.
[12] T. Hyötyläinen, K. Hartonen, Trends Anal. Chem. 21 (2002) 13.
[13] C. Thomsen, E. Lundanes, G. Becher, J. Sep. Sci. 24 (2001) 282.
[14] C. Thomsen, H. Leknes, E. Lundanes, G. Becher, J. Anal. Toxicol. 26 (2002) 129.
[15] M. Söderström, K. Nylund, U. Järnberg, T. Alsberg, L. Asplund, Chemosphere 58 (2005) 355.
[16] C. Thomsen, H. Leknes, E. Lundanes, G. Becher, J. Chromatogr. A 923 (2001) 299.
[17] O. Päpke, P. Fürst, T. Herrmann, Talanta 63 (2004) 1203.