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COMBINED EXTRACTION / CLEAN-UP STRATEGIES FOR FAST DETERMINATION OF PCDD/Fs AND WHO-PCBs IN FOOD AND FEED SAMPLES USING ACCELERATED SOLVENT EXTRACTION

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Introduction

Today, there is an increased demand for cost-efficient, fast analytical methodologies for determination of polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs) and dioxin-like PCBs (WHO-PCBs) in food and feed samples with a minimum of sample handling. Such methodologies will increase sample throughput both in times of crisis and in environmental monitoring programs. Improved control of the occurrence of dioxins in food and feed will lead to safer food and thereby increased human health. The average exposure of the European population (8-21 pg/kg body weight[1]) is occasionally higher than the total tolerable weekly intake for dioxins and dioxin-like PCBs. Consequently, there is a driving force from the European Commission to decrease the overall intake. An important tool to achieve this goal is to increase monitoring (Commission Directive 2002/69/EC). Lately, built-in clean-up procedures in accelerated solvent extraction (ASE) have been developed by placing a small amount of fat-retainer in the cell after the sample. Thereby, selective extractions have been achieved for PCBs in food and feed[2-5]. Björklund et al. investigated various fat retainers and found sulfuric acid impregnated silica to give the cleanest extracts[2]. Later, Müller et al. tested this fat retainer on a number of food and feed samples with good results[3]. In this work, the developed on-line clean-up method for selective extraction of PCBs[5] was tested also for dioxins. Additionally, a new extraction strategy with new assemblies, designed in-house to fit into the commercially available ASE cells, were tested for the first time.

Methods and Materials

All solvents were of high purity (pesticide grade or glass distilled). Silica, Na_2SO_4 and sulphuric acid were supplied by Fluka (Fluka Chemie GmbH, Germany). The vegetable oil and fish oil were from an on-going European project[6]. Prior to extraction, the oils were fortified with ^{13}C-labelled internal standards. All extractions were carried out on a ASE 300^{TM} (Dionex, Sunnyvale, CA) at Lund University (LuU). Two extraction-packing strategies were tested (Figure 1).

Extraction Strategy 1 extracted all PCBs and dioxins from the sample in one step using *n*-heptane while the fat was destroyed by the sulphuric acid in the extraction cell. The amount of fat in relation to fat retainer (fat to fat retainer ratio, FFR) is important and should be somewhere in the range 0.025-0.05[2-3,5] depending on how much co-extracted fat that is acceptable. In this case, a FFR value of 0.05 was used (3.0 g of oil and 60.0 g of fat retainer) in 100 mL extraction cells. The amount of coextracted fat was ca 5 mg, which was removed by a miniaturized clean-up step.

Extraction strategy 2 included three consecutive extractions in a 34 mL cell. The first step (A) was extraction of fat and bulk-PCBs with pure *n*-heptane. The second step (B) utilized a combination

of *n*-heptane/acetone (1:2.5, v/v), alternatively *n*-heptane/dichloromethane (1:1, v/v), to extract more polar fat residues together with mono-*ortho* PCBs. Finally the extraction cells were turned up-side down and non-*ortho* PCBs and dioxins were back-eluted with toluene (C).

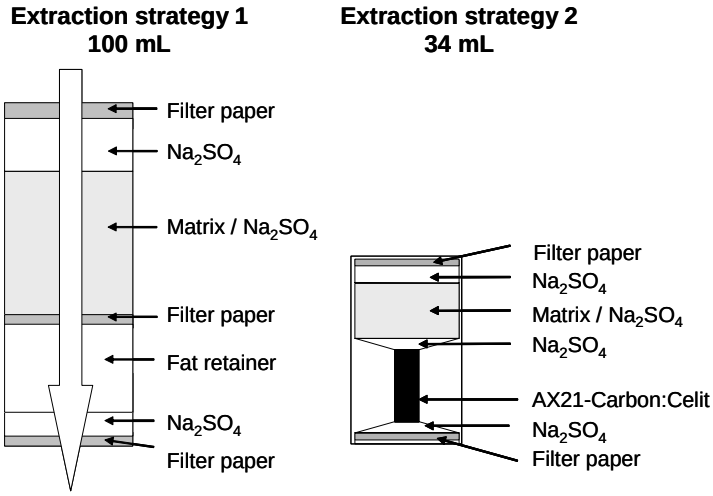

Figure 1. Packing of the extraction cells using two different extraction strategies.

Further clean up and instrumental analysis was performed at Umeå University (UmU) according to validated methods. The extracts obtained with Strategy 1 were fractionated into three fractions on a carbon (AX21) column. The mono-*ortho* PCBs were eluted in fraction 2 with *n*-hexane: dichloromethane (1:1, v/v) and PCDD/Fs and non-*ortho* PCB were eluted in fraction 3 with toluene. The final clean up step was a miniaturized multilayer silica column. The three fractions obtained from the newly designed extraction cells (Strategy 2) were also cleaned up on multilayer silica columns, which were designed to handle the fat content of each fraction. The analysis was performed on a GC-HRMS (VG 70-250S). Reference standards were from Wellington (PCDD/Fs) and Cambridge Isotope Laboratories (PCBs) and the quantification was made according to the isotope dilution method. Extracted fat was determined gravimetrically.

Results and Discussion

The quantitative results from Extraction Strategy 1 are presented in Table 1. Results from two reference laboratories, which used classical extraction and clean-up procedures in combination with GC-HRMS, are also included. It is obvious that ASE with on-line fat removal performs equally well to the conventional methods and could replace these for fat containing matrices.

Recoveries obtained in the Strategy 2-experiment are shown in Table 2. For bulk-PCBs the methods worked well since the recoveries were close to 100% of the spiked values, and no bulk-PCBs were found in fraction B. The mono-*ortho* PCBs appeared in Fraction A, while they preferably should have eluted in Fraction B. This might be due to too high temperatures and too

short carbon trap. Also the non-*ortho* PCBs eluted too early since they appeared in Fraction A and B, while they should have eluted in Fraction C. Finally, the PCDD/Fs appeared in Fraction C as expected. The low recoveries of the lower-chlorinated dioxin congeners indicated that these eluted partly in fraction B. Even so, the results are promising and it should be possible to get clean dioxin extracts on-line, which require a minimum of additional clean up before analysis.

Table 1. Levels of PCDD/Fs and WHO-PCBs in vegetable oil and fish oil (pg/g oil). The results were obtained by using ASE with on-line fat removal (LuU + UmU, Extraction Strategy 1) and by using conventional extraction methodologies with external clean-up (Lab A and Lab B).

	Vegetable oil (pg/g oil) (n=1)			Fish oil (pg/g oil) (n=6)			Fish oil
	LAB A GC-HRMS	**LAB B GC-HRMS**	**LuU+UmU ASE+GC-HRMS**	**LAB A GC-HRMS**	**LAB B GC-HRMS**	**LuU+UmU ASE+GC-HRMS**	**LuU+UmU Rel. std (%)**
PCB-81	3,1	2,9	2,6	2,5	2,2	3,2	14
PCB-114	<100	32	23	<100	110	88	2,9
PCB-167	370	260	200	440	330	1800**	2
PCB-189	<200	27	51	<200	61	98	10
PCB-105	1200	1300	1200	2100	2100	2100	2,2
PCB-118	5100	4800	4100	7400	5600	6100	2,1
PCB-156	370	270	310	730	580	690	2
PCB-157	<100	29	30	240	177	210	4
PCB-77	1000	1100	990	79	64	73	3,9
PCB-126	22	20	21	39	28	38	4,9
PCB-169	5,7	5,7	6,6	13	10	14	4,4
2378-TCDD	0,33	0,33	0,25	0,32	0,34	0,29	13
12378-PeCDD	0,58	0,66	0,57	1,3	1,3	1,5	6,9
2378-TCDF	0,33	0,31	0,47	6,3	4,4	5,9	6,7
12378-PeCDF	0,73	0,61	0,59	2,1	1,1	1,6	13
23478-PeCDF	2,1	2,0	1,9	5,7	4,7	5,8	6,7
123478-HxCDF	0,68	0,68	0,53	0,38	0,42	0,45	14
123678-HxCDF	0,66	0,62	0,54	0,65	0,41	0,45	18
234678-HxCDF	0,68	0,61	1,0	0,59	0,45	0,55	19
123789-HxCDF	<0.03	0,51	0,94	0,03	0,31	0,41	27
WHO-TEQ*							
PCDD/PCDF	2,5	2,5	2,6	5,5	4,7	5,8	7,5
PCB	3,3	3,0	2,9	5,5	4,2	5,3	3,3
SUM TEQs	5,8	5,5	5,5	11,0	8,9	11,1	5,3

Remarks:
*Upperbound (the limit of detection was used for congeners below the detection limit).
**coleluted with PCB-128

Conclusion

The previously developed on-line clean-up method for PCBs in food and feed (Extraction Strategy 1) was demonstrated to work nicely also for PCDD/Fs. The method will be tested on a number of certified reference materials to demonstrate a wider applicability. Regarding the newly designed extraction cell (Extraction Strategy 2), it showed a good potential for the future. However, some parameters need to be optimized. These involve extraction temperature, type of active carbon and length of the carbon trap.

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Table 2. Recoveries of ^{13}C-labelled PCDD/Fs and selected PCBs extracted from spiked fish oil using the newly designed extractions cell (Extraction strategy 2).

	Fraction A Sample 1-4 heptane	Fraction B Sample 1-2 DCM:hept	Fraction B Sample 3-4 aceton:hept	Fraction C Sample 1-2 toluene	Fraction C Sample 3-4 toluene
Bulk-PCBs					
PCB-47	106	0	1	NA	NA
PCB-52	111	0	0	NA	NA
PCB-101	136	0	1	NA	NA
PCB-138	112	0	1	NA	NA
PCB-153	127	0	1	NA	NA
PCB-180	105	0	1	NA	NA
Mon-ortho PCBs					
PCB-105	112	0	1	NA	NA
PCB-118	138	0	1	NA	NA
PCB-156	103	0	1	NA	NA
PCB-157	90	0	1	NA	NA
Non-ortho PCBs					
PCB-77	96*	3*	5*	0	0
PCB-126	90*	6*	12*	0	0
PCB-169	72*	13*	42*	1	2
PCDDs and PCDFs					
2378-TCDF	NA	NA	NA	46	59
2378-TCDD	NA	NA	NA	44	62
12378-PeCDF	NA	NA	NA	62	82
23478-PeCDF	NA	NA	NA	63	92
12378-PeCDD	NA	NA	NA	67	105
123478-HxCDF	NA	NA	NA	97	119
123678-HxCDF	NA	NA	NA	90	100
234678-HxCDF	NA	NA	NA	96	112
123789-HxCDF	NA	NA	NA	92	109
123478-HxCDD	NA	NA	NA	99	128
123678-HxCDD	NA	NA	NA	85	90
123789-HxCDD	NA	NA	NA	88	102
1234789-HpCDF	NA	NA	NA	95	96
1233678-HpCDD	NA	NA	NA	81	83
OCDF	NA	NA	NA	72	64
OCDD	NA	NA	NA	67	58
Fat recovery (%)	98.7	0.7		0.1	

***-caculated as percentage of total recovery in Fraction A and B**
DCM-dichloromethane

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