Determination of PBDEs in human adipose tissue by large volume injection narrow bore (0.1 mm id) capillary gas chromatography-electron impact low resolution mass spectrometry

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Summary. Human adipose tissue (n=20) from Belgium was analysed for PBDEs using large volume injection narrow-bore (0.1 mm id) capillary gas chromatography-electron impact low resolution mass spectrometry. Concentrations of PBDEs (sum of BDE 28, 47, 99, 100 and 153) range between 1.7 and 10.1 ng/g fat and are similar to reported values from Spain and Sweden.

Introduction. Due to their persistance and bioaccumulation potential, polybrominated diphenyl ethers (PBDEs) are found in the environment and in humans[1]. They have been measured in adipose tissue samples from Spain[2], Sweden[3] and Finland[4]. Because concentrations of PBDEs in humans are in the order of ng/g lipid weight, most of the analytical work has been carried out by highly sensitive systems as gas chromatography-high resolution mass spectrometry[5] (GC/HRMS) or gas chromatography-negative chemical ionization low resolution mass spectrometry[6] (GC/NCI-LRMS).

Electron impact low resolution mass spectrometry (EI-LRMS) was used mostly for the determination of PBDEs in samples with relatively high concentrations[7], but its use for humans was limited due to lower sensitivity. Here we show that a combination of large volume injection (LVI), narrow-bore (0.1 mm id) capillary column and EI-LRMS can be used for the determination of PBDEs in human adipose tissue. This is the first report on PBDE levels in Belgian population.

Methods and instrumentation. Human adipose samples (n=20) were obtained by autopsy from the Universitary Hospital of Antwerp, Belgium. One gram of each sample was accurately weighted and mixed with anhydrous Na_2SO_4 till a fine floating powder was obtained. After addition of 100 µl of internal standards (13.06 pg/µl in iso-octane from a mixture of ^{13}C-BDE 47, 99 and 153), the powder was extracted by automated hot Soxhlet for

2 hours with 75 ml of hexane : acetone : dichlormethane = 3:1:1 (v/v). After concentration and determination of lipid content, the extract was subjected to clean-up on 2 successive solid phase cartridges containing acid silica and acid silica : neutral silica : deactivated basic alumina (from top to bottom), respectively. PBDEs (and PCBs) were eluted with 50 ml hexane. The eluate was concentrated to almost dryness and 100 µl of the recovery standard (bromobiphenyl (PBB) 80 – 18pg/µl in iso-octane) was added, after which it was reconcentrated to approximately 60 µl.

A Hewlett Packard 6890 GC was connected via a direct interface with a HP 5973 mass spectrometer. A 10m x 0.10mm x 0.10µm AT-5 (5% phenyl polydimethyl siloxane) capillary column (Alltech) was used with helium as carrier gas at constant flow of 0.4 ml/min. Twenty µl (4x5 µl) were injected in a Gerstel (CIS 4) PTV in solvent vent mode (vent flow 100 ml/min for 1 min, injector at 70°C for 1.1 min and then heated with 700°C/min to 270°C) with the split outlet opened after 2.1 min. The interface temperature was set at 300°C. The temperature program of the AT-5 column was 2.2 min at 70°C, 40°C/min to 230°C, 25°C/min to 280°C, 5 min at 280°C. The LRMS was operated at 70 eV in selected ion monitoring (SIM) mode. Dwell times were set at 10 msec. Two most abundant ions were monitored for each level of bromination for native and labeled PBDE. Retention times, ion chromatograms and ratio of the monitored ions were used as identification criteria. A deviation of ion ratios of less than ±20% from the theoretical value was considered acceptable.

Results and discussion. The targeted compounds eluted from the GC column between 6.1 and 9.2 min. The short retention times are due to the use of a narrow-bore capillary column (id=0.1mm), which offers the resolution power of conventional column (id=0.25mm), but decreases the analysis time with more than 50% (Figure 1). Furthermore, the smaller id results in a smaller peak width and an increased mass sensitivity (higher S/N ratios for the same amount injected). Thus, for human samples with relatively high concentrations of PBDEs (adipose tissue), it is possible to use EI-LRMS with sufficient accuracy. Moreover, with the introduction of extract volumes up to 20 µl, low detection limits (DL) can be achieved (Table 1). A good linearity (r^2>0.995) was achieved for each compounds between 2 x DL (calculated for a S/N=3) and 10 ng/g fat. Recoveries of internal standards, ^{13}C-labeled BDEs (calculated based on PBB 80 added prior to injection) were between 81 and 103% with a standard deviation of less than 21% (Table 1). Analysis of two samples of biota (eel and porpoise liver) used for the first interlaboratory test on PBDE[8], showed a variation of 10-15% from mean values.

Concentrations of PBDEs in Belgian adipose tissue were ranging between 1.70 and 10.13 ng/g fat. This is in concordance with concentrations reported in other countries[2-4]. Interestingly, BDE 47 was not always the most abundant congener. Higher values of BDE 153 were obtained in some samples. Blanks were run to check for interferences, but no significant contribution to these high values was observed. Similar trends were seen in some samples (n=13) from Spain[2] (Table1). Following the addition of appropriate internal standards, the extraction and clean-up procedure allowed also the determination of PCBs and DDTs on the same sample aliquot. Low correlation coefficients were obtained between PBDEs and PCBs (r^2=0,34) or PBDE and age (r^2=0.07). Meneses[2] has observed in 13 samples that the highest as well as the lowest levels of PBDEs correspond to elder men, while for other persistent organohalogenated contaminants (PCBs, DDTs) older persons were found to have higher levels due to bioaccumulation and long-half lives of the compounds.

Table 1. Acquisition parameters and concentrations of targeted compounds in Belgian human adipose tissue samples (n=20).

Target compounds	RT (min)	Ions	Recovery (%)	DL (ng/g fat)	Mean ± SD (ng/g fat)	Range (ng/g fat)	Spain[2] (ng/g fat)
BDE 28	6.18	406, 408		0.05	0.11 ± 0.07	nd – 0.26	
^{13}C-BDE 47*	7.03	496, 498	81 ± 12				
BDE 47	7.03	484, 486		0.1	1.45 ± 1.01	0.54 – 4.71	1.36
^{13}C-BDE 99*	7.94	576, 578	84 ± 17				

BDE 99	7.95	564, 566		0.15	0.29 ± 0.38	nd – 1.61	0.42
BDE 100	7.72	564, 566		0.15	0.48 ± 0.34	nd – 1.50	0.51
^{13}C-BDE 153*	9.16	496, 498	103 ± 21				
BDE 154	8.92	484, 486		0.25	nd	nd	nr
BDE 153	9.17	484, 486		0.25	1.47 ± 0.70	nd – 2.79	1.83
Sum BDE					3.73 ± 2.11	1.70 – 10.13	
Sum PCBs					830.8 ± 428.5	263.4 – 1757.4	

nd-not detected, nr-not recorded

When compared with HRMS, EI-LRMS are instruments easy to maintain and operate. It also allows the use of 13C-labeled standards as internal standards (this procedure is not possible when using NCI-LRMS). Moreover, the higher selectivity of EI is important compared to NCI where often only Br ions can be measured. However, the response factors in EI are very different for congeners with different degree of bromination. Thus, detection of highly brominated congeners is problematic in EI-LRMS, due to low concentrations to be measured and poor sensitivity. It is possible to tune manually the MS to obtain increased sensitivity for higher masses. The problem can be overcome by using large volume injection which allows the introduction of a larger amount of extract, thus increased sensitivity. However, the clean-up procedure should be very efficient as interferences may easily disturb the chromatogram.

Conclusions. GC/EI-LRMS in combination with LVI and narrow bore capillary column was found suitable for the determination of major PBDE congeners in 20 human adipose tissue samples from Belgium and may serve as an alternative for GC/NCI-MS and GC/HRMS.

References

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Figure 1. Selected ion chromatograms of a human adipose tissue extract: ^{13}C-labeled BDEs (chromatogram A) and target PBDEs (chromatogram B).