Solid phase extraction of PCDDs/PCDFs and dioxin-like PCBs from oils and fats

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Introduction

At present, the analysis of polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF) and dioxin-like biphenyls (DL-PCB) has become a routine procedure performed in hundreds of laboratories in different countries. Sample preparation is done manually according to principles of US EPA Method 1613 or using various automatic or semi-automatic systems. A common practice of dioxin sample preparation is a three-stage clean-up procedure: first on a column filled by silica gel impregnated by sulfuric acid and potassium silicate for the acid-base destruction of labile matrix components, and then fractionation on alumina and carbon columns. This scheme is the gold standard for samples with a relatively small content of extractable organic compounds, *e.g.* environmental samples. But when a sample contains several grams of fat, a large amount of sorbents is needed to chemically decompose the matrix and there is a likelihood of carbonization or "sticking together" inside the column. The procedure of the fat matrix chemical degradation can be avoided by using a sorbent having high selectivity and efficiency with respect to the substances to be detected and eluting with a relatively small amount of solvent. For PCDD/PCDF, these properties have AX-21 carbon (Anderson Development). Unfortunately, AX-21 is not commercially available for more than 15 years; its analogue - PX-21 (Amoco), as well as the FAS-MD carbon brand synthesized at the Institute of Physical Chemistry of the Russian Academy of Sciences [1], are also inaccessible. We have previously used AX-21 and FAS-MD carbons for quantitative extraction of dioxins from samples containing more than 50 g of fat with elution by 5-10 ml of hot toluene. Method 1613 recommends Carbopack C, but it is only suitable for fine clean-up at the last stage and does not allow for the extraction of PCDDs/PCDFs from fat-containing solutions.
The sorption properties of Carboxen 1000 and 1016 carbons (Supelco) in relation to PCDDs/ PCDFs and dioxin-like PCBs were previously reported [2]. That data gave us reason to assume that these sorbents can extract dioxins and dioxin-like compounds from fatty solutions.

Materials and methods

We used dual-layer glass columns containing 100 mg of adsorbents Carboxen 1016 and 1000 in the form of 0.18-0.25 mm spherical granules (Supelco 28399). Such a design should provide sorption and desorption of both planar compounds and mono-substituted PCBs. The first ones are adsorbed by Carboxen 1016 micropores, and are easily desorbed with toluene. Carboxen 1000 mesopores retain both PCDD/PCDF and mono-ortho substituted PCBs, but elution from it is considerably complicated [2]. The column was conditioned with 10ml toluene and hexane, displacing air; then 5-6 g fat sample diluted with 2-6 volumes of hexane (depending on the viscosity) passed through the column; next we wash column by 20 ml hexane. Finally, the column was placed into a custom-made oven and eluted by 40 ml toluene in back flow at 110±5°C.
The eluate was rotary evaporated to dryness, dissolved in 2 ml of hexane and cleaned up on a custom made system consisting of two columns (12 and 6 cm length, ID 9 mm) directly connected to each other and a solvent reservoir. The bottom one was filled with 4 g of activated basic aluminum oxide (600°C, 12 hours), the upper column contained two layers of H_2SO_4 on silica gel (44%) and potassium silicate, separated by anhydrous sodium sulfate.
The system was conditioned with 20 ml of hexane before use; a sample was applied, washed with 25 ml of hexane, discarding the eluate. After that we elute non-planar PCB by 25 ml dichloromethane:hexane mixture

(1:19 v:v); next remove the upper column and elute and PCDDs/PCDFs and coplanar PCBs by 50 ml dichloromethane: hexane (3:2 v:v) from alumina. The solvents were passed through the columns under a slight pressure, providing a flow rate of 3-4 ml/min.
The GC-MS analyses were performed on a Waters AutoSpec Premier HRMS mass-spectrometer equipped with a Thermo TR-5MS column. Isotopically labeled standards were purchased from Wellington Laboratories and CIL.
The main criteria for assessing the quality of purification were the stable retention times and the absence of chromatographic peak distortions in comparison with pure standards.

Results and discussion:
To assess the possibility of solid phase extraction of dioxins and dioxin-like compounds from fat matrixes by Carboxen 1000/1016, the following objects were chosen: fish oil, milk fat, unrefined sunflower oil, refined rapeseed oil and soya lecithin. All samples were spiked by $^{13}C_{12}$ PCDDs/PCDFs, WHO-PCBs, indicator PCBs and PBDEs.
The eluate from the Carboxen column usually had an intensive coloration, but contained only small amounts of matrix residues that were easily removed by subsequent dual-column cleanup, giving practically perfect chromatograms for both fractions (fig 1 and 2).
Unfortunately, the results for indicator PCBs and PBDEs should be considered as negative, as the recovery in some cases drops below 10% and is strongly depended on substituent pattern. Recoveries for PCDDs/PCDFs and WHO-PCBs are presented in Table 1.
According to the literature data [2], 60 ml of toluene at room temperature elutes 90-95% PCDDs/PCDFs from Carboxen 1016 and 65-80% from Carboxen 1000, which is in agreement with our data obtained for the standard solutions under eluting with hot toluene, however, in experiments with fats, lower values were observed. To determine the causes of losses, we elute the column repeatedly, reinstalled the column upside down and checked the "breakthrough". Summarizing all results, it can be assumed that for the fat's solution the mesopores of Carboxen 1016 and 1000 are not effective enough and sorption predominantly occurs on micropores of the Carboxen 1000 and nearly irreversible sorption is the main reason of lowered recoveries.
We consider the optimum recovery level for 2,3,7,8-TCDD is about 85%, however, according to US EPA Method 613 a wide range from 16 to 279% of recoveries is allowed. The obtained recoveries cannot be called optimal, and this approach cannot be recommended for samples requiring the ultimate sensitivity. But this method can be useful in the food and feed fats analysis, since the amount of sample is usually not limited, and the loss of sensitivity can be compensated for by a large sample size.

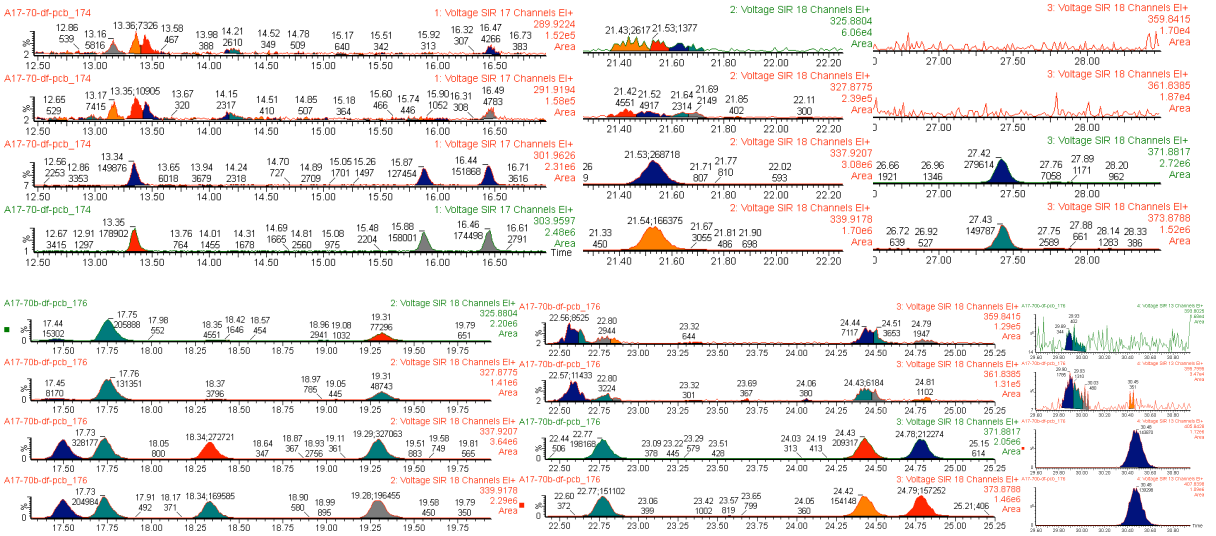

Fig. 1. Mass chromatograms of WHO-PCBs in soya lecithin sample ("planar" fraction – three chromatograms at the top, "PCB" fraction – three chromatograms at the bottom)

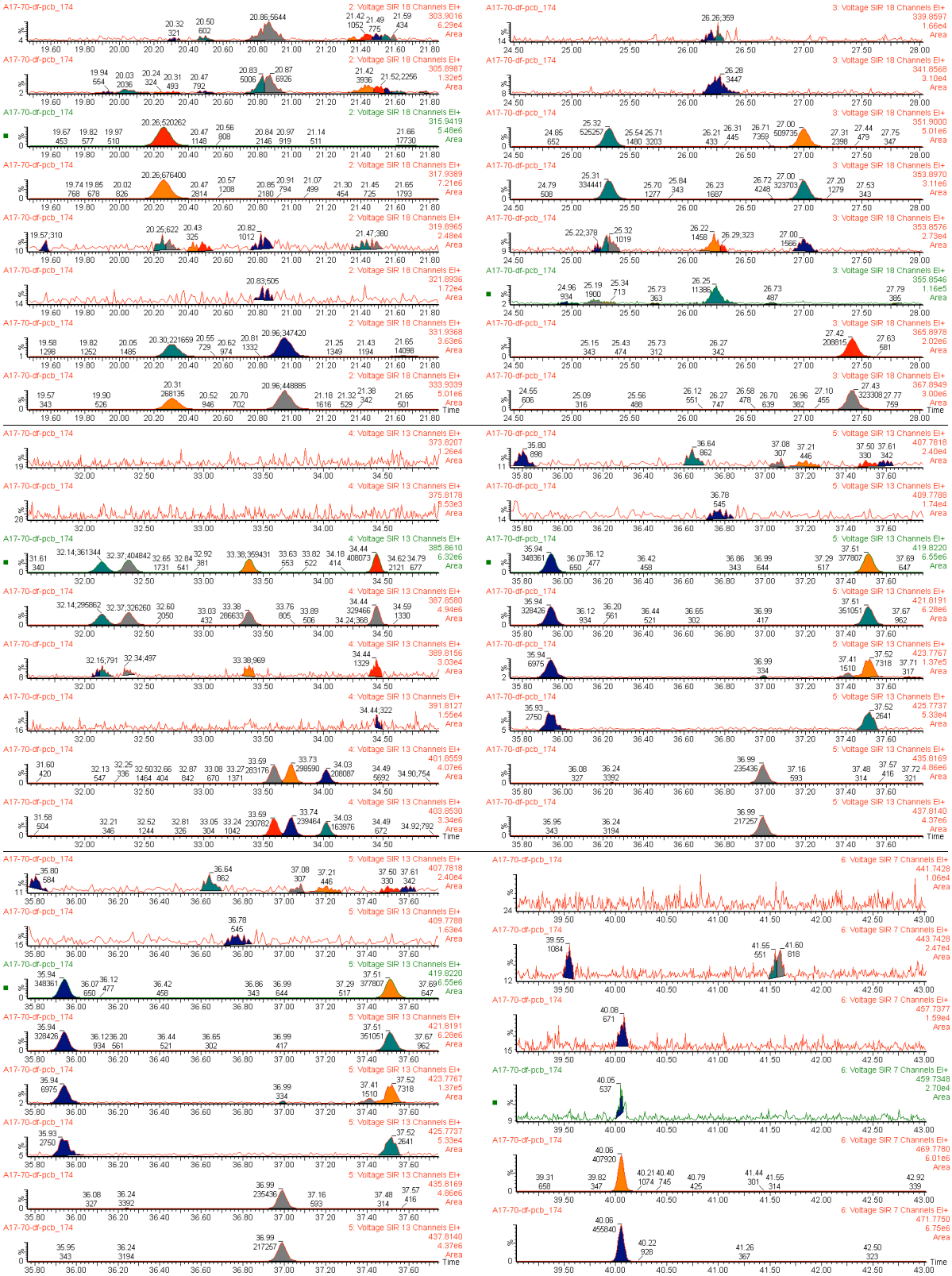

Fig. 2. Mass chromatograms of PCDDs/PCDFs in soya lecithin sample

The use of a carbon column in the first step significantly reduces sorbents and solvents consumption, and the total cleanup time is only about two hours if we use the scheme described above. Also the method can be integrated into the FMS EZprep family systems, or you can use traditional multilayer and alumina columns according to US EPA Method 1613.

We can assume that optimizing the method will result in more stable and high recovery, but right now the proposed scheme with Carboxen 1000/1016 carbon column as the first step followed by cleanup on small multilayer column and basic alumina column provides acceptable sample preparation quality for dioxins and WHO-PCBs from a variety of fatty matrix at relatively low time and solvent costs.

Table 1: Recoveries for PCDD and dioxin-like PCBs from oils and fats

	Fish oil	**Milk fat**	**Sunflower oil**	**Rapeseed oil**	**Soya lecithin**
2,3,7,8-TCDD	78	59	50	56	70
2,3,7,8-TCDF	65	53	43	50	73
1,2,3,7,8-PeCDD	74	50	35	43	56
1,2,3,7,8-PeCDF	71	57	42	49	66
2,3,4,7,8-PeCDF	64	45	27	38	59
1,2,3,4,7,8-HxCDD	62	38	26	33	54
1,2,3,6,7,8-HxCDD	64	39	27	34	54
1,2,3,4,7,8-HxCDF	59	42	28	36	60
1,2,3,6,7,8-HxCDF	57	40	29	35	61
2,3,4,6,7,8-HxCDF	55	40	27	34	59
1,2,3,7,8,9-HxCDF	63	53	51	51	67
1,2,3,4,6,7,8-HpCDD	72	34	32	32	42
1,2,3,4,6,7,8-HpCDF	59	27	28	31	44
1,2,3,4,7,8,9-HpCDF	68	43	37	37	52
OCDD	73	36	45	44	56
PCB-77	81	65	55	49	76
PCB-81	66	47	47	41	71
PCB-126	79	91	44	53	68
PCB-169	55	88	47	49	60
PCB-105	33	49	30	28	81
PCB-114	27	47	25	24	69
PCB-118	34	54	29	28	81
PCB-123	32	50	28	27	77
PCB-156	29	65	33	30	99
PCB-157	30	68	36	29	99
PCB-167	28	66	28	26	99
PCB-189	21	61	23	23	90

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