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Analytical Methods

Using a membrane technique (SPM) for high fat food sample preparation in the determination of chlorinated persistent organic pollutants by a GC/ECD method

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ABSTRACT

This paper focuses upon the use of semipermeable membranes (SPM) as a clean-up method for the determination of 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, Aldrin, Dieldrin, Isodrin, Lindane (γ-HCH), 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TCB), Pentachlorobenzene (PeCBz), Hexachlorobenzene (HCB), and Hexachlorobutadiene (HCBD) in high fat food samples. Pork fat, beef fat, butter, egg yolks and chocolate were all used as high fat food samples. The procedure consists of three steps: the first is dialysis in an SPM tube, using *n*-hexane as an external solvent. The second step is a clean-up procedure using a silica gel column, and the third step is GC/ECD analysis. This experiment shows that recovery values obtained for individual compounds were in the range of 55–100%. The conclusion drawn is that the SPM technique is an efficient method of preparation of high fat food samples for the determination of POPs by GC/ECD methods.

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1. Introduction

Persistent Organic Pollutants (POPs) are toxic chemical compounds of which the most recognised adverse biological activity is endocrine disruption. They persist in the environment over extended time periods and biomagnify as they move up through the food chain (Tanabe, 2004). Their persistence in human body is due to their strong lipophilic character, causing accumulation over time in living, especially fatty, tissue. They are resistant to degradation, and their persistence in various mobile media facilitates their transport over potentially long distances to remote geographical regions where they have never been a feature of the local environment (Jones & de Voogt, 1999). The manufacture and consumption of POPs are controlled by the directives of the Stockholm Convention which was passed on the 23rd of May 2001. This convention regulates twelve of the most hazardous organic contaminants, including nine pesticides (Kretchik, 2002).

Many of the papers describe the determination of POPs in various matrices using gas chromatographic methods. Pesticides have been determined in plant materials by Niessner, Buchberger, and Eckerstorfer (1999) and Lino and Noronha da Silveira (1997), who used Solid Phase Ectraction (SPE) and FLORISIL® for sample clean-up. Gançalves and Alpendurada (2002) determined four groups of pesticides in soil and drinking water using Solid Phase Microectraction. Therdteppitak and Yammeng (2003) determined organochlorine pesticides by gas chromatography in fish (also a high fat content samples), and used a column with FLORISIL® as a clean-up method. FLORISIL® was also employed by Rekha, Naik, and Prasad (2006) for determination of pesticides in flour and rice. Contents of chloroorganic pesticides in fish were determined by Falandysz et al. (2004) who did not use any method of sample clean-up; instead, they made only a sample extraction by *n*-hexane. Until now little work has been done in the application of SPM as a clean-up method in the determination of POPs in high fat food samples by gas chromatography. Bergqvist, Strandberg, and Rappe (1999) have undertaken research on lipid removal during the analyses of PCDDs, PCDFs and PCBs (Bergqvist, Strandberg, Bergek, & Rappe, 1993) in environmental samples, e.g. animal food samples, using a Semipermeable Membrane technique.

The Semipermeable Membranes (Huckins, Tubergen, & Manuweera, 1990) are layflat polyethylene tubing of 80 μm thickness and 26 mm width. Polymeric films are commonly referred to as nonporous, or dense, despite the presence of transient cavities with diameters up to about 1 nm being formed by random thermal motions of polymer chains in them. These cavities permit the diffusional-jump

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transfer of organic contaminants dependent on the concentration gradient (Huckins, Manuweera, Petty, Mackay, & Lebo, 1993).

The use of the SPM technique for sample clean-up is based on dialysis rules, i.e. analyte diffusion from the extract introduced inside the membrane to the solvent placed in outer side of the membrane (external solvent). Molecules of analytes which are smaller in size move from the matrix to the external solvent through transient cavities of diameters up to 1 nm, however, the fats and the other larger sized molecules of matrix components remain inside the membrane. An importantly feature of this method is that it allows a non-destructive preparation of the sample. This means that the SPM may be effective as a sample clean-up method even for highly reactive compounds or compounds which are not chemically resistant. It also means that some of non chemically resistant compounds in the sample may be determined as well. This may be of greater importance in cases where the total mass of the sample available for the analysis is relatively low.

In this paper a method of high fat food sample preparation for POPs determination using the SPM clean-up technique is reported, and the results of POPs determination in real samples of chocolate, butter, egg yolk, pork and beef fats are presented.

2. Experimental

2.1. Reagents and materials

Anhydrous sodium sulphate (J.T. Baker, Deventer, The Netherlands), *n*-hexane and dichloromethane (POCh, Gliwice, Poland) used were of high purity and free from all traces of pesticides.

Silica gel (70–230 mesh ASTM, Merck, Warszawa, Poland) was heated at 200 °C in dish drainer for 24 h and cooled in a desiccator. Elution solvents:*n*-hexane, dichloromethane (4:1) and *n*-hexane, dichloromethane (3:17) were prepared daily.

Semipermeable Membranes (SPM – Exposmeter, Sweden) were conditioned for 48 h in *n*-hexane before they were used.

2.1.1. Stock standard solution

The stock standard solution was prepared by weighing and dilution of the 14 POPs individual compounds in *n*-hexane obtaining the concentration of 1 mg ml^{-1} of each compound: 1,2,4,5-tetrachlorobenzene (1,2,4,5-TCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TCB), pentachlorobenzene (PeCBz) of 98% purity (Aldrich); 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,3-trichlorobenzene (1,2,3-TCB), Isodrin, DDT, DDE, DDD of 98.3–99.0% purity (Pestanal); hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), Lindane (γ -HCH), Dieldrin, Aldrin of 98.2–99.9% purity (Supelco, USA) and stored at 4 °C.

2.1.2. Fortifying standard solution

A fortifying standard solution at a concentration of 100 ng ml^{-1} was prepared by dilution of the stock solution (see Section 2.1.1).

2.1.3. Internal standard solution

The tribromobenzene (TBB) used as an internal standard was of laboratory grade.[3] The internal standard solution (1 mg ml^{-1}) was prepared by weighing and dilution of TBB in *n*-hexane. The concentration of 100 ng ml^{-1} was prepared by dilution of the internal standard solution and stored at 4 °C.

2.1.4. Precision and recovery standard solution

1,4-dichlorobenzene (1 mg ml^{-1}) was used as a precision and recovery standard, and was prepared by weighing and dilution of 1,4-dichlorobenzene in *n*-hexane. The concentration of 100 ng ml^{-1} was prepared by dilution of the standard and stored at 4 °C.

2.1.5. Calibration standard solution

Fourteen POPs solutions in hexane (0.2, 0.5, 1, 2, 5, 10, 50 ng ml^{-1} for each compound) used for apparatus calibration were prepared by dilution of the stock solution (see Section 2.1.1).

2.2. High fat food samples

High fat food samples: chocolate, butter, egg yolks, pork and beef fats, were used in the experiments. After extraction and dissolution with *n*-hexane and dichloromethane (DCM) the extracts were stored at 4° C.

2.3. Equipment

2.3.1. Gas chromatographs

Gas chromatographic separation of 14 POPs was conducted using a gas chromatograph (VARIAN CP-3800) with electron capture detection (ECD) system equipped with CP-Sil 5 CB column (Supelco) (30 m × 0.32 mm × 0.25 μm). The injector temperature was 250° C and the following temperature program was applied: 60° C (1 min) – 3° C min^{-1} –110° C – 5° C min^{-1} – 210° C. Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. ECD conditions: temperature 300° C and make-up gas 60 ml min^{-1}. Sample (1 μl) was split injected.

A chromatographic separation of real fat content food samples was also conducted by application of a gas chromatograph (CE Trace 2000) coupled to a Finnigan GCQ Plus GC–MS/MS detector. A ZB 5 MS column (ZEBRON) was applied (60 m × 0.25 mm × 0.25 μm). The following temperature program was used: 80° C (3 min) – 15° C/min – 280° C (5 min) – 20° C/min – 300° C (10 min). Helium was employed as a carrier gas at a flow rate of 1 ml min^{-1}. The injector, ion trap and transfer line temperature were 250° C, 200° C and 280° C, respectively. 2 μl of sample was splitless injected.

2.4. Analytical procedure

2.4.1. High fat food sample extracts

The method of sample preparation varied between fat samples. Pork and beef fat samples were cut into very small pieces and melted at 150 °C in a dish drainer. The butter was also melted in the dish drainer at a temperature of 105 °C, but was kept at this temperature overnight to allow for water evaporation. Egg yolks were extracted in dichloromethane in a Soxhlet set for 6 h. A bar of chocolate was dissolved in 250 ml of *n*-hexane and was left for 24 h to allow for cocoa powder deposition. A yellowish *n*-hexane layer containing fat was separated and transferred to a flask for solvent evaporation. Afterwards, all fat were dissolved in *n*-hexane and dichloromethane (1:1) to obtain a fat concentration of 0.4 g ml^{-1}. All extracts were stored at 4° C.

2.4.2. SPM membranes dialysis

Portions (8 ml) of high fat food extracts, and 5 ml of internal standard (TBB) were adjusted to 10 ml with *n*-hexane and placed in the SPM tube. A membrane was put in the glass tube containing 80 ml of *n*-hexane. The dialysis was conducted for 48 h. The solvent was replaced after 24 h with a new *n*-hexane portion. Each of the two dialysate portions were concentrated in a rotary evaporator to ca. 1 ml. After concentration the extracts were introduced to the silica gel column. The column was subsequently eluted with 50 ml of dichloromethane:*n*-hexane (20:80, v/v) and 20 ml of dichloromethane: *n*-hexane (85:15, v/v), and both fractions were collected. The volumes of these fractions were reduced to 1 ml using a rotary evaporator, and then to 50 μl under nitrogen stream

[3] A gift from Organic Chemistry Laboratory, Cracow University of Technology, Poland.

following addition of 2 μl of precision and recovery standard, and 50 μl of nonane. Afterwards, the analysis of 14 POPs was conducted using the GC/ECD chromatograph. The SPM conditioning time (48 h), the composition of external solvent, time of dialyse and the type of the preparation column were selected during the experiments described in the following paragraphs.

The identities of detected POPs were confirmed by GC/MS/MS analysis.

2.5. SPM dialysis parameters

A series of experiments were performed for the determination of the best conditions for POPs analysis in high fat food samples.

2.5.1. External solvent selection

The selection of the composition of the external solvent was decided by the analysis of the dependence between the content of the external solvent, and the recovery value of POPs from the standard solution. *n*-Hexane (100%), 20% DCM in *n*-hexane, and 60% DCM in *n*-hexane, were used as the external solvent. A mixture of 8 ml of the butter extract, 1 ml of the POPs standard solution, and 1 ml of *n*-hexane, were inserted into three membranes which were then placed in glass tubes in the perpendicular position. The dialysis was conducted for 48 h in darkness and the external solvent was replaced after 24 h. After concentration the dialysates were cleaned-up in the silica gel columns, and were analysed by GC/ECD.

2.5.2. A dialysis time determination

8 ml of each food product, with 1 ml of the 14 POPs standard solution (100 ng/ml), and 1 ml of *n*-hexane, were placed separately in SPM tubes. 80 ml of *n*-hexane was used as an external solvent. The prepared tubes were left for 72 h in the perpendicular position, and in the darkness. After 24 and 48 h the external solvent was replaced by a new portion of *n*-hexane. Dialysates were concentrated separately, and cleaned-up on the silica gel columns before being analysed by GC/ECD.

2.5.3. Membrane conditioning time determination

Three variants for membrane conditioning time were tried. The membranes used were either not conditioned at all, or conditioned by keeping them in *n*-hexane for 24 h, or 48 h. Non-conditioned membranes were just rinsed both within and without in *n*-hexane. Only the extracts of butter and pork fat were used in these experiments.

2.5.4. Lipid carry-over experiment

For each of the analysed matrices the extracts of fats (containing 1 g of fat), for each analysed product, were placed in four membranes, each conditioned for 48 h in *n*-hexane, and diluted by *n*-hexane up to the volume of 10 ml. The prepared membranes were inserted into a glass tube filled with 80 ml of external solvent, and left for 72 h in the perpendicular position in the darkness. After 24 h the external solvent was replaced with a new solvent portion, and the dialysates were separately placed in 100 ml flasks. The dialysates were evaporated in a rotary evaporator down to ca. 1 ml, quantitatively transferred to the weighed 1 ml empty glass vessels, and completely dried under a nitrogen stream. After drying the vessels were weighed again, and the residue (as a fat mass) was recorded for the lipid carry-over calculation.

2.6. Preparative column selection

The following prepared columns (20 or 30 cm length and 10 mm i.d.) were analysed so the most useful could be selected:

A. Neutral silica gel column: completely filled with 0.5 g anhydrous Na_2SO_4, 5 g of neutral silica gel and 5 g anhydrous Na_2SO_4.
B. Acid–alkali silica gel column: completely filled with 0.5 g anhydrous Na_2SO_4, 4 g silica gel modified by NaOH (30 wt%), 1 cm of neutral silica gel, 6 g silica gel modified by H_2SO_4 (44 wt%), 0.5 g anhydrous Na_2SO_4.
C. Neutral and acid (44 wt%) silica gel column: completely filled with 0.5 g anhydrous Na_2SO_4, 5 g neutral silica gel, 2 g silica gel modified by H_2SO_4 (44 wt%), 0.5 g anhydrous Na_2SO_4.
D. Neutral and acid (14 wt%) silica gel column: completely filled with 0.5 g anhydrous Na_2SO_4, 5 g neutral silica gel, 2 g silica gel modified by H_2SO_4 (44 or 14 or 7 wt%), 0.5 g anhydrous Na_2SO_4.
E. Neutral and acid (7 wt%) silica gel column: completely filled with 0.5 g anhydrous Na_2SO_4, 5 g neutral silica gel, 2 g silica gel modified by H_2SO_4 (7 wt%), 0.5 g anhydrous Na_2SO_4.
F. FLORISIL® column: completely filled with 0.5 g anhydrous Na_2SO_4, 5 g FLORISIL® and 0.5 g anhydrous Na_2SO_4.
G. FLORISIL® column: completely filled with 0.5 g anhydrous Na_2SO_4, 5 g FLORISIL® and 0.5 g anhydrous Na_2SO_4.

The columns A–F were conditioned before the application of sample extracts by rinsing with 30 ml of *n*-hexane preventing the column from drying. The column G was conditioned before the application of a sample by rinsing with 30 ml 2% DCM in *n*-hexane thus preventing the column from drying.

1 ml of the POPs standard solution, and 50 μl of the internal standard (TBB), was applied to each of the tested columns. Columns A–G were eluted with 50 ml of eluent I (40 ml *n*-hexane: 10 ml DCM). In order to Dieldrin elution a column was additionally eluted with 20 ml of eluent II (17 ml DCM: 3 ml *n*-hexane). Column F was eluted by 35 ml with eluent III (2% DCM in *n*-hexane) and 20 ml of eluent IV (5% DCM in *n*-hexane). In each case the fractions were pooled, concentrated to 50 μl and analysed by GC/ECD. The obtained results were used for the determination of the recovery of particular POPs.

3. Results and discussion

3.1. SPM dialysis parameters

3.1.1. The external solvent selection

The recovery values obtained for each composition of the external solvent were similar (57–100%). Nevertheless, when a mixture of *n*-hexane and DCM was used the lipid carry-over was larger than when an external solvent containing only *n*-hexane was applied. Meadows, Tillitt, Huckins, and Schroeder (1993) studied the composition of the external solvent, they compared cyclopentane, *n*-hexane, as well as a mixture of *n*-hexane and DCM (80:20 v/v), and reported higher recovery rates for *n*-hexane and cyclopentane (80% ± 3), and low lipid carry-over. Therefore it was thought that the employment of *n*-hexane would be the most appropriate combination of price and performance for this study.

3.1.2. Time of dialysis

The largest recoveries for all samples were obtained after the first 24 h of dialysis, and the lowest ones after next 48–72 h of dialysis. Levels of POP recovery did not exceed 10% after the longer time period. Taking into account the increasing lipid carry-over as a result of the prolongation of the dialysis time, and the associated difficulties during the subsequent sample clean-up, it was decided to perform the dialysis for 48 h with only one exchange of external solvent.

3.1.3. Membrane conditioning time

The best recovery values were obtained for membranes conditioned for 48 h (Fig. 1). These were 63–93% for butter extract, and 71–97% for pork fat extracts.

The membranes were also conditioned by the application of the initial Soxhlet extraction. Huckins et al. (1990) suggested that 2-h Soxhlet extraction could replace keeping a membrane in solvent before its application for dialysis. The method seemed to be uncomplicated and of interest due to potiential time savings. However, one of two membranes were destroyed in most of the tests performed in this study, moreover, the recoveries of POPs (from the standard solution) were unsatisfactory in the application of the non-destroyed membranes.

3.1.4. Lipid carry-over

The values for lipid carry-over were small, from 0.35% for pork fat extract to 0.65% for the butter extract after the first 24 h of dialysis. The values of the lipid carry-over determined after the second 24 h were in the range of 0.13–0.26%, the values of lipid carry-over after the third 24 h being virtually the same as those after second 24 h. It was concluded that the largest mass of lipid is transferred through the membrane during the first 24 h. Moreover obtained results showed that the values of the lipid carry-over are different for each kind of the analysed fat content food sample. Similar conclusions were drawn by Meadows et al. (1993) after analysis of a lipid carry-over for extracts of carp, egg yolks and peanuts. Moreover, Standberg, Bergqvist, and Rappe (1998) noticed that the higher the dialysate mass the lower lipid carry-over value.

3.2. Preparative column selection

The largest values of POPs recovery (87–107%) relative to the other analysed columns were obtained for Column A (Table 1). This column did not cause any loss of any analysed compounds. Column B performed poorly due to a loss of Aldrine, Isodrine and Dieldrine. The recovery values obtained for 4,4′-DDE, 4,4′-DDD and 4,4′-DDT were lower then the values obtained by from the other tested columns. Column C was also shown to be performing poorly. Column C seemed to cause the loss of Aldrine, Isodrine and Dieldrine in a similar way to Column B, and the recovery rates of 4,4′-DDE, 4,4′-DDD and 4,4′-DDT were about 86–107%. Unfortunately for Column C lower values of recovery were also observed for chlorobenzenes. The reduction in the acidity of silica gel seemed not bring any desirable effects. The use of Column D caused a loss of Isodrine and Dieldrine, and the recovery of Aldrine was only 16%. Column E saw an improvement of Aldrine recovery (84%), however the recovery of Isodrine was still low (34%), and Dieldrine was lost altogether. Columns F and G also performed poorly. Near total loss of Dieldrine (1% recovery) was observed for Column F. Column G caused a loss of Dieldrine and 4,4′-DDT, as well as a substantial decrease of HCBD recovery (down to 13%). Column A was considered the best in this particular application.

Table 1
The POPs recoveries for each of the tested chromatographic columns

POPs	Column						
	A[a]	B	C	D	E	F	G
1,2,4-TCB	89	84	66	82	95	97	99
1,2,3-TCB	87	68	59	85	96	86	92
HCBD	94	89	67	78	97	86	13
1,2,4,5-TCB	97	96	70	95	115	98	61
1,2,3,4- TCB	95	95	72	98	117	101	81
PeCBz	98	101	75	88	107	100	72
HCBD	107	98	76	97	112	90	87
Lindan	103	102	92	109	117	96	80
Aldrin	93	1	1	16	96	99	88
Isodrin	95	1	1	1	42	102	109
Dieldrin	97	1	1	0	0	102	0
DDE	89	72	91	107	115	1	112
DDD	92	63	99	116	121	104	83
DDT	96	73	107	114	111	104	0

[a] A, neutral silica gel column; B, acid–alkali silica gel column; C, neutral and an acid (44 wt%) silica gel column; D, neutral and an acid (14 wt%) silica gel column; E, neutral and an acid (7 wt%) silica gel column; F, FLORISIL® column I; G, FLORISIL® column II.

3.3. Selectivity of GC/ECD analysis

The ability to differentiate between each compound in a mixture of compounds, both with, and without the influence of a fat matrix is termed selectivity analysis. This was studied using the correlation curves of the relative retention times (with TBB as the internal standard) of particular POPs in the standard solution, with the relative retention times obtained for spiked samples. The retention times for the all analysed food samples were almost identical. The calculated correlation coefficients (r) were close to 0.999, and the values of slope for each POP for each regression of retention time for a fortified sample fitted to the retention time for an unfortified sample close to 1. The value of the slope could only be 1 when the relative retention time of a particular POPs obtained in the standard solution was equal to the relative retention

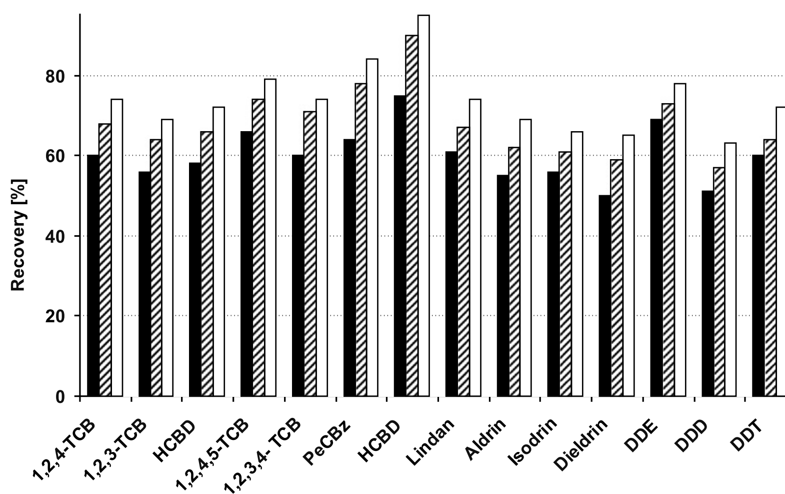

Fig. 1. The dependence between the POPs recoveries and time of membrane conditioning for a fortified butter sample (black bar, 0 h; dashed bar, 24 h; white bar, 48 h).

Table 2
Calibration curves ($y = ax + b$) parameters, coefficients of determination (R^2), limits of detection (LOD) and limits of quantification (LOQ10)

POPs	a	b	R^2	LOD (pg)	LOQ10 (pg)
1,2,4-TCB	0.00133	0.0063	0.9974	0.70	2.13
1,2,3-TCB	0.00220	0.0064	0.9959	0.25	0.74
HCBD	0.00715	0.0112	0.9976	0.12	0.38
1,2,4,5-TCB	0.00232	0.0125	0.9957	0.49	1.48
1,2,3,4-TCB	0.00427	0.0015	0.9983	0.30	0.90
PeCBz	0.00716	0.0122	0.9986	0.10	0.30
HCB	0.00804	0.0140	0.9991	0.14	0.42
Lindan	0.00863	0.0394	0.9988	0.11	0.33
Aldrin	0.00803	0.0026	0.9959	0.08	0.25
Isodrin	0.00340	0.0126	0.9974	0.95	2.88
Dieldrin	0.00807	0.0026	0.9963	0.15	0.45
DDE	0.00810	0.0007	0.9966	0.10	0.30
DDD	0.00562	0.0114	0.9990	0.13	0.40
DDT	0.00430	0.0040	0.9942	0.19	0.59

Table 3
The recoveries of 14 POPs for the analysed high fat food samples

POPs	Sample				
	Butter *W* (%)	Pork fat *W* (%)	Beef fat *W* (%)	Chocolate *W* (%)	Egg yolks *W* (%)
1,2,4-TCB	69 ± 2	76 ± 3	60 ± 2	77 ± 1	60 ± 1
1,2,3-TCB	65 ± 2	76 ± 4	58 ± 4	72 ± 1	61 ± 1
HCBD	66 ± 2	73 ± 2	55 ± 3	71 ± 2	59 ± 2
1,2,4,5-TCB	75 ± 3	86 ± 3	74 ± 1	100 ± 3	81 ± 2
1,2,3,4-TCB	72 ± 2	89 ± 1	71 ± 2	100 ± 4	81 ± 2
PeCBz	80 ± 2	93 ± 5	79 ± 2	88 ± 4	95 ± 5
HCB	94 ± 2	97 ± 2	89 ± 3	96 ± 2	100 ± 2
Lindan	70 ± 2	81 ± 2	77 ± 2	85 ± 2	79 ± 1
Aldrin	63 ± 4	76 ± 2	61 ± 1	62 ± 2	63 ± 2
Isodrin	67 ± 1	75 ± 3	75 ± 3	72 ± 3	65 ± 2
Dieldrin	60 ± 1	69 ± 3	61 ± 3	74 ± 2	62 ± 2
DDE	75 ± 1	99 ± 3	84 ± 2	59 ± 1	95 ± 1
DDD	62 ± 2	75 ± 1	62 ± 1	67 ± 1	61 ± 1
DDT	69 ± 3	98 ± 1	78 ± 4	72 ± 2	87 ± 3

time of a particular POPs obtained in the fortified sample. Therefore it can be concluded that the proposed method of POP analysis in fatty food samples is highly selective, and the components presented in the fat matrix do not interfere with the selectivity of the method.

3.4. POPs quantitative analysis by GC/ECD

Calibration curves were calculated on the basis of those data observed during the analysis of the 14 POPs. 1 ml mixtures at each of eight concentration levels: 0.2, 0.5, 1, 2, 5, 10, 50, 100 ng ml^{-1}. Three replicates were made for each concentration. Calculated values of a and b as well as the correlation coefficients are presented in Table 2.

It is clear (Table 2) that the calibration curves obtained for particular compounds explained most of the variability, and that the determined calibration curves are a sufficient fit for the determination of particular POPs in the fat content samples. The linearity of the ECD detector signal response for the described method was in the range of 0.2–100 ng ml^{-1} for each POP. The limits of detection and quantification of each of the POPs are also presented in Table 2.

The content (ng) of each POP in each analysed sample was calculated by from the mass of each POPs in 1 μl of injected sample, obtained on the basis of suitable equations of a calibration curve, this value was multiplied by the final volume of analysed sample. In principle this volume should be equal to 50 μl for the objective of measuring the particular POPs content in the analytical sample. However, due to problems with the precise reduction of the sample volume from 1 ml to 50 μl during the evaporation process, precision and recovery standards were added. This allowed us to determine the true final volume of analysed sample. The values of recovery of each POP were also taken into account (Table 3) as they did not equal 1 (100%).

For an analysis of the precision and accuracy of the method 3 g of pork fat and 3 g of chocolate were spiked with 1 ml of standard solutions containing 5 ng/ml and 25 ng/ml of each of the 14 POPs. two samples of pork fat, and two samples of chocolate, were prepared in total, and each sample was analysed three times by GC/ECD.

The accuracy of the proposed analytical procedure was checked, and the values for the content of each POP in the analysed extracts of fatty food samples were found to be equal to their known content. This was only not observed for HCBD and PeCBz (one of the samples fortified with 1 ml 5 ng/ml standard solution) and for HCBD and HCB (sample no. 1 fortified with 1 ml of 25 ng/ml standard solution).

The precision of the determination of each of the 14 POPs in the fortified samples was expressed by the relative standard deviation (RSD). It was calculated on the basis of 6 replicates for a particular concentration (5 ng/ml and 25 ng/ml). Analysis of the obtained re-

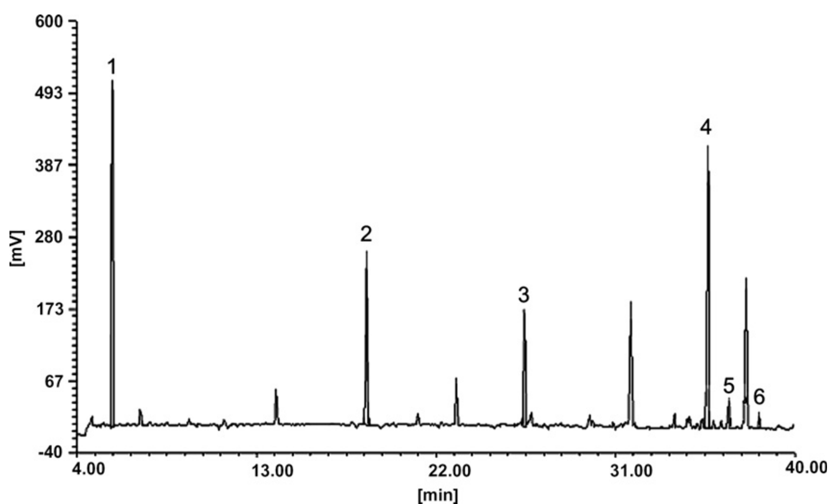

Fig. 2. A chromatogram of beef fat (1 – 1,4-DCB, 2 – TBB, 3 – HCB, 4 – DDE, 5 – DDD, 6 – DDT).

Table 4
The concentrations of 14 POPs in the analysed high fat food samples

Sample	Concentration of POPs (ng g^{-1}of fat)													
	1,2,4-TCB	1,2,3-TCB	HCBD	1,2,4,5-TCB	1,2,3,4-TCB	PeCBz	HCB	Lindan	Aldrin	Isodrin	Dieldrin	DDE	DDD	DDT
Butter 1	–	–	–	–	–	–	1.97	–	–	–	–	8.34	–	0.74
Butter 2	–	–	5.09	–	–	–	0.61	–	–	–	–	1.03	0.23	
Butter 3	–	–	0.93	–	–	–	1.88	–	–	–	–	11.77	0.19	0.87
Chocolate 1	–	–	–	–	–	–	0.94	4.93	–	–	–	0.73		
Chocolate 2	–	–	–	–	–	–	0.46	3.63	–	–	–	5.00	0.62	0.55
Chocolate 3	–	–	–	–	–	–		0.68	–	–	–	–	–	–
Chocolate 4	–	–	–	–	–	–	0.28	3.25	–	–	–	2.65	0.23	0.33
Pork fat 1	–	–	–	–	–	–	0.52	–	–	–	–	4.13	0.34	2.80
Pork fat 2	–	–	–	–	–	–	–	–	–	–	–	5.15	4.86	5.69
Pork fat 3	–	–	–	–	–	–	–	–	–	–	–	8.29	0.55	5.26
Beef fat 1	–	–	–	–	–	–	5.90	–	–	–	–	20.51	0.37	0.94
Beef fat 2	–	–	–	–	–	–	3.75	–	–	–	–	19.01	1.05	1.12
Egg yolk 1	–	–	–	–	–	–	0.45	0.80	–	–	–	2.93	0.17	2.48

–, not detected, below the limit of detection (LOD).

sults showed that all values of RSD were below 10%. It was concluded that the proposed method of analysis of selected 14 POPs gave satisfactory accuracy in the case of each of the analysed matrices.

3.5. Analysis of real samples

The preparation procedure of high fat food samples for POPs determination by the chromatographic method outlined above was employed for the POPs determination in the real samples of chocolate, butter, egg yolks, beef fat (Fig. 2) and pork fat.

The concentrations of the individual compounds detected in the real samples were obtained on the basis of the calibration curves (Table 2). The results are presented in Table 4.

The most commonly detected POPs in the real samples were HCB, 4,4′-DDD, 4,4′-DDD and 4,4′-DDT, however their concentration was relatively low. The largest number of POPs were detected in the butter sample.

GC/MS/MS analysis was also conducted to confirm the identification of those POPs detected by the GC/ECD method in the real food samples. The spectra of these particular POPs were compared with their mass spectra presented in the NIST library (National Institute of Standards and Technology).

4. Conclusions

These results demonstrate the usefulness of the SPM technique for the preparation of high fat food samples for the determination of 14 POPs using the GC/ECD technique. An advantage of this method is that a wide range of chlorinated organic compounds can be determined in the same sample in one analytical procedure, which is especially important when the total mass of the sample available for the analysis is low. The best results were obtained when a semipermeable membrane was conditioned for 48 h in *n*-hexane, *n*-hexane was used as an external solvent, the time of high fat food sample dialysis was 48 h, the extract clean-up was performed on the neutral silica gel chromatographic column, and the internal standard (TBB) and the precision and recovery standard solution (1,4–dichlorobenzene) were used for the reduction of errors related to GC analysis and a sample volume reduction.

The proposed method is characterised by a good accuracy, precision and selectivity as well as low limit of detection and limit of quantification of each POPs. It should also be emphasized that the recoveries for all of the 14 POPs obtained in this work were between 55% and 100% and the lipid carry-over was not greater than 0.4%.

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