

Development of a multi-residue analytical method for TBBP-A and PBDEs in various biological matrices using unique reduced size sample

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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. Numerous studies related to the detection of tetrabromobisphenol A (TBBP-A) and polybrominated diphenylethers (PBDEs) have been developed over the last few years; they were mainly based on GC-ECD¹, GC-NCI-MS² or GC-EI-HRMS³, and recently GC-EI-MS/MS^{4,5}. The sample treatment is usually derived from the analytical methods used for dioxins, but recently some authors proposed the utilisation of solid phase extraction (SPE) cartridges^{6,7}. In this study, a new analytical strategy is presented for the multi-residue analysis of TBBP-A and PBDEs from a unique reduced size sample. The main objective of this analytical development is to be applied for background exposure assessment of French population groups to brominated flame retardants, for which, to our knowledge, no data exist. A second objective is to provide an efficient analytical tool to study the transfer of these contaminants through the environment to living organisms, including degradation reactions and metabolic biotransformations.

Material and methods

MSTFA was provided by Fluka (Buchs, Switzerland). *n*-Nonane was provided by Sigma (Steinheim, Germany). Other solvents (ethyl acetate, acetonitrile, *n*-hexane, cyclohexane, dichloromethane) and concentrated sulphuric acid were purchased from LGC Promochem (Picograde®, Wesel, Germany). Reference standards were purchased from Cambridge Isotope Laboratories or Wellington Laboratories were TBBP-A (13C-labelled or native), triBDEs (30, 32, 17, 25, 28, ¹³C-28, 33, 35, 37), tetraBDEs (75, 49, 71, 47, ¹³C-47, 66, 77), pentaBDEs (100, 119, 99, ¹³C-99, 116, 118, 85, 126), hexaBDEs (155, 154, ¹³C-154, 153, ¹³C-153, ¹³C-139, 138, 166), heptaBDEs (183, ¹³C-183, 181, 190), octaBDEs (196), nonaBDE (206) and decaBDE (209, ¹³C-209).

For GC-HRMS measurements, an HP 5890 gas chromatograph (Palo Alto, CA, USA) was used. Before injection, TBBP-A was derivatised using *n*-nonane/MSTFA (9:1, v/v) (ambient temperature, 15 min), leading to the diTMS derivative. Volumes of 2 µL were injected in the splitless mode (280 °C, purge splitless 1 min). The gas chromatograph was fitted with a capillary

column, 15 m x 0.25 mm id. x 0.25 μm film thickness, coated with a diphenyl(5%)-dimethylpolysiloxane(95%) stationary phase (UB5-Premium). Helium was used as carrier gas at 0.82 mL.min⁻¹. The temperature gradient was from 120 °C (held for 2 min) to 280 °C (10 °C.min⁻¹) and then to 320 °C (20 °C.min⁻¹, held for 8 min). Mass spectrometric data were acquired on a SX-102A (Jeol, Tokyo, Japan) double focusing electromagnetic instrument, using perfluorokerosene (PFK) as calibration reference and resolution set at 7000. Source temperature was set at 230 °C. The electronic beam energy was set at 70 eV.

The sample treatment procedure is presented in Figure 1. Fresh blood serum or plasma (1-10 g), liquid fat (0.5-1 g, heated at 150 °C) or lyophilised colostrum or placenta (1 g) were spiked with ¹³C-labelled internal standards (TBBP-A and PBDEs 28, 47, 99, 154, 153, 183, 209) and kept one night for equilibration. For serum samples, a first liquid/liquid extraction with ethyl acetate was performed. Thus, for all samples, a liquid/liquid extraction and simultaneous partitioning was applied using a CH₃CN/*n*-hexane mixture. The acetonitrile phase, which contains TBBP-A and potential hydroxylated or conjugated metabolites, was subjected to an enzymatic hydrolysis (*Helix pomatia*) at pH 5.2 and 50 °C during 4 hours, followed by two purification steps using SPE cartridges. First, an Oasis[®] HLB cartridge (Waters, Milford, USA) (500 mg of stationary phase) was used in reverse phase with a CH₃CN/H₂O solvents system for the washing step and dichloromethane for elution. Secondly, a SiOH (UCT, Bristol, UK) (1 g of s. p.) was used with a DCM/*n*-hexane solvents system. The *n*-hexane phase which contains PBDE congeners was then submitted to two further SPE purification steps. First, an Oasis[®] HLB (500 mg of s. p.) was used in normal phase with a DCM/*n*-hexane solvents system. Secondly, a multilayer column packed with 1 g, 3 g and 4 g of neutral, 22% and 44% of sulfuric acid activated G60 silica, between two layers of anhydrous sodium sulfate. Analytes were eluted with *n*-hexane. All the extracts were reconstituted into 10 μL of *n*-nonane/MSTFA (9:1, v/v) containing the external standard (¹³C-BDE-139) before injection.

SAMPLING, CLEAN-UP AND SEPARATION

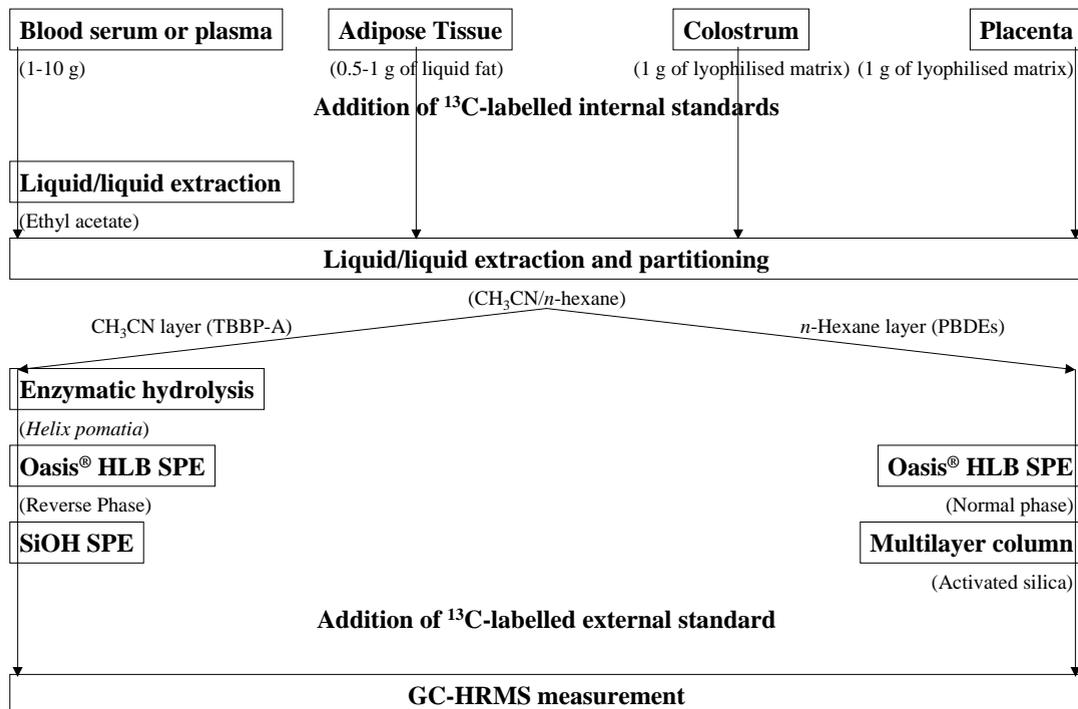


Figure 1 : Analytical strategy for the analysis of TBBP-A and PBDEs in biological matrices.

Results and discussion

The recovery yields were estimated in the 30-60% range for TBBP-A and tetra- to heptaBDEs in serum, adipose tissue and placenta samples. A full validation is planned soon to determine the performances of the method.

A major advantage of this method is the relatively weak consumption of solvents, involving a reduced methodological contamination contribution. Compared to the 300-500 mL commonly used in existing methods, only 50-80 mL of organic solvents are susceptible to be concentrated into the final extracts.

Figure 2 shows characteristic ion chromatograms corresponding to samples spiked with the ¹²C-native congeners. The detection limits deduced from these data (based on fresh weight) were in the 5-25 ppt range from 1-10 g of sample.

The further objective of this study is to apply the developed analytical protocol to human matrices obtained after deliveries under caesareans, such as placenta, cord and maternal blood serum, colostrum or maternal adipose tissue. Furthermore, the transplacental transfer of brominated flame retardants in the pregnant woman have to be characterised in view to study the risks for the newborn. At last, the search of potential metabolites in these matrices will be investigated.

Conclusion

A new analytical method was developed for the multi-residue analysis of TBBP-A and 32 tri- to decaBDE congeners in various biological matrices, including one or two liquid/liquid extractions and two purifications by SPE. This method is compatible with small sample size. Its main characteristics are its excellent sensitivity and the low environmental noise due to limited amounts of solvents used all along the protocol. Work is now in progress concerning the analysis of samples from a French population group.

References

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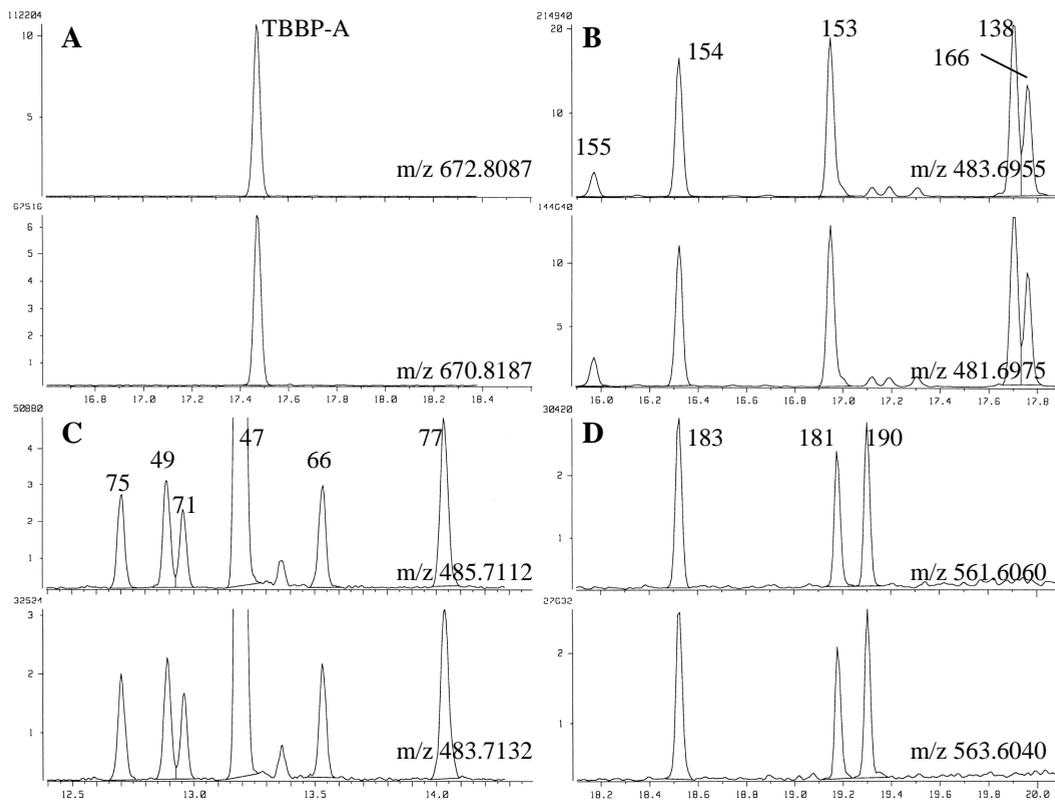


Figure 2 : GC-HRMS SIM diagnostic ion chromatograms of **A** TBBP-A for a spiked plasma (800 pg / 1 g), **B** hexaBDEs for a plasma (800 pg / 1 g), **C** tetraBDEs for a human adipose tissue pool (400 pg / 1 g liquid fat) (BDE-47 corresponds to the fortification level and a real contamination to 8 ppb) and **D** heptaBDEs for a human placenta pool (1000 pg / 1 g dried weight).