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ANALYSIS OF POLYBROMINATED DIPHENYL ETHERS IN FOODSTUFFS - EXTENSION OF AN EXTRACTION METHOD FOR PCDD/Fs AND PCBs TO INCORPORATE PBDEs

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Introduction

Polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) have been recognised as ubiquitous environmental pollutants for several decades. However, due to emission controls and legislative measures taken to reduce environmental pollution, the levels of many organochlorine compounds including PCBs and PCDD/Fs in the environment have decreased[1]. In contrast, levels of polybrominated diphenyl ethers (PBDEs) in the environment and food have been increasing[1], due to their widespread and ongoing use as flame retardants in many man-made materials. Understanding of the toxicology and abundance of PCBs and PCDD/Fs is appreciable due to widespread research. Scientific interest is now also focused on PBDEs, which have also been highlighted as toxic[2], ubiquitous, persistent, organic pollutants[3].

Methodology for the determination of ortho and non-ortho substituted PCBs (o-PCBs and n-PCBs respectively) and PCDD/Fs is very well documented and extensively reviewed[4,5]. The utilization of activated carbon columns allows the fractionation of these analytes into discrete groups with good efficiency based on the physical characteristics, principally relative planarity of each analyte. Several methods have been published for the singular analysis of PBDEs in the environment and food[6]. However, the adaptation of in-situ methodology to include this important class of contaminants allows a single extraction process for a range of analytes. This leads to the reduction of solvent usage and total extraction time, as well as the generation of results for PCBs, PCDD/Fs and PBDEs from exactly the same sample aliquot. An existing method involving the on-line extraction, clean-up and analysis of o-PCBs, n-PCBs, PCDD/Fs in foodstuffs has been extended to incorporate PBDEs and is outlined in this paper with typical profiles for some food types from preliminary data.

The method, including the extension to PBDEs, has been accredited to the ISO 17025 standard.

Methods and Materials

Sample preparation and extraction:
All samples were homogenised and lyophilised prior to extraction along with a certified reference material for quality control purposes. $^{13}C_{12}$ mass labelled and native standards, were produced by either Cambridge Isotope Laboratories or Wellington Laboratories.

The sample, typically 5-50 g, was weighed out to give an approximate total fat weight of 5g per sample. The sample was fortified with a mixture of $^{13}C_{12}$-mass labelled internal surrogate standards. This mixture consisted of six 2,3,7,8 substituted PCDDs; nine 2,3,7,8 substituted

PCDFs; n-PCBs IUPAC numbers 77, 81, 126 and 169; o-PCBs 28, 52, 101, 118, 138, 153, 180 and 194; and PBDEs 28, 47, 99, 153, 154 and 183.

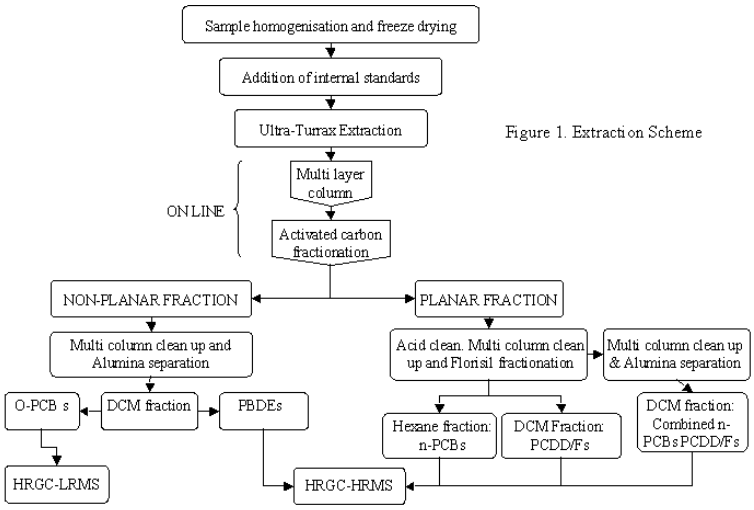


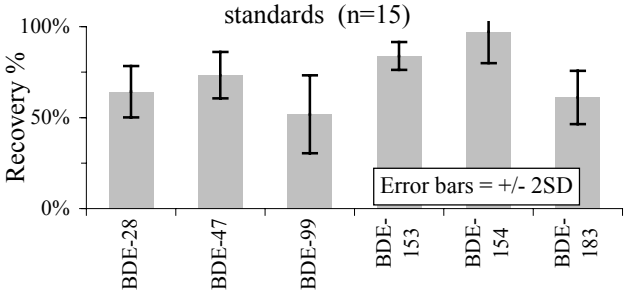
Figure 1. Extraction Scheme

The sample was extracted with hexane using high speed blending. The blended sample was transferred to the top of a mixed bed extraction column packed with a series of acid and base modified silica gels. The outlet of the extraction column was connected in line to a glass column packed using glass-fibre frits loaded with activated carbon. The extract was eluted through the two column system using a mixed solvent (Dichloromethane/hexane) under nitrogen head pressure.

Planar analytes clean-up:
The planar fraction was eluted from the carbon using toluene. The extract was concentrated then further purified by chromatography on an acid and base modified silica column followed by alumina or Florisil. The cleaned extract was concentrated and spiked with a ^{13}C surrogate dioxin syringe standard (1,2,3,4-T_4CDD & 1,2,3,7,8,9-H_6CDD) for quality control purposes.

Non planar analytes clean-up:
The extract was concentrated and cleaned using acid and base modified silica gel column chromatography and finally separated on activated alumina. Analytes were removed from alumina using dichloromethane/hexane, this gave a fraction containing the o-PCBs and PBDEs. This was concentrated and fortified with a syringe standard keeper containing ^{13}C surrogate compounds (PCB-77, PCB-202 and PBDE-139), which was used to calculate analytical recovery for quality control purposes.

Figure 2. Recovery of 13C PBDE Internal standards (n=15)



Analysis:
Analysis of PCDD/F, n-PCBs and PBDE fractions was carried out using HRGC-HRMS (Autospec Ultima, Micromass) as described elsewhere[4], o-PCBs were analysed by HRGC-LRMS[7]. Separation was carried out using a DB5-MS 60 m x 0.25 mm i.d., 0.25 µm film. Programmed temperature volatilization (Gerstel CIS-4) was used to increase injection volumes and to reduce PBDE degradation. Instrumental linearity was demonstrated for 18 PBDEs (di to hepta brominated) in the range 0.75-3750 pg on column. R^2 values ranged from 0.997-1.000. Analyte identification and

quantitation acceptance criteria followed were the same used for dioxin analysis[8].

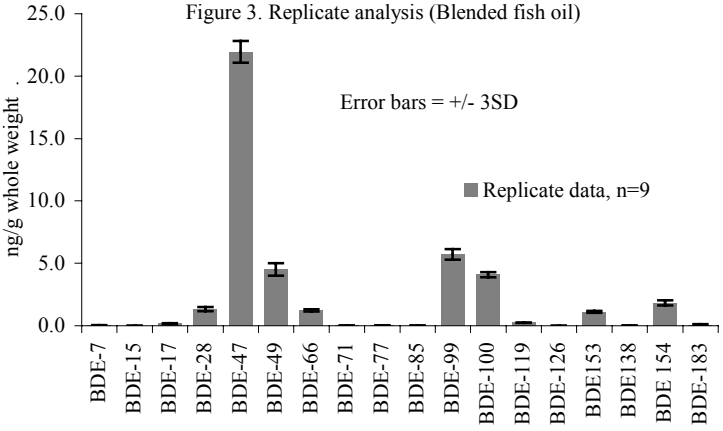


Figure 3. Replicate analysis (Blended fish oil)

Results and Discussion

The method was applied to the analysis of a variety of food stuffs and biological tissues. Repeatability and reproducibility of in-house reference material (fish-oil homogenate) results was good (Figure 3) with acceptable recoveries (Figure 2). Repeated analyses of certified reference materials (CRMs) using the extended method incorporating PBDEs gave results in agreement with published consensus values for n-PCBs, o-PCBs and PCDD/Fs. Unfortunately there was no CRM available at present for PBDEs. IRMM-445 Pork Fat reference material (BCR) quotes an indicative PBDE concentration for BDE-47 as 3.9 ng/g. Replicate analyses of this material over a period of several months produced results in agreement with the published indicative value (Table1), in addition to values in agreement with consensus values for PCBs for this material.

Table 1. Analysis of IRMM-445 Pork Fat

Extract #	A	B	C	D	Mean	St Dev	CV (%)
BDE-47	4.28	4.02	3.89	3.89	**4.021**	0.18	4.6
BDE-99	0.53	0.43	0.21	0.29	**0.367**	0.14	38.2
BDE-100	0.18	0.14	0.05	0.14	**0.123**	0.06	48.3

As further validation of the methodology, samples of various foodstuffs (vegetable oil, milk, egg, lamb, fish, fish oil & animal feed) were fortified with unlabelled (native) PBDEs at concentrations ranging from 0.05-50 ppb and analysed in duplicate. Corrected, measured results for these sample matrices agreed to within ±10% of the theoretical fortified concentration across the range, giving further confidence in this approach.

A working limit of determination was derived from blank determinations over a period of months and is given as LOD = 0.05 µg/kg lipid except BDE 183 = 0.1 µg/kg lipid. In all charts, levels <LOD are recorded as LOD, i.e. upper bound.

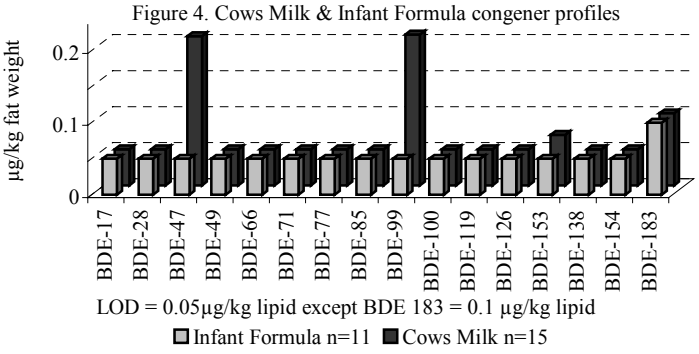
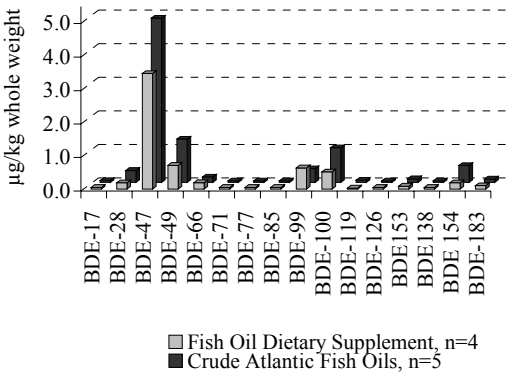


Figure 4. Cows Milk & Infant Formula congener profiles

The method was used to analyse a variety of samples including; cows milk, infant formula, fish oils and dietary supplement fish oils. Whilst this data is far from comprehensive for each sample type, it does serve to provide useful indicative congener profiles (Figures 4-5). Interestingly, congener profiles in a range of biota were, in general, found to have similarities but not exactness to the profiles of certain polybrominated diphenyl ether

commercial technical products such as Bromkal 70-5DE[9].

Figure 5. Fish Oil congener profiles



Conclusions

The incorporation of PBDEs into the existing method[4] for PCDD/Fs and PCBs does not decrease the efficacy of the analytical procedure for PCBs and PCDD/Fs, neither does it provide any significant interferences for any of the analytes of interest.

The method is applicable to a wide range of foodstuffs, as indicated by a series of validation experiments. The method was shown to be robust, reproducible, repeatable and precise for fortified, in house as well as reference material. Material with endogenous contamination gives the best estimation of extraction performance. Furthermore, the provision of a food based CRM for the analysis of PBDEs, or indeed the inclusion of PBDE consensus values for established PCB and PCDD/F CRMs, would help to confirm the accuracy of the method for PBDEs.

Further work is underway to establish human, wildlife and environmental exposure to PBDEs, and will be reported in due course.

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