A NEW METHOD FOR DETERMINATION OF BROMINATED FLAME RETARDANTS IN HUMAN MILK USING SOLID-PHASE EXTRACTION

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Introduction

Human milk monitoring is a convenient, non-invasive method of determining body burdens of persistent organic pollutants (POPs). It has been used to estimate infants' exposure to POPs from nursing and for elucidating spatial and temporal trends in environmental pollution levels.[1,2] While concentrations of polychlorinated POPs such as DDE, PCBs and PCDDs/PCDFs in human milk have decreased over time in several countries, a recent Swedish study revealed an exponential increase of polybrominated diphenyl ethers (PBDEs) in human milk.[3] PBDEs and other brominated organics are widely used as flame retardants (FRs) in many products such as plastics, foams and textiles, and have been shown to occur in various environmental samples such as indoor air, sediments and wildlife.[4] Human exposure may arise from food consumption or inhalation of contaminated air.[5]

The aim of this study was to develop a rapid and simple method for determination of phenolic and neutral brominated flame retardants (BFRs) in human milk using solid-phase extraction (SPE) with lipid decomposition by concentrated sulphuric acid directly on the SPE column. Gas chromatography coupled to electron capture mass spectrometry (GC-ECMS) was chosen for quantification of the BFRs due to high selectivity and sensitivity towards halogenated compounds.

Materials and Methods

Chemicals

2,4,4'-Tribromodiphenyl ether (BDE-28), 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), 2,2',4,4',6-pentabromodiphenyl ether (BDE-100), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154), 2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE-183) and ^{13}C-3,3',4,4'-tetrabromodiphenyl ether (BDE-77) were kindly supplied by Wellington Laboratories (Guelph, Ontario, Canada). Tetrabromobisphenol-A (TBBP-A) and chlorotribromobisphenol-A (CtriBBP-A) were gifts from the Wallenberg Laboratory (University of Stockholm, Sweden). 2,4,6-Tribromophenol (TriBP) and tetrabromo-*o*-cresol (TBCr) were purchased from Aldrich (Milwaukee, WI, USA). 1,3,5-Tribromobenzene (TriBB) was obtained from Fluka (Buchs, Switzerland), and 3,3',4,4'-tetrabromobiphenyl (BB-77) from AccuStandard Inc. (New Haven, CT, USA). All solvents were pesticide grade from Labscan (Dublin, Ireland).

Milk samples

For validation, unprocessed cow's milk was used for reasons of easy availability and low contamination level. The milk was homogenized by sonication before weighing sub-samples of 5.0 g into glass bottles. These were kept frozen until analysis.

Sample preparation
Samples of 5.0 g were spiked with an internal standard solution containing TBCr, BDE-77, BB-77 and CtriBBP-A in ethyl acetate. Samples used for method validation were further spiked with standard solutions at six different levels (I-VI) of TriBP, PeBP, TCBP-A, TBBP-A, and the BDEs 28, 47, 99, 100, 153, 154 and 183. Concentrations were in the range 1.8 - 180 pg/g milk, except for TriBP (0.65 – 65 pg/g milk), PeBP (2.5 – 250 pg/g milk) and TCBP-A (3.6 – 360 pg/g milk). After spiking, the samples were left to equilibrate overnight in a refrigerator. Oasis™ HLB SPE columns (500 mg, Waters Corporation, Milford, MA, USA) were used for extraction. The procedure for sample preparation is outlined in Figure 1.

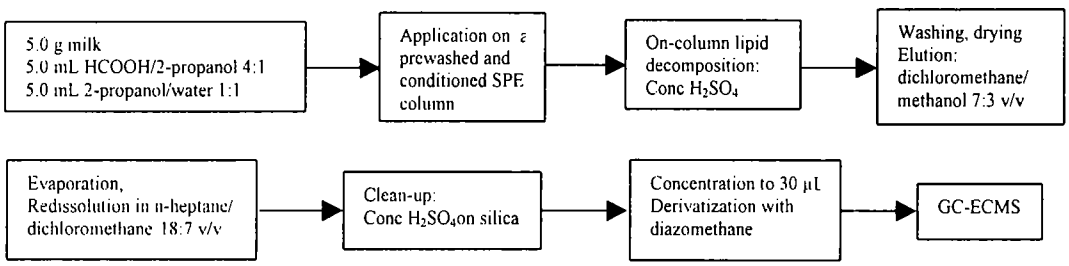

Figure 1. Procedure for sample preparation.

After derivatization, the samples were added 15 µL of a TriBB solution used as GC-MS quantification standard. Analysis was performed by capillary GC electron capture MS, detecting the brominated and chlorinated compounds at *m/z* 79/81 and 35/37, respectively. Instrumental aspects have been discussed in details elsewhere.[6,7]

Results and Discussion
The combination of SPE with on-column lipid decomposition and further clean-up using sulphuric acid-silica columns resulted in clean extracts for both cow's and human milk, avoiding problems with chromatographic interferences (Figure 2). The estimated limits of detection (LOD, S/N = 3) were in the range of 0.3 - 1.0 pg/g milk for all compounds, except for TCBP-A, for which the LOD was 6.7 pg/g milk.

Due to a pronounced matrix effect,[7] the validation samples were quantified against calibration curves made from standards in extracts of non-spiked milk. At spike levels I, III and V, four replicates were extracted, while at levels II, IV and VI, only one sample was prepared at each level. In addition, three replicates of human milk spiked at level III were analyzed two months after the validation experiment. The results are presented in Figures 3 and 4. Absolute recoveries were calculated using the GC-MS quantification standard, while the accuracy was determined as recovery with respect to internal standards.

Reasonably good recoveries and accuracies were obtained for all compounds, except for TCBP-A and TriBP. Due to a high native content of TriBP in the validation samples, this compound could not be quantified at the two lowest levels. The cow's milk used for validation also contained low native concentrations of some of the other BFRs (Figure 5), and corrections were made.

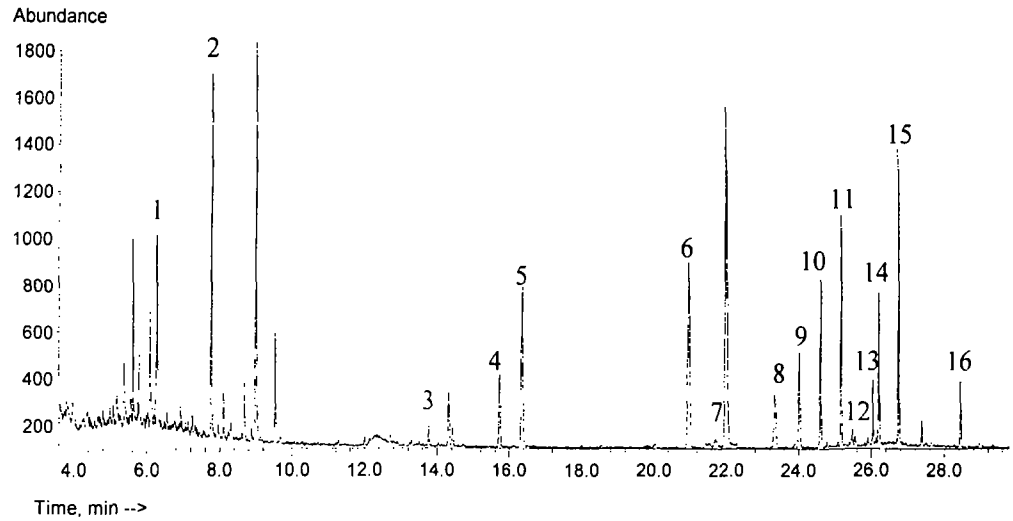

Figure 2. Chromatogram of a 5.0 g human milk sample spiked at level III. Retention order 1: TriBB, 2: TriBP, 3: TBCr, 4: BDE-28, 5: PeBP, 6: BDE-47, 7: TCBP-A, 8: BDE-77, 9: BB-77, 10: BDE-100, 11: BDE-99, 12: CtriBBP-A, 13: TBBP-A, 14: BDE-154, 15: BDE-153 and 16: BDE-183.

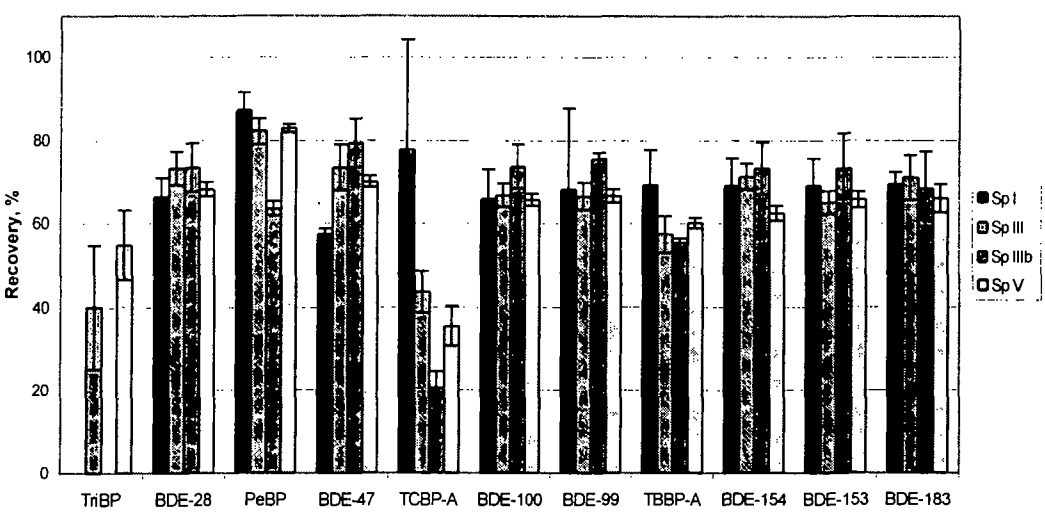

Figure 3. Absolute recovery of the halogenated flame retardants at three spike levels (I, III, V). Sp IIIb represents human milk spiked and analyzed two months after the validation experiment. Standard deviations of four replicates (three at level I) are shown as error bars in the diagram.

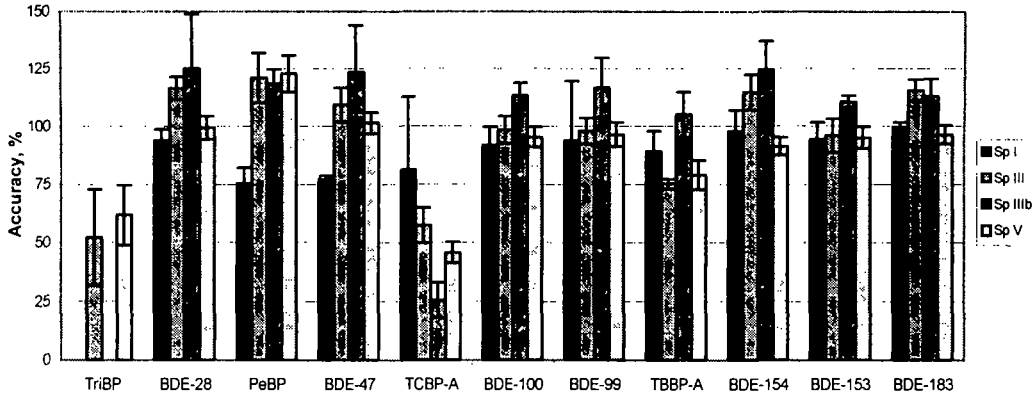

Figure 4. Accuracy, determined as recovery with respect to internal standard. Sp IIIb represents human milk spiked and analyzed two months after the validation experiment. Standard deviations of four replicates (three at level I) are shown as error bars in the diagram.

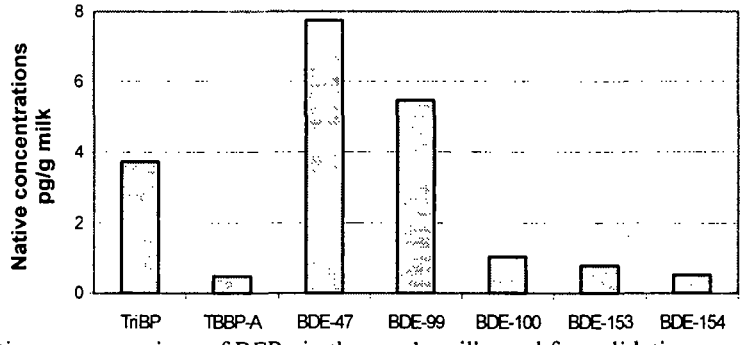

Figure 5. Native concentrations of BFRs in the cow's milk used for validation.

Acknowledgements

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References

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