FOOD SAMPLES CLEAN-UP USING SEMIPERMEABLE POLYETHYLENE MEMBRANES (SPM) FOR THE DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS

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1. Abstract

Dialysis with semipermeable membranes (SPMs) made of low density polyethylene is an efficient lipid removal method in the determination of bioaccumulative, persistent, halogenated organic compounds. In this study the use of SPMs for the preconcentration of polybrominated diphenyl ethers in food samples is described. Chocolate, butter, chicken egg, pork and salmon fat samples were dialyzed to investigate percentage lipid carry-over values (%LC). For the determination of recovery values, 6 natural PBDE internal standards were used for the fortification of chocolate and butter samples. Finally, SPM sample clean-up was used for the determination of PBDEs in pork fat samples.

2. Introduction

Flame retardants are chemicals that are added to polymers which are used in plastics, textiles, electronic circuitry and other materials to slow down the burning process and prevent fires. Some of the technical flame retardant products contain brominated organic compounds. The most used brominated flame retardants (BFRs) are polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A) and polybrominated biphenyls (PBBs)[1].

The commercial PBDEs products predominantly consist of penta-, octa-and decabromodiphenyl ether products. At present, decabromodiphenyl ether is the most used PBDEs product. Polybrominated diphenyl ethers (PBDEs) are additive to wide range materials at concentrations up to 30% by weight[2]. The major uses are in high impact polystyrene, flexible polyurethane foam, textile coatings, cable insulation, electrical and electronic equipment[3].

Polybrominated diphenyl ethers are nonreactive flame retardants, so are not incorporated into the polymeric materials by bonding between the polymer and the flame retardant. Therefore, they may separate from the surface of their product applications into the environment[4].

Polybrominated diphenyl ethers are persistent, lipophilic and bioaccumulating chemicals. They are toxic for human and animals [5].

In the analysis of PBDEs in food samples an important role plays sample preparation. Food samples require highly efficient methods of purification before the final analysis. Dialysis with semipermeable membranes in an organic solvent leads to separation of organohalogen contaminants such as PBDEs from lipids.

SPM is a nondestructive method of separation, used for the preconcentration of organic chemicals in animal or plant fat samples. Membranes are made of low density polyethylene foil, which have pore sizes of about 1 nm. This make the restriction for dialysis of molecules bigger than this size[6]. The membrane is formed like a tube, sealed in one of its end. The polyethylene foil thickness is about 80 μm[7].

3. Experiment

3a. Materials, reagents and apparatus

SPM membranes were purchased from Exposmeter, Sweden.

Certified PBDE standard solutions of 5 μg/ml concentrations were purchased from Wellington Laboratories (Ontario, Canada).

Solvents, adsorbents and reagents were purchased from POCH, Gliwice, Poland.
Acidic silica is prepared by mixing 20% of the total mass of H_2SO_4 with appropriate mass of silicagel to obtain a uniform powder which is placed in a 10mm of inside diameter glass column.

Varian CP3800 gas chromatograph equipped with electron capture detection (GC/ECD) was used. A CP-Sil5 CB capillary column (30m x 0.32mm i.d., 0.25 µm film thickness) was employed for the chromatographic separations. Split-less injection were used and the split was opened 1 min after injection. The column oven temperature was programmed as follows:120˚C(1min), 10˚C/min up to160˚C, 20˚C/min up to 250˚C, 10˚C/min up to 300˚C (5min).

3b. Procedure:

To investigate lipid carry-over (%LC), butter, chocolate, chicken egg, salmon and pork lipid samples were dialyzed. 1g of animal or plant fat was dissolved in 15 ml of 10% (v/v) of methylene chloride in hexane solvent. Before the use, the empty membrane was pre-washed by placing for 72 hours in a bottle, filled with n-hexane. Then, the fat solution was transferred into the membrane tube using a Pasteur pipette. Membrane after sealing the top with glass stopper was placed in a bottle containing 80 ml of n-hexane for dialysis. The food sample fat solutions were dialyzed for 24 hours. After this time dialysate was transfered to round bottom flask and concentrated to 1 ml in a rotary evaporator. Finally, dialyzate was evaporated to dryness in a stream of inert gas in a small conical flask and the residue was weighted.

For the analyte recovery control, the chocolate and butter fat solutions were fortified with addition of 2,5 ng of each of selected 6 PBDEs: 2,4,4'-TriBDE (28), 2,2',4,4'-TeBDE (47), 2,2',4,4',5-PeBDE (99), 2,2',4,4',5,5'-HxBDE (153), 2,2',4,4',5,6'-HxBDE (154), 2,2',3,4,4',5',6-HpBDE (183). Those compounds were not present in the investigated samples. Membranes were prepared and used as it is described as follows: 1g of fat samples were dissolved in 15 ml of 10% v/v dichloromethane in hexane and were quantitatively transferred to SPM membrane. The membrane was sealed and placed in a bottle filled-up with 80 ml of n-hexane. The dialyzate was cleaned-up using a combination of two columns filled-up with: first column: 5g of acidic silicagel and the second column: 5g of basic Alumina, activated overnight in 200^0C. Finally, to the eluate form Alumina 50 µl of dodecane was introduced as a keeper and the solvent excess was evaporated in an inert gas stream.

This method was used for the determination of PBDEs in pork fat samples. The pork samples was prepered in the same way like chocolate and butter fat samples.

4. Results and Discussion

SPM clean-up method for animal fat samples was used to investigate lipid carry-over (%LC). Butter, chocolate, chicken egg, salmon and pork fat samples were dialyzed. The results (%LC) obtained for animal or plant fat were listed in Table 1.

Table1. Percentage lipid carry-over (%LC) for 1g of animal fat samples using SPM method

FOOD SAMPLE	FAT MASS BREAKTHROUGH [g]	% LC	AVERAGE %LC
BUTTER	0.0162	1.31%	**1.10%±0.3%**
CHOCOLATE	0.0079	0.88%	
CHICKEN EGG	0.0120	1.15%	
SALMON	0.0102	0.83%	
PORK	0.0170	1.35%	

Mass of lipid after dialysis is so small that we can use SPMs for efficient method lipid removal and further clean up may be effective when performed on acidic silica gel.

In this study was used recovery standard contains six congeners PBDEs. These congeners were found in highest level in environmental samples. To investigate value recovery of the internal standard used chocolate and butter fat. The values recovery standard were similar for two type of lipid. The recovery standard range was between 51 - 70%.

The results were listed in Table 2. Conducted studies indicated that food samples can be dialysed with acceptable recoveries of PBDEs internal standards.

Table2. Recovery of PBDE standards in butter and chocolate fat samples

CONGENER	RECOVERY OF PBDEs STANDARDS	
	BUTTER FAT	CHOCOLATE FAT
2,4,4'-TriBDE (28)	64%	70%
2,2',4,4'-TeBDE (47)	66%	70%
2,2',4,4',5-PeBDE (99)	67%	66%
2,2',4,4',5,5'-HxBDE (153)	64%	54%
2,2',4,4',5,6'-HxBDE (154)	69%	62%
2,2',3,4,4',5',6-HpBDE (183)	51%	57%

Among the PBDEs congeners determined in pork samples, BDE-47, BDE-99 and BDE-100 were in approximately 90% of the total PBDEs mass. In pork samples investigated in this study the total level of the three PBDEs congeners were completly dominated by BDE-47. The total PBDEs mass concentrations in pork samples were calculated at a 35 pg/g of fat level.

5. Conclusions

SPMs method can be used for efficient and not expensive animal fat samples clean-up. The method is nondestructive and varied fat samples can be dialysed with acceptable recoveries of PBDEs internal standards. SPM sample clean-up can be used in the determination of the other environmental persistent pollutants such as pesticedes, polychlorinated biphenyls(PCBs) and polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs).

6. References

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