RECENT ADVANCES IN LOWERING THE COST OF DIOXIN ANALYSIS

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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), solvent concentration with rotary evaporator 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 al¹, and Yang et al² 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 DioniunTM 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.

Concentrating samples can be a laborious, time consuming step, however with the Rocket Evaporator, twelve samples coming off the Cape clean-up columns can be to the instrument in 1-1.5 hours. We will show how it is possible to prepare and analyze six samples in a day.

Materials and methods

Samples: Salmon oil, commercially available Reagents: Native and ¹³C-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 SGE HT8 column, 60 m x 0.25 mm i.d., proprietary film thickness ASE 350 Accelerated Solvent Extractor, Thermo Scientific Instruments Rocket evaporator, Genevac Inc.

The Rocket Evaporator is designed to concentrate solvent from multiple samples simultaneously. Up to six 400 mL samples or eighteen 60 mL samples can be concentrated at one time. Similar to a rotary evaporator, it uses reduced pressure. However, rather than rotating (60-150 rpm) a boiling flask in a water bath, samples are placed in a two chamber centrifuge and spun at 1800 rpm. The centrifugal forces prevent solvents from bumping.

Thirty-six 60 mL glass vials containing 50 mL of toluene were fortified with 0.5-1.0 ng of fifteen 13C-PCDD/F. Six of the vials were then

Figure 1. The Rocket Evaporator



fortified with 2-20 ng of seventeen toxic PCDD/F congeners (2 ng for TeCDD/F, 20 ng for OCDD/F, and 10 ng for the remainder). Additional sets of six vials were fortified with 0.2-2 ng, 20-200 pg, 2-20 pg and 0.2-2 pg of PCDD/F. The last set of six vials were kept as blanks. Six sample pucks (vial holder for three 60 mL vials) were loaded with one each of the two highest concentration samples plus a blank. These were loaded into the



Rocket Evaporator and concentrated using factory settings (mid-boiling solvent). The samples were dry in <20 minutes. A further 15 mL of hexane was added to the vials and they were returned to the Rocket Evaporator, to be concentrated to 2-3 mL. The solvent was transferred to a 15 mL test tube and further concentrated under a gentle stream of nitrogen. The process was repeated for the remaining spiked samples. Final analysis was by GC HRMS.

Figure 2. Positioning of 60 mL vials in Rocket Evaporator.

Eleven 10 g meat samples were fortified with 10 pg each of seventy-two PCB congeners and 2 ng each of twenty-seven ¹³C-PCB congeners. They were extracted by Accelerated Solvent Extraction (ASE) and cleaned up with tandem acid silica gel / carbon columns (Cape Technologies)³. All solvent concentrations were performed on the Rocket Evaporator. Prior to analysis samples were fortified with 2 ng of five 13C-PCB congeners (recovery standards). Samples were analyzed by GC HRMS following EPA 1668C.

Results and discussion:

Fortification											
Level	2-20 ng		0.2-2 ng		0.02-0.2 ng		2-20 pg		0.2-2 pg		All Data
	Ave	RSD	Ave	RSD	Ave	RSD	Ave	RSD	Ave	RSD	Average
	%	%	%	%	%	%	%	%	%	%	%
2378-TCDD	101	7.4	99	3.9	108	4.7	106	10.4	145	18.8	112
12378-PeCDD	90	6.9	98	1.7	115	1.2	108	5.3	121	19.9	106
123478-HxCDD	89	6.5	105	3.3	114	6.3	107	4.8	116	4.9	106
123678-HxCDD	93	7.0	105	2.4	111	3.7	105	5.8	106	11.7	104
123789-HxCDD	92	5.6	97	3.5	100	5.7	96	5.3	111	3.5	99
1234678-HpCDD	85	5.6	97	2.0	100	2.5	94	3.3	118	5.4	99
OCDD	88	5.6	92	2.5	97	5.0	95	3.8	117	8.7	98
-											
2378-TCDF	95	7.2	100	2.3	103	9.6	96	5.4	148	18.2	109
12378-PeCDF	91	6.3	97	1.1	106	4.3	100	2.2	118	8.8	103
23478-PeCDF	92	6.9	96	2.0	105	2.5	101	4.1	119	7.9	102
123478-HxCDF	90	5.9	107	2.6	118	6.1	105	1.5	118	9.7	108
123678-HxCDF	86	6.3	99	2.8	103	5.2	93	2.7	103	7.6	97
234678-HxCDF	88	7.1	103	2.1	109	7.6	96	1.8	110	13.9	101
123789-HxCDF	89	5.5	97	1.6	100	5.9	92	4.1	120	8.5	100
1234678-HpCDF	88	5.0	108	2.9	110	6.5	99	3.2	110	11.4	103
1234789-HpCDF	85	8.3	104	1.8	107	6.9	93	2.3	105	8.7	99
OCDF	107	5.2	101	2.2	99	7.2	86	6.1	99	12.5	98

Table 1. Recoveries and Relative Standard Deviations (RSD) n=6, for samples fortified	with PCDD/F at
various concentrations.	



The Rocket Evaporator was tested for concentrating dioxins and PCBs in both natural and man-made samples. Table 1 gives recovery results and RSDs of a study where PCDD/F was added to toluene at levels ranging from 0.2 pg to 20 ng. The lowest level spike does show a positive bias, however that is attributed more to noise contribution from the instrument rather than a false positive recovery. Overall, the average recoveries for each congener ranged from 97-112%. RSDs were all under 20% and ranged from 1.2% to 19.9%

Method detection limits (MDL) were calculated for both PCBs and dioxins. In the case of PCBs, all congeners with the exception of the Dichlorobiphenyl congeners gave MDLs <0.5 ng/kg. The Dichlorobiphenyls were <1 ng/kg.

Recoveries of the dioxin-like PCB standards ranged from 85-120%, with RSDs below 10%. The level of chlorination standards (LOC – the first and last eluting PCB congeners in each level of chlorination) recoveries are shown in Figure 4. Of special interest are the recoveries of the more volatile standards – 1L, 3L, 4L, 15L, 19L, 54L and 104L. The minimum recovery was >30% in all cases, well above the 5% recovery limit set by EPA 1668C.

Analvte	Ave Conc	Matrix Blank	σ	RSD	MDL
	ng/kg*	ng/kg*	ng/kg*		ng/kg*
2378-TCDD	0.132	0.025	0.011	9%	0.035
12378-PeCDD	0.294	0.074	0.034	11%	0.106
123478-HxCDD	0.254	0.067	0.031	12%	0.097
123678-HxCDD	0.246	0.089	0.052	21%	0.162
123789-HxCDD	0.239	0.029	0.039	16%	0.124
1234678-HpCDD	0.466	0.34	0.059	13%	0.186
OCDD	1.642	1.11	0.119	7%	0.374
2378-TCDF	0.147	0.043	0.019	13%	0.059
12378-PeCDF	0.281	n.d.	0.039	14%	0.123
23478-PeCDF	0.296	0.069	0.033	11%	0.103
123478-HxCDF	0.263	0.045	0.046	17%	0.145
123678-HxCDF	0.254	n.d.	0.025	10%	0.078
234678-HxCDF	0.265	n.d.	0.035	13%	0.109
123789-HxCDF	0.307	0.098	0.047	15%	0.149
1234678-HpCDF	0.287	0.066	0.017	6%	0.053
1234789-HpCDF	0.234	0.053	0.053	23%	0.167
OCDF	0.513	0.243	0.058	11%	0.181

Table 2. MDLs for meat samples fortified with PCDD/F (n=7). Level of Fortification for TCDD/F 0.1 ng/kg, OCDD/F 0.5 ng/kg, all others at 0.25 ng/kg.

The MDLs for PCDD/F are given in Table 2 and range from 0.035 - 0.37 ng/kg lipid weight. This is comparable to other MDL concentrations we have calculated for food. RSDs ranged from 7-23%.

Using the Rocket Evaporator, we were able to process six oil samples from cleanup to instrument within a normal work day. While Cape Technologies cleanup columns were being rinsed, the oil samples were weighed out (5 g) and dissolved in hexane (30 mL). The samples were passed through a wide bore (2.5 cm) 14 g acid silica column using an additional 50 mL of hexane. The samples were concentrated and then eluted through a standard tandem acid silica gel / carbon column. Dioxin and dioxin-like PCB fractions were collected, concentrated with the Rocket Evaporator and transferred to GC vials for overnight analysis by GC HRMS. While we used fish oil for this test, it is possible for one person to process a set of 6-12 extracts through the Cape



Figure 4. The minimum and maximum recovery and RSD for LOC standards (n=11) processed with Rocket Evaporator.

Technologies cleanup procedure and to the instrument within a standard eight hour shift. The same process using conventional concentration techniques would take several days.

References:

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3. Hope D, Pond P, Mudalige W, Del Pozo J, Wright M. (2014); Organohalogen Compounds. 76: 796-799