Integrated PLE-multi step automated clean-up and fractionation for the measurement of dioxins and PCBs in food and feed

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Introduction

Sample preparation-fractionation for the measurement of dioxins and PCBs in biological matrices is a complex field of investigation[1]. Several different approaches are possible, but the common point is to aim for coupling and automation to reduce and simplify the inputs. Three years ago, we started a new project based on the direct coupling of pressurized liquid extraction (PLE) with automated multi-sorbent clean-up and fractionation[2]. The design of the early prototype system has evolved through several generations of changes dictated by a long term testing exercise. This paper reports on the latest data that were obtained using this system for food and feed samples dedicated to dioxin and PCB analyses.

Materials and Methods

Samples. Two types of quality control (QC) samples were tested. Fortified yolk QC samples have been prepared from commercial eggs that were pealed and from which the yolk was separated from the white part before lyophilization. The estimated mean levels were 1.71 pg PCDD/F TEQ/g fat and 0.94 pg NO-PCB TEQ/g fat. The fat content of the lyophilized yolk was 50.53%. Between 4.5 g and 5.5 g of yolk (d.w.) were extracted. The unfortified animal feed QC sample was made of products of vegetable origin. The PCDD/F and PCB content is issued from a small scale inter-calibration study. The levels were 2.54 pg PCDD/F TEQ/g d.w. and 0.05 pg PCB TEQ/g d.w. Between 29 g and 31 g of feed (d.w.) were extracted. Blank levels were measured with sodium sulfate inside the PLE cells. The ^{13}C-labelled standards containing PCDD/Fs and selected PCBs were added to the extraction cell prior to extraction and clean-up.

Extraction and clean-up. Extraction cells were 28 cm length and 2.2 cm of diameter for a volume of approximately 100 ml. Such size permits the extraction of adequate quantities of materials. The stainless steel parts (the main core, the quick connectors, the frits) of the PLE cells were washed after each run. Viton and Peek washers were solvent washed and visually inspected before reuse. The HPLC system (pump and valves) is located in an individual module separated from the low pressure module and can operate at pressures up to 2500 psi. The PLE cell is attached outside the module via quick connectors and the movable oven is closed around it during extraction. The extraction pressure and temperature are recorded by the software during the run and can be reviewed afterwards for QA/QC purposes. The utilization and maintenance of the low pressure part of the system has been described elsewhere[3].

Extract concentration. Extracted and purified extracts are collected in evaporation tubes and the solvent volume is reduced to 200 µl using a Turbovap II workstation (Caliper Life Sciences, Teralfens, Belgium). This volume is transferred in GC vials containing 4 µl and 90 µl of nonane (keeper) in the case of PCDD/Fs and PCBs, respectively. The GC vials are further evaporated of their toluene or hexane-dichloromethane (1:1) mixture content using a RapidVap evaporation system (Labconco, Kansas City, MO, USA).

Measurements. GC-IDHRMS was used. PCDD/Fs and non-*ortho*-PCBs were measured on an Autospec Ultima (Micromass) coupled to an Agilent 6890 Series GC. The column was a 50 m VF-5MS (0.20 mm ID x 0.33 µm df) (Varian). Mono-*ortho*-PCBs and indicator PCBs were measured on an MAT95XL (ThermofinniganMAT) coupled to an Agilent 6890 Series GC. The column was a 25 m HT-8 (0.22 mm ID x 0.25 µm df) (SGE). The analyses were performed under ISO17025 quality criteria.

Results and Discussion

The current plumbing of the system is depicted in Figure 1. The HPLC pump uses hexane during the extraction and

dichloromethane for the wash. The pressure transducer allows computer recording of the pressure during the run. Nitrogen is available for the purge of the PLE cell after extraction. A 60 micron stainless steel filter is present after the PLE cell to ensure particle free solvent after extraction. The pressure relief valve is set at the extraction value (1500 psi) and is responsible for maintaining the pressure to the set point during the extraction. Eluents issued from this valve are directed to the clean-up column to ensure proper transfer of extracts exiting the PLE cell during the static extraction step (Figure 2). Extracts are purified on the multilayer silica column[3].

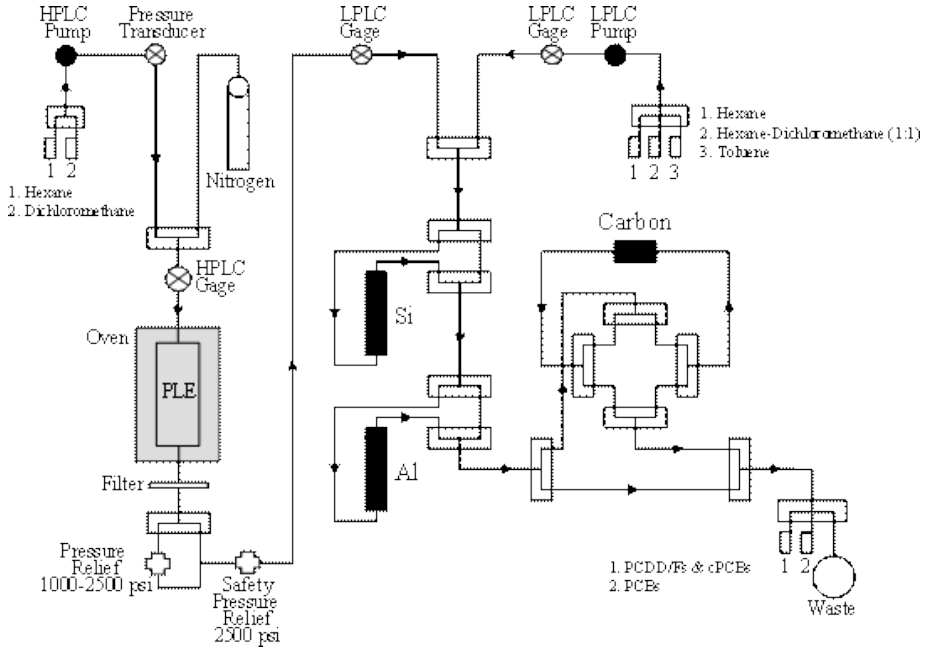

Figure 1: Plumbing diagram of the system.

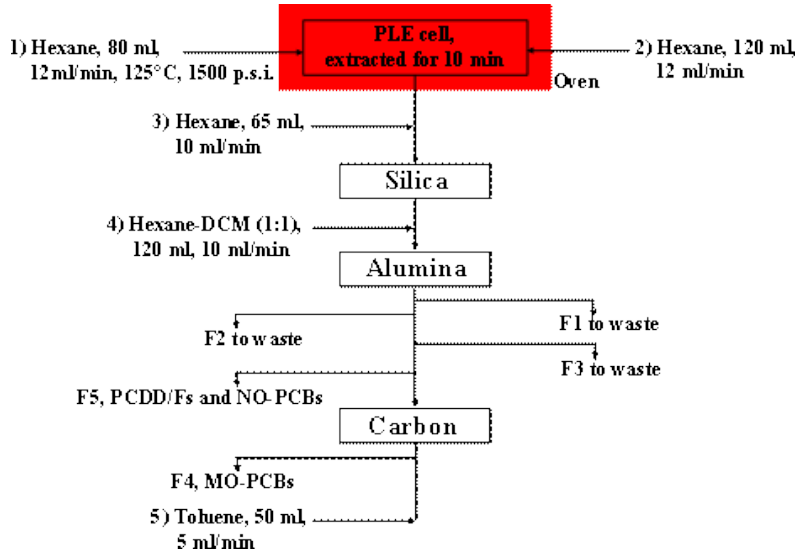

Figure 2: Flow diagram for the validated on-line extraction and clean-up approach.

Dioxins and PCBs are fractionated using the basic alumina and the carbon columns. The entire clean-up run has been simplified and validated. In practice, the entire run time is 1.5h and can be divided in 3 main parts: 1) the column conditioning (30min), 2) the extraction step (30min for 1 extraction cycle), and 3) the clean-up step.

As the PLE cell is filled up with solvent, it is already lined up with both the multilayer silica and the alumina columns to

ensure the transfer of early extracted analytes once the PLE cell is full of solvent and starts to leak out on the multilayer silica column. In the mean time, it is important to ensure that the air content of the PLE cell is not transferred to the clean-up columns for proper retention and fractionation of the analytes. PCB retention on alumina, especially for MO-PCBs, is sensitive to the presence of air inside the column.

Recovery rates for PCDDs, PCDFs, and NO-PCBs are listed in Table 1. They ranged from 62% to 87% with good reproducibility. Table 1 also shows the comparison to the assigned value of the yolk QC on a congener basis in terms of concentrations and TEQs. The larger deviations for NO-PCBs are probably correlated to the clean-up column drying and are under investigation.

Table 1: Concentrations and recovery rates for the yolk QC samples.

	Eggs (n=8)					
	Mean concentration (pg/g fat)	RSD (%)	Assigned value	Relative deviation from assigned value	Recovery (%)	SD
PCDDs						
2,3,7,8 - Tetra CDD	0.48	21	0.45	5	82	7
1,2,3,7,8 - Penta CDD	0.53	17	0.57	6	85	10
1,2,3,4,7,8 - Hexa CDD	0.54	16	0.46	17	63	18
1,2,3,6,7,8 - Hexa CDD	0.70	19	0.54	30	62	10
1,2,3,7,8,9 - Hexa CDD	0.50	15	0.47	6	79	14
1,2,3,4,6,7,8 - Hepta CDD	<LOQ	-	5.12	-	84	11
Octa CDD	<LOQ	-	0.00	-	87	13
PCDFs						
2,3,7,8 - Tetra CDF	0.70	18	0.41	69	63	11
1,2,3,7,8 - Penta CDF	0.63	18	0.52	22	82	5
2,3,4,7,8 - Penta CDF	0.60	16	0.46	30	75	9
1,2,3,4,7,8 - Hexa CDF	0.54	18	0.37	45	84	16
1,2,3,6,7,8 - Hexa CDF	0.55	16	0.51	10	85	13
1,2,3,7,8,9 - Hexa CDF	0.53	19	0.49	8	70	18
2,3,4,6,7,8 - Hexa CDF	0.46	19	0.48	3	84	13
1,2,3,4,6,7,8 - Hepta CDF	0.34	24	0.49	1	66	11
1,2,3,4,7,8,9 - Hepta CDF	0.58	45	0.60	4	-	-
Octa CDF	<LOQ	-	0.36	-	67	14
Total TEQ PCDD/Fs	1.80	18	1.71	5	-	-
NO-PCB						
PCB 77	<LOQ	-	nd	-	74	15
PCB 81	16.43	51	10.36	59	66	17
PCB 126	15.71	75	8.69	81	68	16
PCB 169	12.75	100	6.74	89	70	18
Total TEQ NO-PCBs	1.70	77	0.94	81	-	-
Total TEQ PCDD/Fs & NO-PCBs	3.50	41	2.65	32	-	-

For those yolks, one and 3 extraction cycles were tested and one cycle showed to be sufficient. Purging of the PLE cell after extraction was either carried out using nitrogen or fresh hexane. Both ways were efficient but the use of hexane is preferred because it does not yield to undesirable clean-up column drying. Figure 3 shows the PCDD/F TEQ data versus the assigned value in a QC-type plot showing the 95% confidence interval based on the mean value ± 2SD. None of the new method data felt outside this 95% interval.

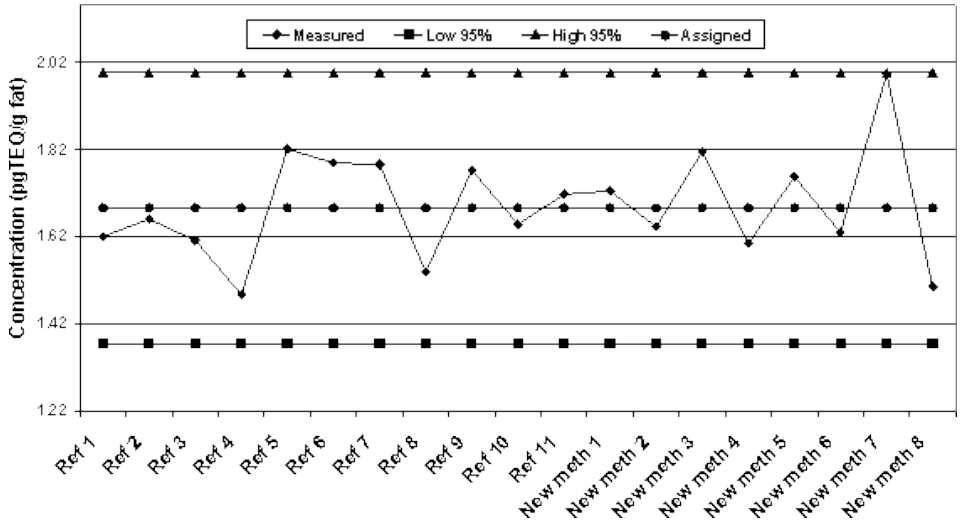

Figure 3: QC chart for the yolk QC samples prepared using the reported method.

For animal feed QC, the extraction solvent was also hexane. Three extraction cycles were performed to ensure efficient extraction. The sum of the PCDD/F and NO-PCB TEQs was 2.19±0.13 ng/kg of dry weight. The assigned value was 2.59±0.39 ng/kg of dry weight, thus the deviation from this value was an underestimation of 16%.

No differences were observed regarding the extract quality compared to other routinely used extraction and clean-up methods in the laboratory.

On a practical point of view, a set of three animal feed samples can be received in the laboratory at 8.00 AM, extracted and cleaned-up by lunch time, evaporated by 3.00 PM, GC-MS injected by 4.30 PM, and reported after QA/QC verifications by 6.00 PM.

Acknowledgements

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