AN AUTOMATED EXTRACTION/SAMPLE PREPARATION METHOD FOR THE DETERMINATION OF PCDD/F, DLPCBs, BDEs AND PCNs IN BIOTA IN A SINGLE ANALYSIS

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Introduction

Human health not only depends on providing good medical care but also on the priority given to prevent exposure to environmental and other health risks. Persistent Organic Pollutants (POPs) are organic compounds typically of anthropogenic origin that resist degradation and accumulate in the food chain and are associated with adverse effects on human health and the environment[1]. Due to their toxicity to humans, at much lower concentration than other pollutants, it is important to monitor compounds like polychlorinated dioxins/furans (PCDD/Fs), dioxin like polychlorinated biphenyls (DLPCBs), polybrominated diphenylethers (BDEs) and polychlorninated Naphthalenes (PCNs). More sophisticated requirements are needed for their analysis[2]. It is well known that the sample preparation is an important and costly step in the analysis of persistent organic compounds.

In the past, extraction and clean-up of POPs present in fish and biota samples were conducted with procedures such as Soxhelt extraction, acid digestion and liquid-liquid extraction[3]. The clean-up of these samples was accomplished through chromatographic columns using different types of adsorption media such as silica, alumina and carbon. These analytical methods used for analysis not only pose risk through human exposure, but are also typically labor intensive (repetitive manipulations), time consuming, and have high costs. In an effort to alleviate some of these problems an automated extraction and clean-up system was investigated.

A previously reported analytical method involved automated sequential extraction performed on the ASE (Accelerated Solvent Extraction) System and automated clean-up performed on the FMS Power-Prep system.[4] While this system produced reliable results, this sequential process was time consuming resulting in lower output of data. Therefore, a new automated method for extraction and clean up was developed for the specific isolation of PCDD/Fs, DLPCs, BDEs and PCNs from fish and biota samples in one extraction, followed by clean up using only two columns to allow for complete fractionation of the target compounds is required.

Materials and Methods

Three to five gram samples were mixed with diatomaceous earth and loaded into an extraction cell. A mixture of Hexane and Dichloromethane solvents were used for extraction on the FMS automated pressurized liquid extraction (PLE) System. (Fig. 1). The Sample extracts were concentrated to ~ 1ml using a rotary evaporator prior to cleanup on the FMS Power–Prep System (Fig. 2).

All analyses were performed on a MicroMass Autospec GC-HRMS. An HP 6890 gas chromatograph interfaced to the mass spectrometer. The PCDDs/PCDFs and coplanar DLPCBs were analyzed as described in MOE[5] method 3418, PCNs were analyzed using method 3431[6] and BDEs were analyzed using MOE method 3430[7].

A number of samples and reference materials were analyzed to validate the new method; QC Freeze Dried fish tissue (CRM-WMF-01 – Wellington Laboratories, Guelph Canada) (Fig. 3) and CRM-CARP-02 (NRC – Ottawa, Canada); and contaminated natural fish certified reference material (CIL, Andover MA) (Table 1). Blank wet fish tissue (Alaskan Pollock) was used for MDL determination. After homogenization the wet fish tissue samples are mixed with drying agent (Diatomaceous Earth) to remove moisture as well as a packing material for the PLE cells. Freeze Dried fish tissue was mixed directly with the drying agent (Diatomaceous Earth). Samples are then spiked with $^{13}C_{12}$ labeled surrogates prior to extraction.

Automated Extraction and Clean-up

Automated extractions were performed on the FMS PLE system, 40 ml stainless steel cells were filled with the sample/diatomaceous earth mix. The total extraction time for 6 samples using two cycles was 120 min and the volume of solvent used was ~ 160-170 ml. The extracted sample was then concentrated to ~ 1ml using a rotary evaporator and quantitatively transferred to 40ml vials diluted to ~35ml with Hexane to start the clean-up procedure. The clean-up procedure was carried out with the Power- Prep system using pre-packed Teflon sealed PCB-HCDS-ABN then PCBC-CCE columns for the fractionation of the target compounds.

Fig .1. General schematic diagram of PLE system

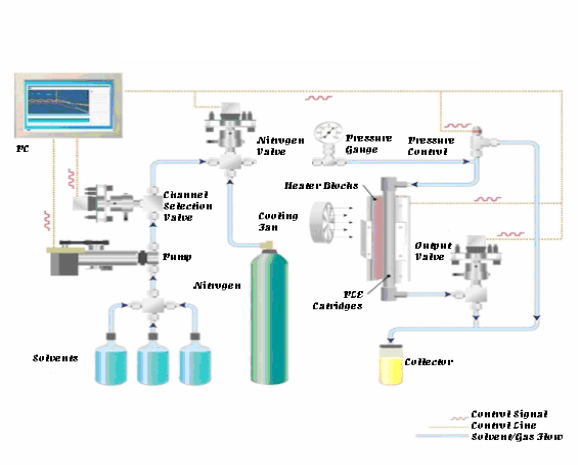
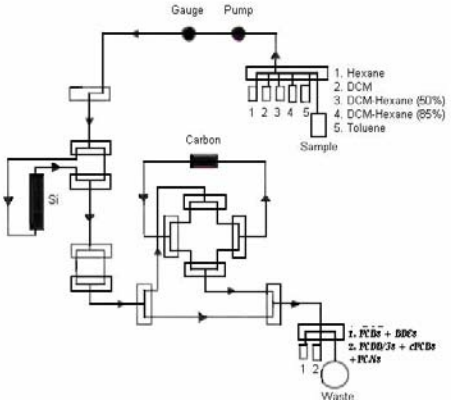


Fig. 2. General schematic diagram of Power-Prep system



Results and Discussion

Method development studies have produced accurate results using real fish samples (see Table 1). Results are well within the acceptable range for the majority of the analytes. Analysis of contaminated natural matrix reference material yielded good recoveries for most of (labeled compounds) ranging between 74% and 101% for dioxins/furans, 103% and 130%, for DLPCBs 61% and 125% for BDEs and 16% to 85% for PCNs. The recovery rates averaged a little lower for the first two PCN congeners due to the fact that they are more volatile than other congeners and this problem will be addressed in the near future. During the study the problem of diphenyl ethers interfering with PCDFs was overcome by adjusting the pressure, temperature and the solvent mix used during the extraction step. The method is able to separate the planar compounds – dioxins/furans, nonortho-PCBs and PCNs into one fraction and non-planar compounds – BDEs and mon-ortho PCBs into another froaction using only silica and carbon columns. The alumina column was eliminated in the cleanup scheme because it was causing the splitting of PCNs into fractions and lower recoveries for the DLPCB and PCNs. High capacity Silica ABN columns were used instead of classical Silica ABN columns enabling the elimination of the alumina stage which was not needed due to the low amounts of interfering analytes in fish samples. All tests carried out during method development studies included Spiked Procedure Blanks (SPBs) for QC purposes. No significant differences were observed between blanks values, also risks of contamination through carry- over on the PLE and Power- Prep Systems was addressed by using samples that had different level of analytes and it was found that a single wash of the systems using a mixture of solvents was sufficient to avoid any carry-over contamination.

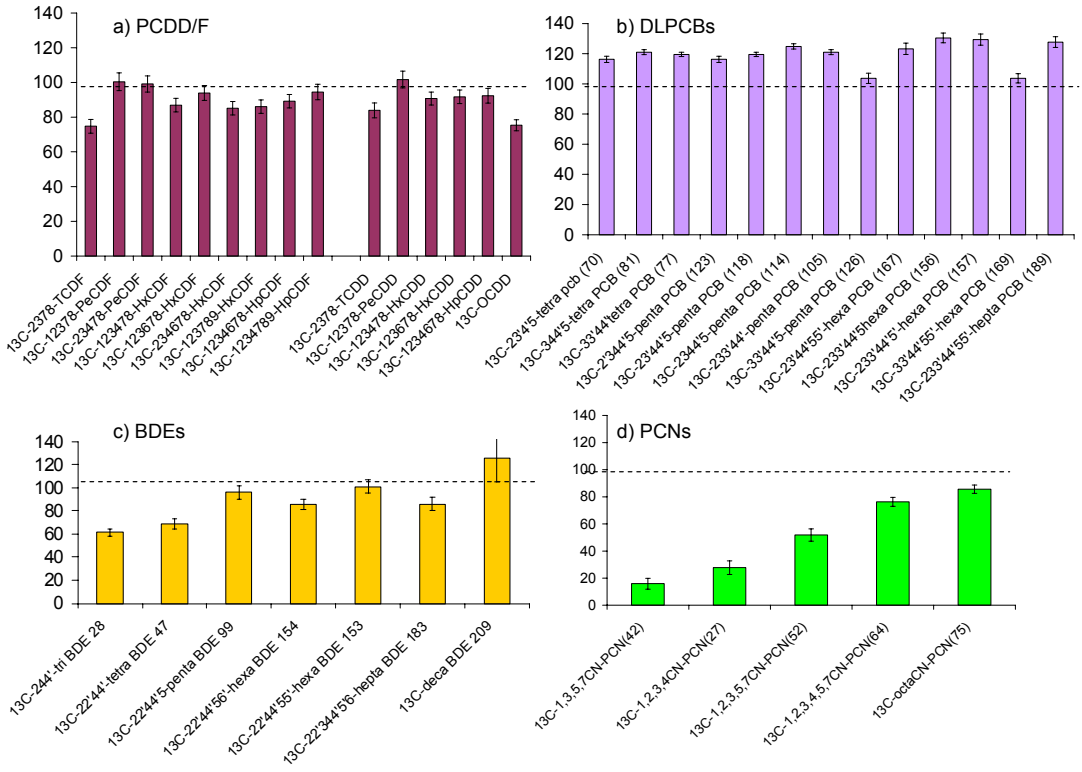
Conclusions

The automated extraction of a sample for PCDDs/PCDFs, DLPCBs, PCNs and BDEs in fish and biota matrices in one extraction followed by an automated clean-up can save time, solvent ,cost and lower the possibility of any health hazards. The automated extraction is less labour-intensive and can run parallel samples unattended. The overall process for this method between extraction and cleanup has demonstrated the ruggedness, accuracy, repeatability and a significant increase in productivity for sample extraction/preparation by reducing the time of sample preparation from about 4-5 working days for each target analyte group to 4 working days for all four POPs in single extraction. Also, this method has lower method detection limits than the separate methods, especially for the PCDD/F, DLPCBs and the PCNs and greater analytical precision and increased surrogate recoveries for BDEs.

Table 1. Accuracy of the new method for reference material RM-2525 and RM-2526

	RM-EDF 2525		RM-EDF 2526	
Compound	**Acceptable range pg/g**	**Result pg/g**	**Acceptable range pg/g**	**Result pg/g**
Polychlorinated dioxins and furans				
2378-TCDF	24.3 ± 4.74	26.7	18.7 ± 5.58	22.8
12378-PeCDF	4.58 ± 1.42	5.00	39 ± 7.36	31.0
23478-PeCDF	14.5 ± 4.04	13.4	37.8 ± 10.2	32.0
123478-HxCDF	5.95 ± 1.52	5.56	83.8 ± 23.0	64.9
123678-HxCDF	1.73 ± 0.54	3.94	62.8 ± 19.6	47.7
234678-HxCDF	1.04 ± 0.30	0.91	58.6 ± 14.2	44.2
123789-HxCDF	0.10 ± 0.20	0.17	57.3 ± 10.9	42.4
1234678-HpCDF	0.59 ± 0.44	0.60	81.6 ± 13.7	59.5
1234789-HpCDF	0.16 ± 0.32	0.24	76.7 ± 26.6	55.8
OCDF	0.38 ± 0.50	0.46	185 ± 57.4	134
2378-TCDD	17 ± 3.9	16.7	19.7 ± 4.18	18.8
12378-PeCDD	3.71 ± 0.90	4.47	39.9 ± 10.6	32.5
123478-HxCDD	0.33 ± 0.18	0.41	54.9 ± 7.8	40.1
123678-HxCDD	2.03 ± 0.60	1.96	51.1 ± 19.3	39.6
123789-HxCDD	0.30 ± 0.14	0.34	52.9 ± 18.1	42.1
1234678-HpCDD	0.48 ± 0.36	0.56	70.7 ± 23.2	57.5
OCDD	1.71 ± 1.38	1.86	181 ± 53.4	150
Polychlorinated biphenyls				
344'5-tetra PCB (81)	161 ± 74.0	199	3.0 ± 5.60	2.5
33'44'-tetra PCB (77)	1850 ± 834	2040	451 ± 179	546
2'344'5-penta PCB (123)	3280 ± 2020	6670	7.38 ± 9.58	25.6
23'44'5-penta PCB (118)	122000 ± 38000	122000	348 ± 392	468
2344'5-penta PCB (114)	3410 ± 1550	3890	7.73 ± 4.36	13.7
233'44'-penta PCB (105)	50100 ± 15700	5600	108 ± 73	178
33'44'5-penta PCB (126)	628 ± 242	654	431 ± 17.9	522
23'44'55'-hexa PCB (167)	7060 ± 3020	8070	12 ± 9.54	21.4
233'44'5-hexa PCB (156)	13100 ± 2620	14000	23.3 ± 23.8	34.2
233'44'5'-hexa PCB (157)	3380 ± 1010	3670	9.3 ± 9.16	12.1
33'44'55'-hexa PCB (169)	52.1 ± 14.0	53.8	512 ± 160	592
233'44'55'-hepta PCB (189)	1440 ± 498	1586	3.51 ± 2.76	5.1
Polybrominated diphenyl ethers				
244'-tri BDE 28	312 ± 202	330		
22'45'-tetra BDE 49	524 ± 274	550		
22'44'-tetra BDE 47	9080 ± 2620	9610		
23'44'-tetra BDE 66	262 ± 81.0	336		
22'44'6-penta BDE 100	1720 ± 566	1540		
22'44'5-penta BDE 99	2280 ± 472	2490		
22'44'56'-hexa BDE 154	2550 ± 1000	2930		
22'44'55'-hexa BDE 153	2030 ± 506	2040		
22'344'5'6-hepta BDE 183	137± 47.8	133		
Deca BDE 209	545 ± 1999	2440		

Fig. 3. Recovery of 10 reference material fish samples (mean ± SE) using fully automated system for extraction and clean-up for a) PCDD/F, b) DLPCBs, c) BDEs, and d) PCNs



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