AUTOMATION APPROACH FOR PBDEs, PCBs, PCDD/Fs IN FOODS AND DIETARY SUPPLEMENTS MADE WITH FISH OILS

Hayward[1] DG and Pisano[2] TS

[1]US Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740
[1]Douglas.hayward@fda.hhs.gov [2]University of Maryland, College Park, MD USA

Introduction

Fish lipids have been of interest in recent years due to their association with lowered risks of cardiovascular disease[1]. Fish consumption has increased along with the use of fish oils[2]. Fish oils are used in animal feed as a means of altering the fatty acid content of animal-derived human food. Fish oils are marketed as dietary supplements in liquid oil or oil capsules. Analytical protocols for PCDD/Fs, PCBs and polybrominated diphenyl ethers (PBDEs) often focus on a single class of compounds or on the congeners with TEFs (dioxin-like PCBs). An automated system (Power-Prep™, Fluid Management Systems) for PCDD/Fs has been modified to handle up to 5g of co-extracted lipids while processing 6 samples simultaneously. On-line extraction with a pressurized liquid extractor (PLE) is available with this system allowing near complete automation. Pirard et al.[3] reported a strategy for preparing samples for PCDD/F, PCB and PBDE analysis utilizing fractions collected at different points during the automated operation of the Power-Prep™. Fractionation is often necessary due to the large concentration ranges found for congeners of the different compound classes[4]. More recently GC^2/TOF was used to separate and measure PCDD/Fs with a broad range of PCB congeners in foods[5]. Another approach receiving attention recently uses a "fat" retainer to remove lipids with PLE by placing a fat retainer like sulfuric acid silica gel in the PLE cell [6,7,8]. Extraction and clean up are combined reducing sample preparation and is often combined with HRMS[8]. Another commonly used clean up for biogenic materials in food is gel permeation chromatography (GPC). GPCs are automated, but handle <1g of co-extracted material with sequential operation. Fast cycling requires a reduction in column size and fat capacity. Desirable aspects for a protocol are: (1) be automated, (2) reliable recovery and chromatography, (3) broad applicability, (4) sufficient sample size to reach needed limits of determination (LODs) for congeners with TEFs. This paper discusses a new method for PBDEs/PCBs/PCDD/Fs in foods combining automated PLE with fat retainers followed by an automated "express" GPC for PCBs/PBDEs with in-line carbon trap for the PCDD/Fs.

Experimental

Food, dietary supplements and feeds were selected that were known or suspected to produce clean up difficulties with PCDD/Fs/PCBs. Seven fish oils were purchased from the supplement section of a supermarket and one from a drug store (4 cod liver oils, one salmon oil, 3 other fish oils; 2 with no PCBs/Hg/Pb on the label). Carrots and green beans were obtained at an outdoor store. The chicken feed sample was a commercial finished feed. Five separate clean up approaches that include automation or could easily be automated completely were tested.

Scheme A: Two grams of fish oil or corn oil for GPC dissolved in 8 mL dichloromethane(DCM) and 5mL aliquots were fortified with 5ng/g $^{13}C_{12}$-PCBs 28, 52, 101, 105, 114, 118, 138, 153, 156, 157, 167, 170, 180, 189 and PBDEs 28, 47, 99, 153, 154. $^{13}C_{12}$-PCBs 77, 126, 169 were fortified at 250pg/g, and 15 $^{13}C_{12}$-PCDD/F, 100pg/g. GPC (70g Bio-Rad SX-3 bio-beads in DCM) clean up was followed by a Pasteur pipet column containing 0.5g k^+silicate and 1g 40% sulfuric acid silica gel (MSBA in figure 1) to check for breakthrough.

Scheme B: Using PLE with fat retainers (45g 44% sulfuric acid on silica gel 60), 2.5 fish oil aliquots were fortified as above (same std mass). Fish and vegetable oils extracted in an ASE 300 with cyclohexane at 1500 psig at 100°C twice for 5min. Extracts were passed through a combination of SPE columns using SCX eluting into two carbon columns. Top carbon column contained 200mg nonporous graphitized carbon black (GCB) while on bottom was 400mg Carbosphere in reversible SPE columns (International Sorbent Technologies). Carbon columns were eluted with 10mL hexane, 15mL 5% DCM/hexane, then separated and eluted with 30mL toluene

Schemes C and D: Freeze dried carrots/green beans (5-10g), fortified as above, are extracted as above with cyclohexane in an ASE 300 100ml cell containing of 45g fat retainers (scheme C). Chicken feed (10g) fortified as above was extracted with cyclohexane in 34mL cell in an ASE 200 without fat retainer as above (scheme D). Chicken feed, green bean and carrot samples were clean up over a triple Pasteur pipet column arrangement. From top to bottom, the columns were MSBA, 20%carbopak C/celite and alumina. Eluted with 6mL hexane MSBA is discarded, then 2mL 50%DCM/hexane (PCB/PBDE fraction) alumina column discarded, then the carbon column eluted with 20mL toluene.
Modified Scheme A: (figure 1). Five g fish oil or corn oil, 5g freeze dried carrots or green beans or 10g fish fillet were fortified as above, and placed in a 100mL ASE 300 cell with 45 or 75g 44% sulfuric acid silica gel and extracted with petroleum ether, exchanged to 5mL 50% DCM/cyclohexane for "express" GPC (1.5cm x 30cm col.) with in-line GCB SPE. PCB/PBDE containing fractions were checked with MSBA, while PCDD/Fs were clean up over super I alumina. For all schemes, PCBs and PBDEs were measured by GC/MS/MS or GC/EI-LRMS respectively as previously described[10]. PCDD/Fs and PCB 77,126 and 169 were analyzed using a Saturn 2000 in tandem MS mode as previously described[9].

Results and Discussion
Fish oil through scheme B produced PCB and PBDE chromatograms with poor chromatography and both low and high boiling interference in the GC/MS total ion current (TIC). While recoveries were generally >50% for most congeners certain PCB congeners such as PCB 28, 52, 101, 138, 153 were well below 50%. Green bean or carrot PCB fractions produced large signal suppression throughout the TIC and poor chromatography, while the PCDD/F fractions were better (scheme C). After just a few extracts, the PCB capillary columns needed to be cut severely or replaced to restore peak shapes. Low and high boiling interferences were present such as waxes which have been reported in plant extracts[12]. Chicken feed extracts produced acceptable chromatography for PCDD/Fs, but PCB chromatograms still contained unacceptable amounts of high boiling material (scheme D). Eight fish oils and a corn oil were measured for PCBs and PBDEs using scheme A. Fish oil or corn oil produced extracts with lower background seen in the TIC and peaks shapes were the same as standards in all GC/MS systems. A recent report of Kelly et al. 2005[12] suggested that a SPE with GCB placed inline to GPC column would recover PCDD/Fs. In a variation to scheme A to separate PCDD/Fs, a GCB SPE column was placed in-line to the GPC. Corn oil fortified with native analytes and processed through this variation of scheme A showed excellent recoveries. Sample size is small for scheme A (1g) for PCDD/Fs. Assuming a LOD for each PCDD/F in HRMS of 50 fg, the upper bound LOD would be 0.75pg/g with 20% injected. This LOD is below a maximum level suggested by EU for edible fish oils, but at a background level in US cream fat[13]. Four fish oils with PCB and PBDE levels and corn oil fortified at 2ng/g for 26 PCBs and 7 PBDEs were reanalyzed using modified scheme A (tables 1&2). Modified scheme A eliminated 99.9% of the co-extractants corn or fish oil. PCDD/F, PCB and PBDE levels and internal standard recoveries for cod liver oil are given table 1. This automated approach appears to be suitable for any food matrix. Foods such as butter, carrots, fish oil, corn oil, and fish fillets have been successfully tested with this approach.

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Table 1. Levels for PCDD/Fs, PCBs (CB =chlorobiphenyl), PBDEs or labeled recoveries ( ) for cod liver oil (ng/g) mean of two determinations (1) scheme A with GCB in-line and (2) through modified scheme A.

$^{13}C_{12}$-TCDD	(60)	CB-77	0.09	CB-66	3.6	CB-28	1.9	BDE-28	0.5
$^{13}C_{12}$-PeCDD	(66)	CB-81	0.005	CB-74	3.6	CB-44	3.9	BDE-47	12
$^{13}C_{12}$-HxCDD1	(71)	CB-126	0.077	CB-105	5.8	CB-49	3.4	BDE-49	1.5
$^{13}C_{12}$-HxCDD2	(74)	CB-169	0.014	CB-114	0.34	CB-52	11	BDE-99	2.3
$^{13}C_{12}$-HpCDD	(81)	$^{13}C_{12}$-CB-77	(92)	CB-118	17	CB-95	6.5	BDE-100	1.5
$^{13}C_{12}$-OCDD	(71)	$^{13}C_{12}$-CB-126	(90)	CB-123	0.14	CB-99	18	BDE-153	0.4
$^{13}C_{12}$-TCDF	(61)	$^{13}C_{12}$-CB-169	(113)	CB-156	1.8	CB-101	17	BDE-154	0.6
$^{13}C_{12}$-PeCDF1	(71)			CB-157	0.58	CB-110	8.1	$^{13}C_{12}$-BDE 28	(93)
$^{13}C_{12}$-PeCDF2	(68)			CB-167	0.71	CB-137	1.6	$^{13}C_{12}$-BDE 47	(92)
$^{13}C_{12}$-HxCDF1	(75)			CB-189	0.67	CB-138	38	$^{13}C_{12}$-BDE 99	(93)
$^{13}C_{12}$-HxCDF2	(80)			$^{13}C_{12}$-105	(81)	CB-149	11	$^{13}C_{12}$-BDE 153	(89)
$^{13}C_{12}$-HxCDF3	(84)			$^{13}C_{12}$-114	(86)	CB-153	66	$^{13}C_{12}$-BDE 154	(101)
$^{13}C_{12}$-HxCDF4	(78)			$^{13}C_{12}$-118	(92)	CB-170	4.2		
$^{13}C_{12}$-HpCDF1	(75)			$^{13}C_{12}$-156	(86)	CB-180	15		
$^{13}C_{12}$-HpCDF2	(87)			$^{13}C_{12}$-157	(89)	CB-187	9.6		
2,3,7,8-TCDF	0.007			$^{13}C_{12}$-167	(87)	$^{13}C_{12}$-CB-28	(72)		
1,2,3,7,8-PeCDF	0.001			$^{13}C_{12}$-189	(82)	$^{13}C_{12}$-CB-52	(78)		
2,3,,4,7,8-PeCDF	0.001			$^{13}C_{12}$-CB-153	(82)	$^{13}C_{12}$-CB-101	(78)		
				$^{13}C_{12}$-CB-170	(82)	$^{13}C_{12}$-CB-138	(85)		
				$^{13}C_{12}$-CB-180	(79)	$^{13}C_{12}$-CB-189	(82)		
WHO-TEQ	0.0012	WHO-TEQ	0.008	WHO-TEQ	0.004	**PCBs**	250	**PBDEs**	19

Table 2. Cod liver oils and salmon oil PBDEs in ng/g oil processed through modified scheme A.

	Cod A	**Cod B**	**Cod C**	**Salmon oil**
BDE-28	0.5	0.27	0.12	0.32
BDE-47	12.4	5.1	4.7	5.8
BDE-49	1.5	0.94	1.0	1.4
BDE-77	nd	nd	nd	nd
BDE-100	1.5	0.78	1.06	0.92
BDE-99	2.3	1.2	0.91	1.2
BDE-154	0.59	0.47	0.62	0.47
BDE-153	0.45	0.20	0.14	0.24
PBDEs	19	9.0	7.6	10

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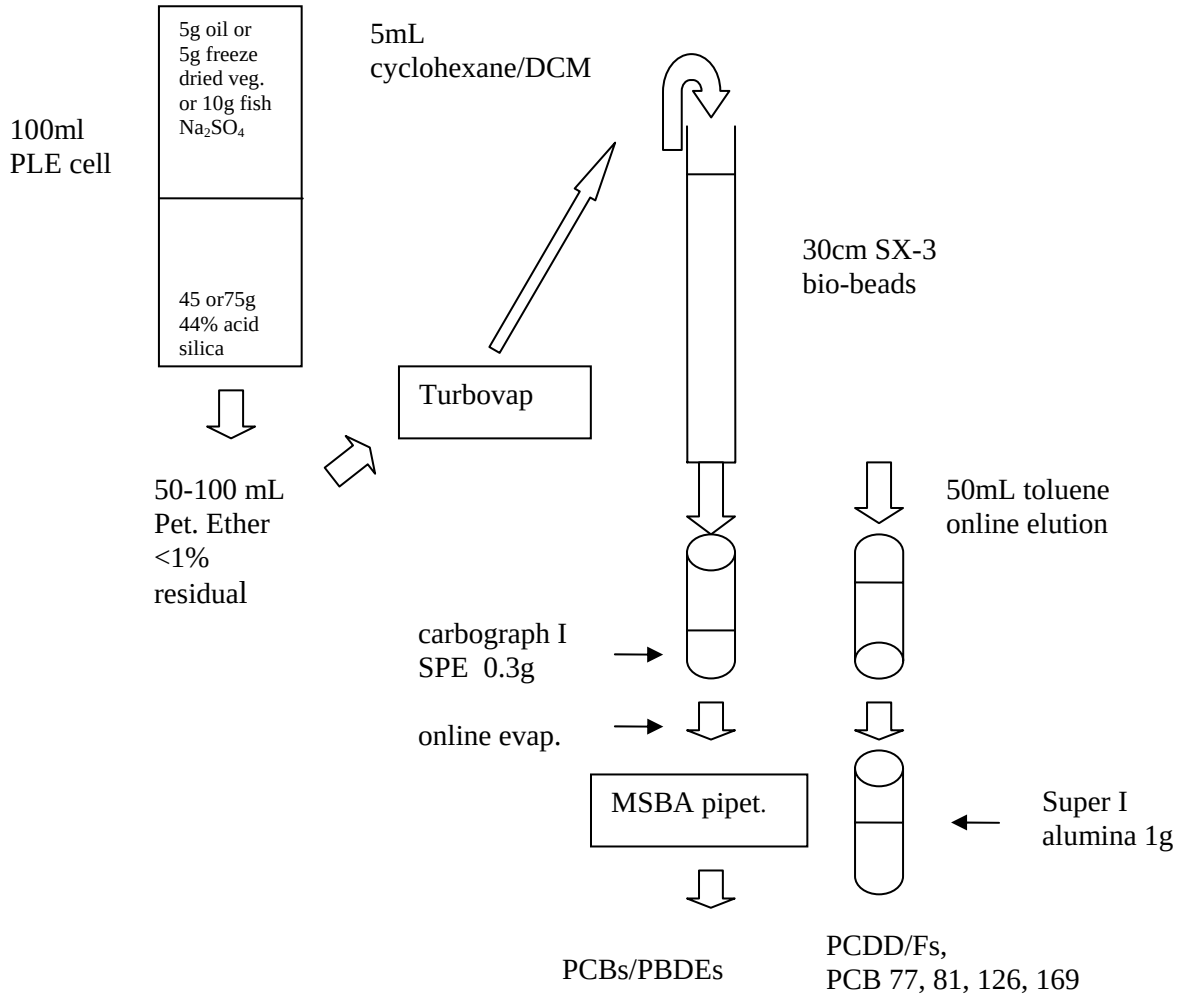

Figure 1. Modified Scheme A using pressurized liquid extraction with fat retainer followed by "express" GPC and SPE carbon.