Cod: 2.2014

FROM SAMPLE TO VIAL: TOTAL SOLUTION FOR POPS ANALYSIS IN SEA FOOD

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Introduction

The occurrence of polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs) and biphenyls (PCBs) in a variety of foods has been amply documented. This includes sea foods such as crabmeat and fish. Some evidence has been found for a relationship between concentrations in sediment and fish tissue.1 PCDD/Fs have been reported in crab meat from around the world, including the Netherlands.2

Analyses of crabmeat and fish samples using US EPA methods 1613 (PCDD/Fs) and 1668 (PCBs) have been carried out worldwide. Traditional Soxhlet extraction and sample clean up are time consuming and can result in data of low quality and reproducibility. As an alternative both the extraction and clean up steps can be automated. This paper describes the automation of Pressurized Liquid Extraction (PLE) and open column chromatography clean up of crab and fish tissues.

Materials and methods

Preparation

5-10 g of sample was mixed with inert material (Hydromatrix)® and spiked with 13C labeled isotope dilution standards. The samples were then transferred to stainless steel extraction cells. Remaining cell volume was topped off with Hydromatrix®.

Pressurized Liquid Extraction

The extraction cells were loaded onto the PLE system. Samples were extracted with a mixture of 50% dichloromethane and 50% hexane. The cells were pressurized to 1500 psi, heated to 120 oC and held at that temperature for 20 min. Afterwards they were cooled to ambient temperature and flushed with extraction solvent. In the final step the cells were purged with nitrogen gas. The samples collected in glass tubes were then reduced in volume under a nitrogen flow and exchanged to hexane.

Automated Column Chromatography Clean Up

The system consists of a control module, valve modules, pump modules, and sample processing modules. A touch screen computer has been built into the system. Programming of the various columns, solvent flows and volumes is done via a plumbing schematic. Chromatographic columns used are pre-packaged and vacuum sealed. A total of 4 columns were used: jumbo acid; regular size acid-base-neutral silica; alumina; and carbon. Solvents used for the sample clean up were hexane, 2%/98% v/v dichloromethane /hexane, 50%/50% v/v dichloromethane: hexane, 50%/50% v/v ethyl acetate/benzene, and toluene. Silica and alumina columns were conditioned with hexane and the carbon was conditioned with each of the solvents. Flow rates varied from 5-10 mLs/min. PCBs were collected in the 2%/98% and 50%/50% dichloromethane/hexane steps, whereas PCDD/Fs were collected in toluene. A typical program contained 25 steps with a total time of 90 min. New developments include using 3 columns (high capacity acid-base-neutral silica, alumina and carbon) instead of 4 and reduced volume and time programs.3

Concentration

Samples were reduced in volume in a 6 position evaporator: pre-heated for 20 min at 45-55 oC, followed by heating under nitrogen at ~ 6-8 psi. The evaporator has built-in sensors that shut off the nitrogen flow when the sample reaches ~ 0.5 mLs of volume. Further nitrogen blow down in a vial evaporator reduced the final sample volume to 10 uL. Recovery standards were then added.

Figure 1. Automated cleanup system, PLE, and evaporator.

Analysis

Samples were analyzed on a high resolution Thermo DFS GC/MS with a Trace 1310 GC containing a 60 m DB-5 like column. Temperature programs used were ~ 35 min for PCBs and ~ 55 min for PCDD/Fs.

Results and Discussion
Table 1 with native fish tissue values, reference material values and 13C-labeled recoveries.

Table 2 with native crab meat values and 13C-labeled recoveries.

PCBs data for fish is shown in Table 1. Excellent agreement was found between the PCBs concentrations measured in our laboratory and the reference values listed for the fortified fish tissue. 13C recoveries of the labeled compounds were very good.

Table 2 shows native PCDD/Fs levels in crab meat and 13C labeled recoveries. Native levels were low with OCDD being the highest at 0.35 pg/g. Excellent 13C labeled recoveries of the isotope dilution standards were observed.

Both crab meat and fish were easily and reliably processed with the combined automated extraction and clean up. With extraction times of ~ 60 min and sample clean up taking only a few hours, same-day analysis of food samples is now possible. Extraction, clean up and analysis by properly trained personnel can be carried out in one day, resulting in low turnaround times for sample batches of any size.

References:
1. Fadaei, H., Watson, A., Place, A., Connolly, J., Ghosh, U. (2015) Environ. Sci. Technol., 49 (20), 12405–12413.
2. Hoogenboom R.L., Kotterman, M.J., Hoek-van Nieuwenhuizen, M., Van der Lee, M.K., Mennes, W.C., Jeurissen, S.M., Van Leeuwen, S.P. (2015) Chemosphere, 123, 1-8.
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Figure 1.

		native	reference value	recoveries
		pg/g	pg/g	%
33'44'-T4CB	77	612.91	451 ± 225	77%
344'5-T4CB	81	1.89	3.0 ± 1.5	68%
233'44'-P5CB	105	105.80	108 ± 54	58%
2344'5-P5CB	114	8.15	7.73 ± 3.86	66%
23'44'5-P5CB	118	298.35	348 ± 174	45%
2'344'5-P5CB	123	39.47		67%
33'44'5-P5CB	126	419.82	431 ± 215	62%
233'44'5-H6CB	156	17.35	23.3 ± 11.6	64%
233'44'5'-H6CB	157	5.58	9.3 ± 4.6	74%
23'44'55'-H6CB	167	12.55	12.0 ± 6.0	78%
33'44'55'-H6CB	169	477.50	512 ± 256	90%
233'44'55'-H7CB	170	29.93		89%
22'344'55'-H7CB	180	99.94	116 ± 58	83%
233'44'55'-H7CB	189	1.84	3.51 ± 1.75	90%

Table 1

	native	recoveries
	pg/g	%
2378-T4CDF	0.05	89%
2378-T4CDD	nd	92%
12378-P5CDF	0.13	94%
23478-P5CDF	0.10	92%
12378-P5CDD	0.13	95%
123478-H6CDF	0.09	92%
123678-H6CDF	0.09	90%
234678-H6CDF	0.08	87%
123789-H6CDF	0.10	93%
123478-H6CDD	0.06	94%
123678-H6CDD	0.08	92%
123789-H6CDD	0.08	
1234678-H7CDF	nd	94%
1234789-H7CDF	nd	95%
1234678-H7CDD	0.09	94%
OCDF	0.06	
OCDD	0.35	93%

Table 2.