INEXPENSIVE RAPID METHOD FOR POPS ANALYSIS OF FOOD USING ASE AND TANDEM ACID SILICA/CARBON COLUMNS

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Introduction

Conventional methods for PCDD/F and dlPCB analysis in food have been laborious and expensive, using soxhlet extraction, multi-column clean-ups (acid/base silica gel, Florisil, alumina, carbon) and ultimately high resolution mass spectrometry for analysis. Automated alternatives have been posed for the extraction and clean-up, however the costs can be prohibitive for smaller labs, especially in a competitive marketplace.

Lee et al[1], and Yang et al[2] have demonstrated the use of tandem acid silica / carbon columns in analyzing environmental samples for PCDD/F and dlPCB. We have taken previously analyzed food samples and developed a method for extraction by Accelerated Solvent Extraction (ASE) using Dioniun™ cells. Samples containing up to 5 g of lipid are cleaned up on acid silica gel columns and by coupling with a carbon column (Cape Technologies), can separate PCBs from PCDD/F.

Materials and methods

Samples:
CARP2, ground whole carp reference material, National Research Council Canada
Horse meat and butter, commercially available
Reagents:
Native and ^{13}C-labelled PCDD/F, dioxin-like PCB (DL-PCB) and marker PCBs (MPCB) standards were purchased from Wellington Labs. Canada.
Solvents used were Pesticide Grade from Caledon Laboratories, Canada.
Tandem acid silica / carbon columns and manifolds were obtained from Cape Technologies, USA.
Apparatus
GC-HRMS: Thermo Scientific Trace GC, DFS HRMS
RX-DIOXIN2 column, 60 m x 0.25 mm i.d., 0.25 µm film thickness
ASE 350 Accelerated Solvent Extractor, Thermo Scientific Instruments

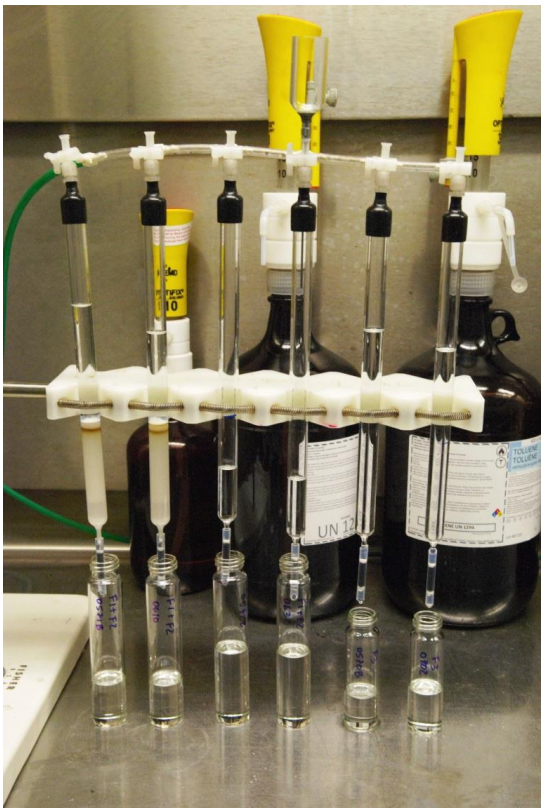
Cleanliness and potential for carry-over on the ASE was evaluated using blanks and fortified samples. Cells were packed with fortified samples containing 1-2 ng of PCDD/F and 1-5 ng of 72 PCB congeners.

Analyte fractionation was determined using standards containing all 209 PCB congeners and seventeen 2,3,7,8-PCDD/F congeners. While many PCBs were eluted with 30 mL hexane, it requires a further 6 mL of 1:1 touene/hexane to elute the 12 dlPCBs. All PCDD/F are eluted by reversing the flow on the carbon column and eluting 30 mL of toluene.

Unfortified samples (butter, horse fat, CARP2) were spiked with 0.5-1.0 ng of fifteen ^{13}C-PCDD/F standards, and twelve ^{13}C-dioxin-like PCB standards, ground with 10 g of diatomaceous earth and then packed into a 100 mL Dionium™ cell as follows: 30 mm cellulose filter, 10 g Dioxex ASE Prep CR Na+ form (sulphonated divinyl benzene/styrene copolymer), sample and top with diatomaceous earth to 10 mm from top of cell. The ASE was run under the following conditions: Extraction Solvent: Hexane; Temperature: 100 °C; Pressure: 1600 psi; Heat Time: 5 min; Static Time: 5 min; Flush Volume: 70%; Purge Time: 120 s; Static Cycles: 3; Total Extraction Time: 25 min per sample. Extracted samples (1-5 g lipid) were rotary evaporated to 10 mL. CAPE Technologies immunoassay tandem acid silica / carbon column sample preparation kits were used for sample clean-up. Carbon columns were prerinsed with solvent in the following order: toluene, dichloromethane and hexane, and then coupled to a prerinsed (hexane) acid silica column. Samples were applied to the acid silica gel column and the system was pressurized to 10 psi with nitrogen to give a flow rate of 1 mL per minute. Thirty mL of hexane was eluted through the tandem column system (F1). The carbon column was removed from the acid silica column and placed on a clean empty glass column. Six mL of 1:1 toluene/hexane was eluted (F2) containing all 12 dlPCBs (see Figure 1).

Lastly, the direction of flow was reversed through the carbon column by inverting it. Thirty mL of toluene was passed through the column and collected (F3) containing all PCDD/F. F2 and F3 were concentrated to near dryness and fortified with injection standards prior to analysis by GC/HRMS. All analyses were performed on a Trace GC coupled to a Thermo Scientific DFS HRMS following protocols set out in EPA 1613b and 1668C.

Figure 1. Cape Technologies tandem acid silica / carbon columns collecting F1, F2 and F3



Results and discussion

Using the clean-up we were able to separate the PCBs from PCDD/F. Further, we were able to isolate the 12 dlPCBs from potential coeluting PCBs of high level of chlorination (e.g. PCB110 and PCB203 elute in F1 and therefore cannot interfer with PCB81 and PCB169 respectively). A total of 61 PCBs elute in F2, leaving 148 in F1. Unfortuneately, not all the marker PCBs are in F1 (PCB28 is in F2). It is interesting to note that the cut off points in each level of chlorination follow the elution order found on an SGE HT8 column (i.e. all tetrachloro congeners eluting on an SGE HT8 column at or before PCB040 are in F1, while those eluting after are found in F2). The first PCB to elute in F2 is: DiCB9, TriCB23, TetraCB57, PentaCB124, HexaCB167 and HeptaCB189). MonoCBs elute in F2, while OcCB, NoCB and DeCB elute in F1.

Figure 2. PCDD/F Analysis of Standard Reference Material CARP2

	Reference Values ng/kg	Average (n=10) ng/kg	RSD
2378-TCDD	6.7-8.1	7.74	5%
12378-PeCDD	4.0-6.6	4.65	13%
123478-HxCDD	1.3-1.9	1.56	12%
123678-HxCDD	5.0-6.6	5.66	10%
123789-HxCDD	0.66-0.90	0.71	32%
1234678-HpCDD	5.5-7.3	6.77	7%
OCDD	7.7-11.1	8.71	10%
2378-TCDF	16.6-19.8	18.5	6%
12378-PeCDF	5.3-5.9	5.97	14%
23478-PeCDF		15.2	8%
123478-HxCDF		4.04	17%
123678-HxCDF		2.56	19%
234678-HxCDF		1.27	32%
123789-HxCDF		0.27	38%
1234678-HpCDF		4.42	11%
1234789-HpCDF		<1	
OCDF		<1	

Ten samples of CARP2 were analyzed for PCDD/F using ASE and Cape columning. NRC lists the lipid content to be approximately 7%. Results on a fresh weight basis (see Figure 2) fall within the range of certification. RSDs were below 20% except when concentrations fell below 1.3 ng/kg.

High lipid food samples (butter and horse fat) were extracted with the ASE and subsequently columned on tandem acid silica / carbon columns (analyzed in replicate, n=5). Previously these samples were extracted using our conventional methodology (dissolving in 3N acid, back extraction with 9:1 dichloromethane/acetone and clean-up on acid/base silica gel followed by basic alumina (control sample). Data for horse meat is presented in Figure 3. The average PCDD/F values for horsemeat matches very well with the Control sample. The relative standard deviation (RSD) for five replicates increases as the concentration decreases. The PentaCB values are lower for the samples analyzed by ASE, while the others are comparable. As the Control sample is only a single data point, it is possible that there was an error in its origonal analysis. ^{13}C-labelled standard recoveries were acceptable for all samples. The average PCDD/F recovery was 81% with an RSD of 15%, while the PCBs recovered at 51% with an RSD of 14%.

Figure 3. Results for naturally contaminated horse meat using ASE/Cape Technologies columns

	Control (n=1)	Average (n=5)	RSD	^{13}C-PCDD/F	RSD
	ng/kg	ng/kg		Recovery	
2,3,7,8-TCDD	0.27	0.18	21%	75	11%
1,2,3,7,8-PeCDD	1.0	0.94	22%	69	8%
1,2,3,4,7,8-HxCDD	1.8	1.7	22%	73	19%
1,2,3,6,7,8-HxCDD	3.8	4.2	9%	-	
1,2,3,7,8,9-HxCDD	0.57	0.42	31%	75	11%
1,2,3,4,6,7,8-HpCDD	33	30	4%	93	16%
OCDD	58	54	8%	36	23%
2,3,7,8-TCDF	0.075	0.11	65%		
1,2,3,7,8-PeCDF	0.087	0.093	90%	81	18%
2,3,4,7,8-PeCDF	0.36	0.32	38%	65	13%
1,2,3,4,7,8-HxCDF	0.29	0.27	25%	64	14%
1,2,3,6,7,8-HxCDF	0.46	0.49	20%	106	19%
1,2,3,7,8,9-HxCDF	0.091	0.085	133%	100	21%
2,3,4,6,7,8-HxCDF	0.20	0.24	53%	89	11%
1,2,3,4,6,7,8-HpCDF	1.97	1.92	9%	101	13%
1,2,3,4,7,8,9-HpCDF	0.076	0.11	69%	103	15%
OCDF	0.39	0.30	55%	87	13%
TEQ (PCDD/F)	**2.47**	**2.29**		-	
PCB 81	<0.5	<0.5		43	12%
PCB 77	<0.5	<0.5		44	14%
PCB 123	21	8.8	22%	51	12%
PCB 118	714	401	5%	51	13%
PCB 114	21	11	30%	56	17%
PCB 105_127	265	150	3%	57	8%
PCB 126	16	9.2	30%	52	19%
PCB 167	61	55	3%	51	13%
PCB 156	91	97	3%	51	11%
PCB 157	24	22	11%	47	14%
PCB 169	<0.5	<0.5		54	19%
PCB 189	14	21	12%	52	11%
Total TEQ (PCDD/F + PCB)	**4.15**	**3.24**			

Butter samples were analyzed in a manner similar to horse meat. In addition, five butter samples were fortified with native PCDD/F and PCBs, then analyzed. The results are presented in Figure 4. The butter was at background concentrations. ^{13}C-standard recoveries for PCDD/F ranged from 23-78% with an average of 42%.

In conclusion, ASE combined with Cape Technologies columns provides an acceptable method for analyzing food samples, including those with high lipid concentrations. ASE provides a rapid extraction of <30 minutes per sample. The Cape columns allow for the separation of PCBs from PCDD/F and provide an inexpensive clean-up procedure, with consumable costs of <$20 per sample.

Figure 4. Results for naturally contaminated and fortified butter using ASE/Cape Technologies columns. Levels of fortification for PCDD/F were 20-200 ng/kg and for PCB were 200 ng/kg

Analyte	Control (n=1)	Average (n=5)	Spiked Sample		Analyte	Control (n=1)	Average (n=5)	Spiked Sample
	ng/kg	ng/kg	Recovery			ng/kg	ng/kg	Recovery
2378-TCDD	<0.03	<0.03	91%		PCB 28	<2	12.8	73%
12378-PeCDD	<0.033	<0.033	94%		PCB 52	5.06	19.1	92%
123478-HxCDD	0.15	0.20	82%		PCB 101	<2	<2	116%
123678-HxCDD	0.56	0.60	87%		PCB 153	61.7	58.4	112%
123789-HxCDD	0.18	0.25	90%		PCB 138	39.6	48.7	109%
1234678-HpCDD	1.21	1.69	96%		PCB 180	25.6	22.0	99%
OCDD	0.88	1.83	95%					
					PCB 81	<1	<1	103%
2378-TCDF	<0.03	<0.03	94%		PCB 77	<1	<1	97%
12378-PeCDF	<0.039	0.07	92%		PCB 123	<1	<1	88%
23478-PeCDF	<0.039	<0.039	90%		PCB 118	51.1	41.7	86%
123478-HxCDF	0.16	0.24	82%		PCB 114	<1	<1	103%
123678-HxCDF	0.12	0.12	86%		PCB 105	<1	<1	99%
123789-HxCDF	<0.056	<0.056	93%		PCB 126	<1	<1	96%
234678-HxCDF	0.11	0.20	80%		PCB 167	<1	<1	103%
1234678-HpCDF	<0.052	<0.052	88%		PCB 156	5.81	4.05	110%
1234789-HpCDF	<0.085	<0.085	87%		PCB 157	<1	<1	105%
OCDF	<0.13	0.20	66%		PCB 169	<1	<1	95%
TEQ	**0.36**	**0.40**			PCB 189	<1	<1	82%

References:

1. Lee TY, Chen YW, We CP, Peng J, Weng YM, Harrison, RO. (2010); *Organohalogen Compounds*. 71: 2967-2972

2. Yang S, Reiner EJ, Kolic TM, Lega R, Harrison, RO. (2010); *Organohalogen Compounds*. 72: 1788-1791