A NEW ACREDITATED METHOD DEVELOPED FOR THE ANALYSING OF POLYCHLORINATED-P-DIOXIN AND POLYCHLORINATED FURANS FROM FOOD AND FEED MATRIXES

Erik Sandell and Juha Kokkonen

VTT/PROCESSES, P. O. Box 1401, FIN-02044 VTT, Finland

Introduction

The need for more accurate determinations of polychlorinated-p-dioxin (PCDD) and polychlorinated furans (PCDF) are more important since the new EU legislation. The legislation stipulates that the dioxin content detected in fish are set to 4 ng WHO TEF/kg (fresh weight) fish. The legislation also pose that the detection limit of determination must be 0.1 ng /kg fish. The analytical method was tested for determination of the PCDD and PCDF content in Baltic Herring. Extraction efficiency of Accelerated Solvent Extraction (ASE) for n-hexane (HEX) and dichloromethane (DCM) were evaluated. The clean up used was based on a column consisting of AX-21 activated carbon on glass fibre [1].

Methods and Materials

Sample preparation included the measuring of length , weight and fat content of each fish used. The fish was then split into two equal specimen (divided in two fillet), one for ASE-extraction with HEX and the other one for ASE -extraction with DCM. The developed and tested method uses certified standard solutions. The certified standards (CRM-614) used were: Solutions S 0-S 5 for calculation of response factors and testing the mass spectrometer linearity, solutions S 6, S 7 for quantitation and solution S 8 for recovery calculations of added ^{13}C-labeled standards. The standards were originally developed for the standard method for emission samples measured using the standardised method ISO SFS EN-1948: Part 1-3.

The ASE-extraction (ASE 300, 34 ml extraction cells) methods used where HEX, 100 ºC, 1500 psi, heat time 5 min, static time 5 min, flush volume 60 %, purge time 90 s, static Cycles 2 (total extraction time 17 minutes)[2] and DCM 125 ºC, 1500 psi, heat time 5 min, static time 5 min, flush volume 60 %, purge time 90 s, static Cycles 2 (total extraction time 17 minutes)[3]. The extracted sample size was 10 g fish and 10 g Na_2SO_4 that was mixed together before the extractions.

The sample size for the Soxhlet extraction was 20 g fish + 20 g Na_2SO_4.

The quantitation standards (S 6 and S 7) were added to the samples before the extractions.

The clean up consisted of basic silica/silica (fish column), AX-21 activated carbon on glass-fibre followed by basic alumina (ICN, Super grade I). The AX-21 column was prepared by cutting Whatman glass-fibre paper (non-bounded) into 0,3-0,5 cm pieces. The glass-fibre was shredded for 30 seconds by a Polytron homogenizer in 150 ml DCM. 50 mg AX-21 activated carbon was added and the mixture was stirred until the carbon was uniformly distributed. The column was packed into a glass column (10 cm long and inner diameter 10 mm).

The column was regenerated by the following solvent sequence 50 ml toluene, 50 ml methanol, 50 ml toluene, 50 ml DCM and 50 ml n-hexane. The sample was loaded in about 10 ml n-hexane and eluted as follows: 40 ml n-hexane, 180 ml DCM (organochloro aromatics OCs, PCBs and PBDEs). The dioxins and NO-PCBs were back eluted from the column by 180 ml toluene in reverse flow. The

samples were analysed after the carbon clean-up. Basic alumina clean up was implied if the samples still showed colour.

Gas chromatographic-high resolution mass spectrometry-selected ion monitoring (GC-HRMS-SIM) analyses were performed on a JEOL SX-102 double focusing mass spectrometer (BE-configuration) equipped with a HP-5890 GC Series II. The ionisation current was 600 µA, ionisation voltage 40 eV. The resolution used was 9000-10000. The capillary column used was a DB-5 (60 m, 0.32 mm id, 0.25 mm phase thickness). The gas chromatographic conditions used were, injection temperature 290 °C, splitless-injection 1.0 minute, transfer line temperature 290 °C and source temperature 250 ° C. The carrier gas used was helium (purity grade ^{3} 4.6) and the injection pressure about 15 psi (at 180 °C oven temperature). The oven temperature program used was, 180 °C(0 min)-4 °C/min-220 °C(12 min)-5 °C/min-235 °C(7 min)- 5 °C/min-330 °C(0 min). Total runtime was 51.0 minutes.

Results and Discussion

The fish samples extracted with the ASE using HEX (samples 1a and 3a) and DCM (sample 1b and 3b) gave the same results. The extraction efficiency was confirmed by the use of direct column extraction on top of the basic silica column (sample 2, so called fish column used by Smith, Stalling and Johnson 1984)[4]. The results are presented in the Table 1.

Table 1. The dioxin content in Baltic Herring, ng / kg fresh fish.

	Sample 1a ASE HEX	Sample 1b ASE DCM	Sample 2 fish column	Sample 3a ASE HEX	Sample 3b ASE DCM	Mean	ST DEV	ST DEV
Dioxin & Furan	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg	%
2,3,7,8-T4DD	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.7.8-P5CDD	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.4.7.8-H6CDD	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.6.7.8-H6CDD	0.48	0.76	0.84	1.6	1.3	1.0	0.45	45
1.2.3.7.8.9-H6CDD	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.4.6.7.8-H7CDD	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.4.6.7.8.9-OCDD	1.8	0.73	< 0.1	0.30	0.42	0.81	6.8E-01	84
2.3.7.8-T4CDF	5.8	7.0	3.2	5.6	6.3	5.6	1.4	26
1.2.3.7.8-P5CDF	1.3	1.9	1.9	2.6	1.1	1.8	0.59	34
2.3.4.7.8-P5CDF	5.0	5.6	7.8	10	10	7.7	2.4	31
1.2.3.4.7.8-H6CDF	< 0.1	< 0.1	0.43	< 0.1	< 0.1	0.43		
1.2.3.6.7.8-H6CDF	< 0.1	< 0.1	0.59	0.69	0.57	0.62	0.064	10
2.3.4.6.7.8-H6CDF	< 0.1	< 0.1	< 0.1	0.60	0.57	0.59	0.021	3.6
1.2.3.7.8.9-H6CDF	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.4.6.7.8-H7CDF	< 0.1	0.83	< 0.1	< 0.1	0.72	0.78	0.078	10.0
1.2.3.4.7.8.9-H7CDF	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
1.2.3.4.6.7.8.9-OCDF	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Sum PCDD & PCDF	14	17	15	22	21	18	3.6	20
WHO-TEF, ng/kg	3.2	3.7	4.5	6.1	6.1	4.7	1.3	28
Fat content. %	9.1	11.5		7.3	7.9			

The limit of determination for the method was 0.1 ng/kg fresh weight

A column packed with AX-21 activated carbon on glass-fibre was tested by analysing all fractions (hexane, dichloromethane and toluene). The added dioxins and furans (both native and ^{13}C-labelled were found in the toluene fraction. None of these compounds could be detected in the other fractions above the limit of determination. The performance of the AX-21 glass-fibre column for samples containing high lipid content was tested, by loading a 5 g fish oil sample diluted in 10 ml n-hexane. The sampling standard solution (20 µl of S 6) had been added to the sample before the application on the AX-21 column for recovery calculations. The column was eluted as described earlier in the method part and the quantitation standard solution (20 µl S 7) was added together with 20 µl dodecane to the injection ampoule (injection 1), bolded figures in the Table 2. After the injection the fish oil sample was cleaned once more using basic alumina (ICN Super Grade I) and 20 µl of S 8 was added as injection standard and 20 µl dodecane (injection 2), normal in the Table 2.

Table 2. The PCDD and PCDF content in the fish oil sample, and the recoveries of the labelled internal standards during the clean-up steps.

	5 g fish oil AX-21 injection 1	5 g fish oil AX-21 injection 2	Recovery of standards
Dioxin & Furan	ng/kg	ng/kg	ng/kg
2,3,7,8-T4DD	< 0.1	< 0.1	98.3
1.2.3.7.8-P5CDD	< 0.1	< 0.1	95.7
1.2.3.4.7.8-H6CDD	< 0.1	< 0.1	115
1.2.3.6.7.8-H6CDD	< 0.1	< 0.1	96.5
1.2.3.7.8.9-H6CDD	< 0.1	< 0.1	Injection std
1.2.3.4.6.7.8-H7CDD	< 0.1	< 0.1	87.1
1.2.3.4.6.7.8.9-OCDD	5.7	4.4	84.4
2.3.7.8-T4CDF	10	12	88.4
1.2.3.7.8-P5CDF	1.0	1.2	**99.3**
2.3.4.7.8-P5CDF	1.8	1.9	92.6
1.2.3.4.7.8-H6CDF	< 0.1	< 0.1	100
1.2.3.6.7.8-H6CDF	< 0.1	< 0.1	88.6
2.3.4.6.7.8-H6CDF	< 0.1	< 0.1	94.5
1.2.3.7.8.9-H6CDF	< 0.1	< 0.1	**103**
1.2.3.4.6.7.8-H7CDF	< 0.1	2.9	91.4
1.2.3.4.7.8.9-H7CDF	< 0.1	< 0.1	**94.4**
1.2.3.4.6.7.8.9-OCDF	2.1	< 0.1	87.1
Sum PCDD & PCDF	21	22	
WHO-TEF, ng/kg fresh fish	2.0	2.2	

The method performed well for all tested samples (over twenty samples)[5]. The first AX-21 column made is still in use. The blank samples run for every 5 sample has proven that the re-used column has no detected memory effects of previous samples. The amount of fat (5 g) did not affect the AX-21 glass-fibre column performance. The AX-21 activated carbon glass-fibre column has the advantages of, very low background, repeated usability (tested for more than 30 samples) and good performance for samples containing high lipid levels (5 g).

ANALYSIS I

The Baltic Herring analysed was from the Turku archipelago. The mean dioxin value found was 4,7 ng TEF/ kg fresh weight. This is 0.7 ng TEF/ kg fresh weight over the new EU legislation limit. Finland and Sweden are allowed an exception until 2006. Fish at higher levels (< 4ng WHO TEF/kg fresh fish) allowed only for sale on the market in these two countries. In the year 2007 the tested values should be 4 ng TEF/kg fresh weight or below.

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Reverences

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