USE OF POLAR APROTIC SOLVENT TO SEPARATE ORGANOCHLORINE COMPOUNDS FROM FATTY FOODS

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Introduction

A simple and effective routine extraction and purification method for organochlorines in fatty foods and simultaneous gas chromatographic determination with ECD is described. Fatty food analysis methods are usually laborious and not fully effective in cleaning up the sample when gel permeation chromatography is not used. In most cases a hexane solution of the sample is extracted with an immiscible solvent, which selectively dissolves organochlorine compounds. Dimethyl sulphoxide (DMSO) is the most used polar aprotic solvent for the separation of the organochlorine compounds from fatty matrices during the liquid-liquid partition extraction. Acetonitrile, dimethylformamide and N-methyl-2-pyrrolidone are used as well in different approaches of the clean-up procedures.[1,2,3,4,5,6]. Simple matrix solid phase dispersion and a single step extraction and clean-up procedure with DMSO/hexane are described. By creating more universal purification procedure for compounds or groups of structurally related compounds, it is possible to reduce costs, increase the sample throughput, and reduce personnel training time.

Materials and Methods

A multiresidual method is developed for the determination of twenty six organochlorine pesticides (HCH (α, β and γ (lindane)), heptachlor, heptachlor epoxide (cis, trans), aldrin, chlordane (cis, trans), DDE (op', pp'), DDD (op', pp'), DDT (op', pp'), isodrin, dieldrin, endrin, methoxychlor (op', pp'), mirex, hexachlorobenzene (HCB), 2,3,4,5 tetrachloronitrobenzene (tecnazene), pentachloronitrobenzene (quintozene), endosulfan (I, II) and six PCB indicating congeners (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180) in different fatty foods. The PCB 209 is used as an internal standard for quality control procedure. In Europe, analytical standard EN 1528 is the reference tool for the determination of OCPs in animal fatty samples[7]. The residues are expressed in milligrams per kilogram of fat. Standard compounds are of at least 95% purity and solvents used are of the highest available grades.

Sample preparation: fat portions are gathered from samples of meat adipose tissues, meat products, milk and milk products, butter, cheese, eggs and other fatty foods either by partitioning extraction or cold centrifugation extraction[7]. Melted fat sample of 0.5g is dissolved in hexane and mixed with 1.5 g portion of Celite to yield a homogenous powder. Celite is used as a solid matrix support and for removal of the bulk of lipids. A stream of nitrogen is used to remove hexane. The sample is prepared for extraction and clean-up procedure.

Extraction and clean-up method: to avoid general drawbacks of a liquid-liquid partitioning in a separating funnel during the analysis, a DMSO based matrix solid phase dispersion extraction and clean-up column chromatography procedure is used. Chromatography alone on a single column filled with Florisil is not enough, so a mini Pasteur column, packed as compactly as possible with the sample-Celite mixture powder, is joined to the second column of 5g 15% water deactivated slurry filled Florisil. For the elution of organochlorines from the first mini column only 5 ml of DMSO are used. When DMSO is present on a second Florisil column, organochlorines are subjected to two types of chromatography. In the upper part, in the DMSO layer, a mechanism of liquid-liquid extraction predominates. The usual adsorption chromatography takes place in the lower part of the Florisil column, where there is no DMSO. After the adsorption chromatography completes, the pesticides are eluted with 75 ml mixture of hexane/diethyl ether (70:5, v/v).

Analysis: a gas chromatograph HP 5890 Serial II equipped with a split/splitless injector is used and a dual column chromatography coupled to two ECDs follows. A deactivated retention gap of 1 m x 0.53 mm I.D. and two capillary columns of different polarities; HP-5MS and DB-1701 (60m x 0.32 i.d.) are simultaneously used to obtain a higher level of confidence in analyte identification. The column oven temperature programme is as follows: 90ºC (0.5 min), 70ºC/min to 180ºC, 1.2ºC/min to 275ºC (15 min) and overall runtime is 95 min. The carrier gas is helium supplied in a constant pressure mode for both columns. Typical GC/ECD/ECD chromatograms are shown in Figure 1.

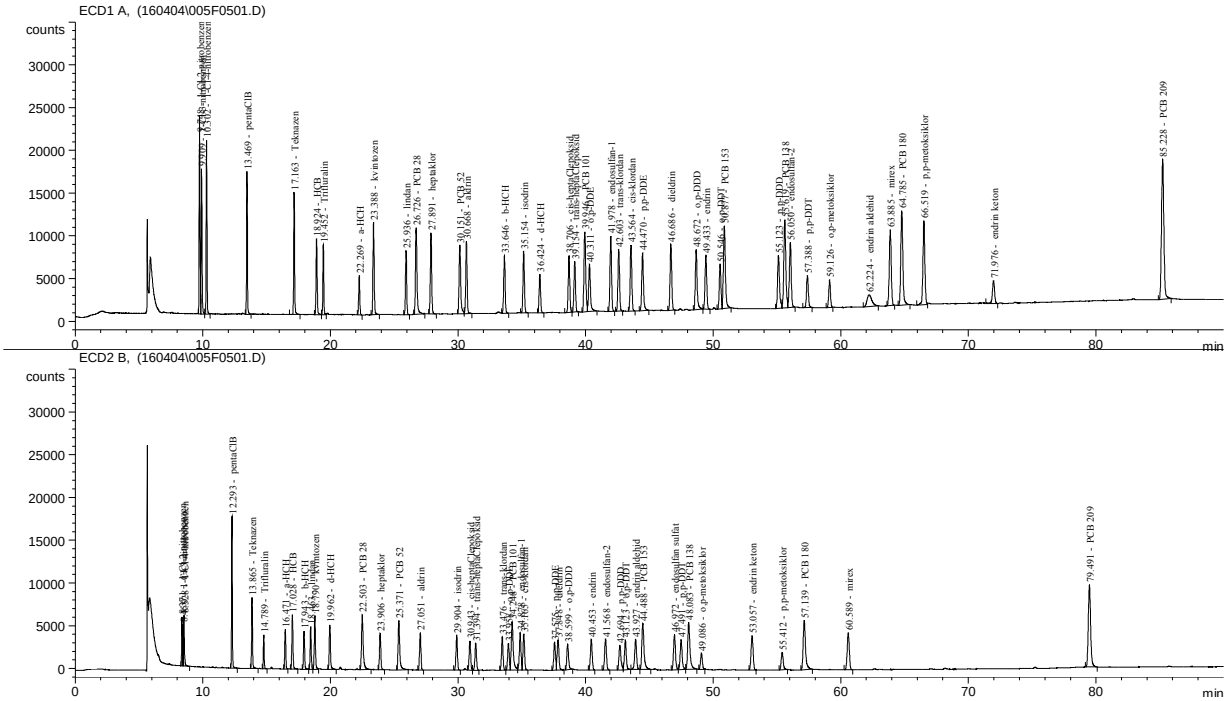

Figure 1. Chromatogram of standard solution of organochlorine pesticides and some PCB indicating congeners performed by dual-column high resolution GC/ECD.

Analytical quality control procedures: optimization of the method is carried out using a spiked fat material, a so-called in-house quality control material. On a daily basis, every batch of samples is accompanied by an analyte-free sample to guard against false positives. Also, a proficiency testing round is considered one of the best tests of an analytical laboratory and is a good evidence that the quality procedures are actually working in practice. For the analytical purpose the FAPAS proficiency tests were using from 2000 to 2005.

Results and Discussion

The method is found to be effective in determining several groups of structurally related compounds (aliphatic chlorinated biphenyls (DDT, DDE, DDD, metoxychlor), HCHs, cyclodienes (aldrin, chlordane, heptachlor, endrin, isodrin, mirex, endosulfan) and PCB residues from the bulk of lipid material. A DMSO extraction and clean-up technique are fast, cheap, robust and does not require any special skills to obtain reproducibility of approximately 15%. Removal of fat from the extracts was sufficiently accomplished by DMSO/hexane partitioning in a wide range of fatty samples and it supplements the extensively used gel permeation chromatography.

The whole procedure takes no more than one hour per sample where a single operator can run several samples at the same time. The results in Table 1 present the average recoveries of twenty-six organochlorine pesticides and six PCBs from 0.01 mg kg^{-1} lipid wt. to 0.1 mg kg^{-1} lipid wt. for the last five years. Limits of determination were in the range of 0.005 mg kg^{-1} lipid wt. to 0.02 mg kg^{-1} lipid wt. Analytical parameters obtained with the method

comply with the application of European Regulation regarding the determination of organochlorines in food of animal origin.
Recoveries for pesticides range from 68% to 94% except for β-HCH, which gives lower, more variable recoveries, and from 81% to 86% for PCBs, with relative standard deviation less than or equal to 16% for pesticides and 10% for PCBs.

Table 1. Recoveries of organochlorine compounds from different spiked fat matrices at different levels performed by the DMSO-based clean-up method with corresponding relative standard deviations.

	Pesticides	Recovery (%) (n= 120)		PCBs	Recovery (%) (n= 120)	
		Mean	RSD (%)		Mean	RSD (%)
1	HCB	76	12	PCB 28	81	7
2	α-HCH	68	15	PCB 52	82	7
3	β -HCH	63	18	PCB 101	86	10
4	γ-HCH (lindane)	72	15	PCB 138	84	10
5	tecnazene	87	11	PCB 153	82	7
6	quintozene	70	10	PCB 180	83	7
7	heptachlor	85	11			
8	heptachlor epoxide-cis	84	8			
9	heptachlor epoxide-trans	85	10			
10	aldrin	80	12			
11	dieldrin	86	10			
12	endrin	94	10			
13	isodrin	82	10			
14	chlordane-cis	86	9			
15	chlordane-trans	89	8			
16	o,p'-DDE	86	12			
17	p,p'-DDE	94	9			
18	o,p'-DDD	82	9			
19	p,p'-DDD	77	10			
20	o,p'-DDT	85	8			
21	p,p-DDT	88	14			
22	endosulfan I	87	10			
23	endosulfan II	79	12			
24	o,p'-methoxychlor	89	11			
25	p,p –methoxychlor	87	16			
26	mirex	84	10			

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