APPLYING PRESSURIZED LIQUID EXTRACTION (PLE) AND AUTOMATED CLEANUP (Power Prep) FOR PBDD/F ANALYSIS IN SOIL

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Introduction

During the last decade brominated dioxins and furans (PBDD/Fs) have been encountered in several matrices, including environmental [1], biological [2] and human samples[3]. PBDD/Fs are structurally similar to the chlorinated homologues but since the bromine atom is much larger than the chlorine atom, the PBDD/Fs molecules[4] are heavier and less polar than the PCDD/Fs and extraction and clean up procedures should be evaluated and adapted to better suit the properties of PBDD/Fs. Furthermore, the analysis of PBDD/Fs is more difficult than PCDD/F analysis since PBDD/Fs can thermally degrade at the injector or in the column. Analyzing PBDD/Fs with increasing numbers of substituted bromines and thereby decreasing polarity is obstructed by on-column degradation and graver interaction with the column phase which yields broader peak shapes, worse chromatography and lower sensitivity.

In this study, a working procedure for PCDD/F analysis using the pressurized liquid extraction (PLE) instrument in combination with the automated clean-up system Power Prep, both from FMS Inc. (Waltham, MA, USA), was evaluated for PBDD/F analysis. Initially the performance of the systems were evaluated on a spiked sample composed of a neutral matrix, sodium disulphate, and later the applicability of the procedure was tested on a real soil sample. Toluene and hexane were tested as extraction solvents. Final extracts were analyzed by gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) by using on-column injection. The presence of PBDEs was monitored to verify that no false PBDF contribution resulted from thermal degradation of PBDEs.

Materials and Methods

Samples and sample preparation

Spiked samples were prepared simply by adding approximately 20 g of disodium sulphate (Merck, Darmstadt, Germany) to the half height of the stainless steel extraction cylinder. A mixture of native and a mixture of mass labeled PBDD/Fs (Wellington Laboratories Inc., Guelph, Canada and Cambridge Isotope Laboratories Inc., Andover, MA, USA) were applied onto the disodium sulphate and were left to evaporate for one hour, before an additional amount of approximately 20 g of sodium sulphate was added to the top of the extraction cell. Soil samples were prepared by filling the bottom of the extraction cells with 10 g of disodium sulphate. Approximately 5 g of dry soil was added and spiked with the mixture of mass labeled PBDD/Fs. The sample was left to dry for one hour, before filling the residual dead volume of the cell with disodium sulphate. In all, two sets of duplicates of spiked samples and two sets of duplicate soil samples were extracted, together with one blank sample for each set of samples (4 blanks). The sediment sample originated from open burning of electronic waste and was part of the 10th round of the international intercalibration study performed during 2005 [5].

Extraction

Prior to extraction a washing method was run to clean the system with the organic solvents that were to be used during extraction. During the wash cycle the pressure later used during extraction was set to 1900 psi. After mounting the extraction cells, the extraction begun by filling the extraction cells with solvent followed by a 1.5 minute long sequence of pressurizing the cell (up to 1900 psi). Then the temperature of the cell was increased to

160°C during a period of 2 min., followed by a final increase to 180°C. The temperature was held at 180°C and the pressure at 1900 psi for 15 minutes before the cells were left to cool for 15 minutes. After cooling the pressure was dropped. This procedure was repeated three times and after the last flush of solvent the extraction cells were purged with nitrogen for 2 minutes. In all, an extract of approximately 75 ml were obtained after three extraction cycles. The extraction efficiency was evaluated for two solvents, toluene and hexane.

Clean up

The hexane extracts were evaporated to 1-5 ml of solvent before injecting them on the Power Prep system. The toluene extracts were evaporated to dryness and then diluted in hexane prior to injection. About 5 ml of concentrated sample was injected, followed by rinsing the sample glass container with 10 ml of hexane. The Power prep was equipped with a series of the following columns; silica column, aluminum oxide (AlOx) column and carbon column. The silica column was eluted with hexane (Hx), the aluminum column were first rinsed with 2% dichloromethane (DCM)/ 98% Hx to extract the non-polar fraction which went directly to the waste cylinder. To obtain the planar fraction the aluminum column was rinsed with a 50% DCM/50% Hx solution. Finally the carbon column was eluted slowly (in the reverse way) with toluene to obtain the final extract in a glass flask already containing 25 µl of tetradecane. The toluene extract was evaporated until the small aliquot of tetradecane was remaining. The sample was transferred to a amber glass auto sampler vial in which the recovery standard (^{13}C-labeled 2,3,7,8-TeBDD) was added. The extracts and standards were stored in -18°C until HRGC/HRMS analysis. Throughout the whole sampling and sample preparation the samples was shielded from UV light to avoid photo degradation.

Instrumental analysis

HRGC/HRMS analysis was performed on a Micromass Ultima operating at >10 000 – 12 000 resolution using EI ionization at 35 eV. All measurements were achieved in selective ion recording (SIR) mode, monitoring the two most abundant ions in the bromine cluster. PBDD/Fs were analyzed by injecting 1 µl of the final extract using the cool on-column technique. The temperature during injection was equal to the set temperature for the oven (i.e. 120°C), but after 0.3 min the injector temperature was ramped to 300°C for 15 minutes. This procedure enhanced repeatability, peak shapes and thereby chromatography. A 25 m BP1 (0.1 µm, 0.25 mm) column (SGE, Australia) coupled to a 3 m fused silica guard column (intermediate polarity) from Supelco (Bellafonte, PA, USA) was used for all PBDD/F analysis. Prior to analysis the guard column was cut by 15- 20 cm to ensure good chromatography. The GC temperature program started at 120°C for 2 min and then the temperature was increased by 20°C/min to 260°C, thereafter to 300°C by 10°C/min. The temperature was held at 300°C for 13 minutes and then increased with 20°C/min to 320°C where it was held for 3 minutes.

Mono- to octa PBDD/Fs were analyzed in all samples. Peaks were identified against ion ratio and the retention times of the congeners presented in Table 1 and 2. The mass labeled mixture used in this study comprise of ^{13}C-labelled: 2,3,7,8-TeBDF, 1,2,3,7,8-, 2,3,4,7,8-PeBDF, 1,2,3,7,8-PeBDD and 1,2,3,4,7,8-HxBDF (Cambridge Isotope Laboratories Inc., Andover, MA, USA). In those cases where sample peaks did not have a match in the standards the identity was primarily based on ion ratios. To fully manifest the PBDF identity of peaks with correct ion ration but lacking a standard match, the following signals were monitored; [PBDF-COBr], [PBDE + 1Br] and [PBDE+2Br]. The fragment obtained when the COBr group leaves the PBDF molecules is formed by EI ionization of PBDFs but not for PBDEs. The other two signals are to make sure that the observed PBDFs are not resulting from thermal degradation of PBDEs (of higher substitution levels of bromine) at the injector or in the column.

Results and Discussion

Initially, a repeatability test was performed where seven injections from the same standard were evaluated. The calculations of recovered concentration of PBDD/Fs were based on the first and last injection of the seven injection mentioned above. The mean value obtained for mono to hepta PBDD/Fs was equal to what was present in the standard but the RSDs (n=5) were above 15% for mono PBDD/Fs and hepta BDF. The OcBDF had a RSD of 50% and the calculated concentration was overestimated to 16 000 pg (correct concentration was 12 500 pg) showing the difficulties with using internal standards of a different degree of bromination (^{13}C-hexaBDF), also showed and discussed by Takahashi et al., 2006 [6].

Table 1. Recovered amounts (pg) of PBDD/Fs in spiked samples.

	Spiked samples		
	Spiked amount (pg)	Hexane fraction	Toluene fraction
Congeners		Rec: 81-101%	Rec: 71-91%
PBDFs			
4-MoBDF	250	44	55
2,7-&2,8-DiBDF	250	160	206
2,3,8-TriBDF	250	190	195
1,2,7,8-TeBDF	250	215	209
1,3,4,7,8-PeBDF	2500	2530	2480
1,2,3,7,8-PeBDF	2500	2580	2480
1,2,3,4,6,7,8-HpBDF	12500	8370	9190
OcBDF	12500	830	1770
PBDDs			
1-MoBDD	250	38	53
2,7-&2,8-DiBDD	250	110	150
2,3,7-TriBDD	250	230	235
1,3,6,8-TeBDD	250	210	225
1,3,7,9-TeBDD	250	230	240
1,3,7,8-TeBDD	250	270	260
1,2,3,4-TeBDD	250	280	280
2,3,7,8-TeBDD	250	240	210
1,2,3,7,8-PeBDD	2500	2660	2540

Spiked samples

The extraction and clean up procedure was not adapted to the lower and the highest brominated (mono to di and hepta to octa) dioxins and furans, see Table 1. For mo-BDD/Fs and octa-BDF congeners, only about 20% of the added amount was recovered. For the case of 2,7-/2,8-DiBDD/Fs and 1,2,3,4,6,7,8-HpPBDF the recovered amounts were 65% and 67%, respectively. For the tri- to penta-substituted congeners more than 90% of the added amounts were recovered in most of the cases. On the other hand, the recovered amounts for OcBDF were very poor. Therefore, in order to enhance the recovered amounts of hepta- and octasubstituted congeners likely the AlOx and carbon column should be eluted by larger volumes of solvent. There were only small differences in the results from extraction with toluene or hexane when using a neutral matrix such as sodium disulphate.

Soil samples

PBDD/Fs of every substitution level were present in the soil sample (Table 2). Hexane was primarily investigated since samples are to exist in hexane when applying them onto the Power Prep system. However, it was found to be insufficient as extraction solvent for PBDD/Fs in soil since the extracted amounts of native PBDD/Fs were significantly lower than in the toluene extracts. On the contrary, the recoveries for the labeled congeners were similar in both cases, between 60-102% in the toluene extracts and 53-92% in the hexane extracts. The mean levels of PBDD/Fs from the two toluene extractions showed good accordance with the results from the 10th intercalibration study (see Table 2). The lower value found for 1,2,3,4,6,7,8-HpBDF compared to the reported value from the intercalibration study are possibly an effect of a too small eluting volume of solvent on the AlOx and/or carbon columns. PBDD/Fs were present in all blanks indicating that the washing procedure of primarily the PLE system was not sufficient. However, the PBDD/F levels in all blanks were below 5% for all congeners measured in this study.

Table 2. Obtained concentrations (ng/g) after extraction and clean up.

	Soil samples		
	Mean value intercal. study 2005 (ng/g) [5]	Hexane fraction (ng/g)	Toluene fraction (ng/g)
Congeners		Rec:53-92%	Rec:60-102%
PBDFs			
4-MoBDF		0.80	2.1
2,7-&2,8-DiBDF		0.10	0.30
2,3,8-TriBDF		2.4	4.9
2,3,7,8-TeBDF	0.66	0.30	0.64
1,2,3,7,8-PeBDF	0.42	0.40	0.43
2,3,4,7,8-PeBDF	0.40	0.40	0.49
1,2,3,4,6,7,8-HpBDF	8.15	2.4	5.1
OcBDF		0.20	0.44
PBDDs			
1-MoBDD		0.05	0.19
2,7-&2,8-DiBDD		0.04	0.11
2,3,7-TriBDD		0.03	0.07
1,3,6,8-TeBDD		0.04	0.09
1,3,7,9-TeBDD		0.09	0.22
1,3,7,8-TeBDD		0.02	0.04
1,2,3,4-TeBDD		0.16	0.24
2,3,7,8-TeBDD	0.02	0.02	0.02
1,2,3,7,8-PeBDD	0.03*	0.03	0.05

*Value after removal of one outlier

Conclusions

The applied method worked well for tri- to hexa-substituted PBDD/Fs when using toluene as extraction solvent. The established concentrations when using toluene as extraction solvent were close to what was reported in the 10th intercalibration study confirming the applicability of the combination of PLE and Power Prep for PBDD/F analysis in a soil sample. However, the methodology needs to be improved for hepta- and octa PBDD/Fs. Possibly this is achieved by increasing the volume of elution solvent for both the AlOx and carbon columns.

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