FAST ANALYSIS OF PCDD/F AND DL-PCBs IN FOOD SAMPLES BY AUTOMATED PLE EXTRACTION AND CLEAN-UP

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Abstract

A fast, selective and sensitive automated PLE extraction followed by a modified clean-up procedure have been optimized for the analysis of dioxins (PCDD), furans (PCDF) and dioxin-like PCBs (DL-PCBs) in fish samples. The extractions were performed on a new extraction apparatus, Power-Prep/PLE, in combination with an automated clean-up system, Power-Prep™ clean-up through multi-layer silica (acidic, basic and neutral), basic alumina and AX-21 carbon columns. Extraction of the wet fish samples mixed with anhydrous sodium sulphate allowed to avoid the freeze-drying step, which is time consuming. During the clean-up process, the DL-PCBs were fully separated from the PCDD/F in order to have all the 12 DL-PCBs together in a separate fraction from the PCDD/Fs. The results showed the feasibility of the extraction of wet fish mixed with anhydrous sodium sulphate using Power-Prep/PLE system, combined with Power-Prep™ automated clean-up, as a new and fast method to carry out the analysis of fish samples in a short time.

Introduction

In the last years, advanced analytical techniques and new strategies have been developed in order to have fast and less tedious methods[1, 2] for the determination of dioxins (PCDD), furans (PCDF) and dioxin-like PCBs (DL-PCBs) in different types of matrices. In addition, the governments have established the maximum contents for this kind of contaminants at low levels that makes constantly the need of develop new analytical strategies. Nowadays the feasibility of the analysis is conditioned to the capability of laboratories to be able to give a quick answer in front of a critical situation or contaminant episode.

The analysis of PCDD/Fs and DL-PCBs is still tedious and time consuming. In the last years, new extraction techniques had emerged and gave such advantages as lower solvent consumption, suitability for automation and less analytical time consuming[3,4]. One of these emergent extraction techniques is the pressurised liquid extraction (PLE), which use high pressure solvent in order to perform the extraction at temperature greater than the normal boiling point of the extracting solvent. As a result, few minutes are needed to perform a quantitative extraction of the samples instead of hours for classical methods such as Soxhlet extraction[5].
The objective of the present work, is to evaluate an alternative method to tedious and time consuming conventional extraction methodologies for the analysis of PCDD/Fs and DL-PCBs in fish samples, using automated extraction and clean up methods.

Materials and methods

PLE system: Pressurised liquid extractions[3,4] (PLE) were performed with an automated Power-prep/PLE extraction system (FMS Inc., MA, USA). Figure 1 shows a diagram of the system used in this work. The extraction cell is made of stainless-steel, and supplied with quick connect Teflon end caps and filters, the PLE cell and end cap filtration is disposable, in order to avoid the carry over. The PLE system is controlled by means of a PC using software (DMS6000) that shows in real time the pressure, temperature, pump, flow rate, solvent, time, valves state and cooling system. This parameters can be programmed, controlled, monitored and recorded during the extraction run and can be revised afterwards.

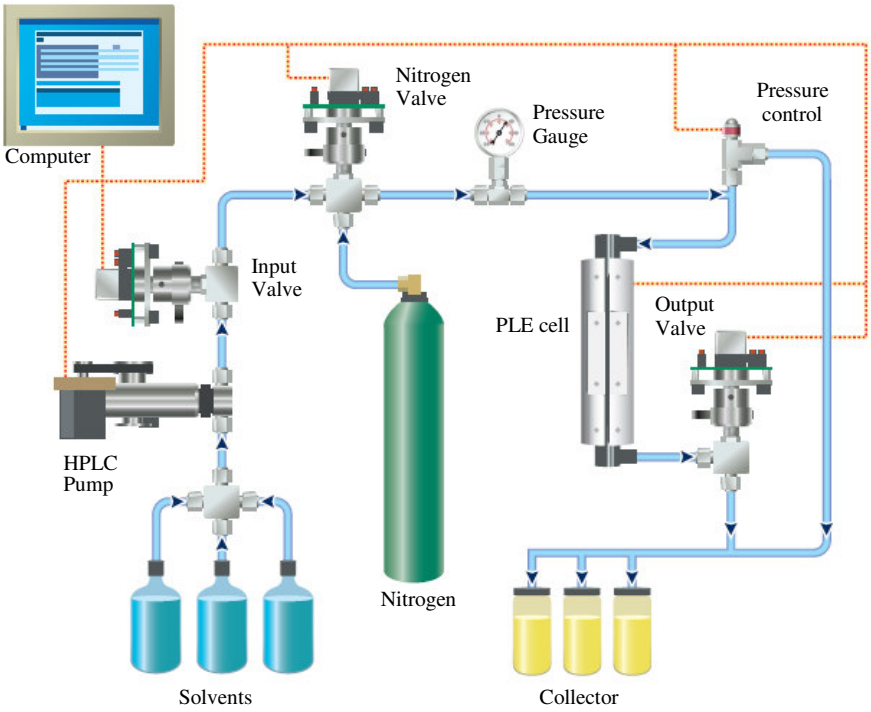

Figure 1 . Diagram of the Power-Prep/PLE system.

Fish samples: Six fish samples were analysed in order to evaluate the automated Power-prep/PLE extraction system (FMS Inc., MA, USA). About 60 g of fresh fish sample was mixed with anhydrous sodium sulphate. The extraction cell was filled with the sample ensuring no free space inside the cell. The PLE system parameters were: hexane:dichloromethane (1:1) as extraction solvent, 125ºC and 1500 psi. Two static extractions of 12 min were performed. The extract was pushed out of the cell with N_2. Afterwards Organic components, fat and other interfering substances were removed by treating the n-hexane extracts by a silica gel column coated with sulphuric acid. The extracts were then concentrated and filtered prior to the next clean-up step.

Clean-up: Purification was accomplished by automated Power-Prep™/Sample Clean-up system ((FMS, Fluid Management Systems, inc., MA, USA). Hexane extracts were loaded and pumped through individual sets of multilayer silica followed by a basic alumina column with n-hexane. All 12 DL-PCBs were eluted from the alumina column with hexane:dichloromethane (9:1), this step is a modification of the standard program to rich DL-PCBs in the same fraction. PCDDs/Fs were eluted from the alumina column and transferred to the PX-21 carbon column with hexane:dichloromethane (1:1). The interferences were eluted on carbon column using ethyl acetate:toluene (1:1) in the forward direction, and PCDDs/Fs were collected from the carbon column in the reverse direction with toluene[5].

The criteria for ensuring the quality of dioxin analysis include the application of some quality control (QC) and quality assurance (QA) measures, such as a continuous monitoring of laboratory contamination based on the determination of a blank sample covering the whole analytical procedure, including extraction, clean-up and quantification.

Instrumental analysis: Instrumental analysis performed by HRGC/HRMS (Autospec Ultima NT, Waters, Manchester, UK), a high resolution 60m x 0.25 mm i.d. x 0.25µm film thickness DB-5ms fused silica column (J&W Scientific, CA, USA) were used.

Results and discussion

The Power-Prep/PLE System is more versatile and rugged than conventional extraction techniques[3,4]. To determine the feasibility of the proposed Power-Prep/PLE system combined to a Power-Prep™ clean-up system, six fish samples were selected for his high level of PCDD/F and DL-PCBs in comparison with others food samples. In addition, a new fractionation solvent mixture on the alumina column of the Power-Prep clean-up system was studied in order to obtain all DL-PCBs and fully separated from the PCDD/F fraction.

Table 1. Results of fish samples.

	Fish 1		Fish 2		Fish 3		Fish 4		Fish 5		Fish 6	
Congener Dioxins/Furans	pg/g f. w	R C^{13} (%)	pg/g f. w	R C^{13} (%)	pg/g f. w	R C^{13} (%)	pg/g f. w	R C^{13} (%)	pg/g f. w	R C^{13} (%)	pg/g f. w	R C^{13} (%)
2,3,7,8-TCDF	0.015	61	1.141	86	0.917	74	0.341	70	0.018	68	0.086	67
1,2,3,7,8-PeCDF	0.005	70	0.129	78	0.120	75	0.064	72	0.007	71	0.034	78
2,3,4,7,8-PeCDF	0.004	74	0.189	74	0.177	75	0.027	73	0.005	76	0.016	83
1,2,3,4,7,8-HxCDF	0.003	70	0.019	80	0.022	77	0.010	65	0.003	77	0.016	76
1,2,3,6,7,8-HxCDF	0.003	68	0.022	82	0.032	77	0.008	66	0.003	78	0.011	77
2,3,4,6,7,8-HxCDF	0.003	70	0.037	74	0.050	72	0.023	65	0.004	75	0.013	76
1,2,3,7,8,9-HxCDF	0.002	73	0.010	72	0.005	77	0.003	70	0.003	74	0.002	79
1,2,3,4,6,7,8-HpCDF	0.005	66	0.026	64	0.062	73	0.062	64	0.009	68	0.011	70
1,2,3,4,7,8,9-HpCDF	0.002	61	0.009	61	0.009	69	0.006	58	0.002	63	0.002	66
OCDF	0.004	n.p.	0.016	n.p.	0.031	n.p.	0.075	n.p.	0.004	n.p.	0.004	n.p.
2,3,7,8-TCDD	0.002	65	0.052	89	0.042	78	0.016	72	0.002	71	0.015	69
1,2,3,7,8-PeCDD	0.005	84	0.073	76	0.068	78	0.035	83	0.003	77	0.008	89
1,2,3,4,7,8-HxCDD	0.002	73	0.011	82	0.011	75	0.013	69	0.004	75	0.002	80
1,2,3,6,7,8-HxCDD	0.002	73	0.046	79	0.053	75	0.032	69	0.003	78	0.013	80
1,2,3,7,8,9-HxCDD	0.002	n.p.	0.013	n.p.	0.016	n.p.	0.018	n.p.	0.004	n.p.	0.006	n.p.
1,2,3,4,6,7,8-HpCDD	0.010	62	0.035	67	0.078	75	0.142	63	0.013	64	0.014	69
OCDD	0.024	55	0.086	50	0.129	69	0.421	50	0.034	63	0.027	57
WHO-TEQ (pg/g fresh weight)	0.01		0.36		0.32		0.11		0.01		0.05	
PCBs No Ortho												
PCB # 81	0.02	74	0.7	66	0.6	55	0.1	46	-	85	0.06	91
PCB # 77	0.42	74	20.3	73	17.6	66	3.0	57	0.52	83	1.38	90
PCB # 126	0.12	77	8.1	80	6.8	76	0.2	72	0.23	80	0.47	85
PCB # 169	0.02	91	1.6	65	1.4	80	-	80	0.04	82	0.09	97
PCBs Mono-ortho												
PCB # 123	0.25	72	25.6	74	24.3	85	0.8	62	0.9	74	0.6	84
PCB # 118	16.25	74	541.6	65	475.3	76	61.9	60	37.4	75	44.9	86
PCB # 114	0.31	79	31.5	70	24.8	77	0.4	56	0.8	74	0.4	98
PCB # 105	6.10	77	419.1	63	372.6	69	20.1	54	14.1	76	13.8	89
PCB # 167	0.95	81	104.4	71	85.6	76	2.4	72	2.3	81	3.56	85
PCB # 156	1.60	83	149.3	56	123.7	65	1.7	57	3.7	82	4.2	89
PCB # 157	0.39	82	43.0	51	35.3	57	1.2	51	0.9	82	1.4	88
PCB # 189	0.16	92	14.7	70	12.8	85	0.1	77	0.4	101	0.6	91
WHO-TEQ (pg/g fresh weight)	0.015		1.16		0.97		0.036		0.032		0.057	

The rule of this work was to perform a rapid, sensitive and selective analytical method for the determination of PCDD/Fs and DL-PCBs in fish samples, the freeze-drying step was avoid, allowing us to save time. However, the samples had to be mixed with an equal amount of anhydrous sodium sulphate to remove the excess of water. Table 1 shows the results obtained for PCDD/Fs and DL-PCBs with the proposed method. As it can be observed in table 1, the recoveries were between 50 and 90 % for PCDD/Fs and between 60 and 90% for DL-PCBs. In addition, to the recovery rates, preliminary control test and QC samples analyzed showed in a good agreement with the classical extractions methods for dioxins and DL-PCBs analysis.

The fractionation of the PCDD/Fs and DL-PCBs was study. The proposed fractionation in the automated Power-prep™ clean-up system use n-hexane:dichloromethane at 9:1 instead of 98:2 as a mixture of solvent for the elution of the alumina column. All DL-PCBs elutes in this fraction and the PCDD/Fs elutes from carbon column together. With this fractionation, recoveries achieved were between 60 and 90 % for DL-PCBs and between 50 and 90% for PCDD/Fs. Recovery rates found with this modification allow us to improve the analysis of PCDD/Fs and DL-PCBs since only two MS analysis are required instead of three MS analysis with the older fractionation.

The proposed extraction and clean-up system allows a single operator to carry out the extraction and clean-up of PCDD/F and DL-PCBs analysis in about two days. And not more than 3 days from the beginning of the entrance of the sample to the laboratory until to final analysis results. The effectiveness of the new Power-prep/PLE system combined with the robustness of the Power-Prep System™ make this combination a powerful tool for analysis of food samples.

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