ONE STEP CLOSER TO A 'COOK BOOK' METHOD FOR DIOXIN ANALYSIS PART 2: ANALYSIS OF FOOD SAMPLES

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Abstract
The application of an integrated sample preparation and analysis method for fish samples is presented. The first paper[1] describes the global procedure and the equipment while this paper illustrates the performance on real biological samples. The development of a fast 'cook book' procedure would be useful in the food processing industry to avoid down time in production lines.

Introduction
Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are lipophilic chemicals and tend to bioaccumulate up the food chain to humans. PCDDs, PCDFs, and PCBs can be measured directly in the lipid stores in human samples such as adipose tissue and the lipids in blood. Most epidemiological studies now measure the internal dose of these chemicals by quantifying them in blood. Human exposure to these chemicals is primarily through the consumption of food of animal origin. Since 95% of human exposure is via this route, stringent regulations have been set for foodstuffs. Food safety programs in many countries require the screening of various food products for PCDDs, PCDFs, and PCBs as well other chemicals. The application of a new totally integrated rapid sample preparation and analysis system for fish oil and fish meal will be described in this paper. The details of the system has been described in another paper presented at this conference.[1]

Materials and Methods
The integrated automated sample extraction (PLE), clean-up (power-Prep), and automated evaporation systems and the step by step dioxin analysis procedure have all been described in detail in another paper presented at Dioxin-2009 and will not be repeated here.[1] The automated PLE and Power-Prep systems are shown in Figure 1 and a flow chart of the fractionation procedure during sample clean-up is shown in Figure 2. The sample extracts were analyzed by isotope-dilution high resolution gas chromatography/high resolution mass spectrometry (ID-HRGC/HRMS).

The fish oil samples were all from the same lot of a commercially processed material and the fish meal was from the same lot of commercially processed material. Samples had approximately 12 % water content.

Results and Discussion
Table 1 presents the PCDD, PCDF, and coplanar PCB results for the analysis of four samples of fish oil all from the same lot of material. Two of the samples were 1 g and two were 2 g of fish oil. These samples were processed only through the automated Power-Prep system (Figure 1) and fractionated as outlined in Figure 2. The fraction reported in this paper is fraction 4 in Figure 2. As can be seen in Table 1, the recoveries of the internal standards through the procedure are good and the analytical CVs of the measurements are excellent, particularly considering the very low levels of the analytes in this fish oil.

Table 2 presents the results for samples of the same lot of fish oil processed through both the automated PLE and Power-Prep systems using automated extract evaporation (Figure 1). As can be seen in Table 2, the recoveries through this entire integrated system are quite good. The analytical CVs are excellent for these very low levels of

analytes. The measured values in these 8 fish oil samples from the same lot processed through only the Power-Prep system and through the total integrated system compare very well (Tables 1 and 2).

Table 3 presents the results for samples of the same lot of fish meal processed through both the automated PLE and Power-Prep systems using automated extract evaporation (Figure 1). As can be seen in Table 3, the recoveries through this entire integrated system are excellent. The analytical CVs are excellent for these very low levels of analytes.

Table 1. Power Prep Sample Clean-up and Automated Extract Evaporation of Fish Oil with ID-HRGC/HRMS

Analyte	Fish Oil 1g		Fish Oil 1 g		Fish Oil 2 g		Fish Oil 2 g				
	PPT	% Recovery	PPT	% Recovery	PPT	% Recovery	PPT	% Recovery	Mean	SD	CV
2378D	0.5	51.9	0.40	68.1	0.38	65.4	0.35	86.4	0.40	0.043	10.8
12378D	1.31	50.7	1.09	64.8	1.0	61.8	1.1	82.2	1.13	0.128	11.3
123478D	0.24	57.2	0.22	73.9	0.17	70.2	0.19	93.1	0.20	0.032	15.7
123678D	0.80	47.4	0.68	59.5	0.67	56.2	0.73	74.3	0.72	0.057	7.9
123789D	0.15	56.2	0.22	71.9	0.18	67.6	0.21	89.4	0.19	0.033	17.4
1234678D	0.71	57.7	0.51	69.4	0.51	65.4	0.51	89.6	0.56	0.099	17.7
1234679D	0.03	57.7	0.0	69.4	0.02	65.4	0.02	89.6	0.02	0.006	27.5
OCDD	1.5	44.0	1.3	60.3	1.0	53.3	0.91	70.2	1.20	0.269	22.4
2378F	8.5	52.2	8.0	67.0	7.7	65.5	8.4	84.9	8.15	0.371	4.5
12378F	1.2	50.3	1.0	65.9	1.0	62.4	1.1	81.6	1.09	0.109	10.0
23478F	4.08	52.7	3.7	67.4	3.8	62.4	4.0	85.1	3.89	0.194	5.0
123478F	0.22	35.6	0.32	67.6	0.30	65.4	0.38	85.3	0.31	0.067	21.8
123678F	0.34	54.1	0.21	69.3	0.26	64.5	0.29	87.9	0.27	0.057	20.7
123789F	0.00	55.8	0.00	71.0	0.00	68.3	0.00	88.5	0.00	0.000	.
234678F	0.49	52.5	0.53	67.4	0.48	62.8	0.51	85.1	0.50	0.023	4.5
1234678F	0.38	58.2	0.31	70.7	0.32	67.0	0.34	90.0	0.34	0.030	8.8
1234789F	0.00	58.2	0.00	70.7	0.00	67.0	0.00	90.0	0.00	0.000	.
OCDF	0.0	46.4	0.0	63.1	0.00	59.5	0.07	81.3	0.02	0.037	.
3344P	193.8	32.1	179.5	41.8	174.4	42.4	188.2	53.9	.	8.661	.
3445P	14.6	26.3	13.8	34.6	12.1	34.5	13.2	44.4	13.44	1.079	8.0
33445P	38.9	42.7	34.8	55.7	35.9	56.0	38.3	69.7	36.97	1.949	5.3
334455P	7.46	50.1	6.2	64.4	6.8	63.9	7.1	79.6	6.88	0.529	7.7

Table 2. PLE Sample Extraction, Power-Prep Sample Clean-up, and Automated Extract Evaporation of Fish Oil with ID-HRGC/HRMS											
Analyte	Fish Oil 1g		Fish Oil 1 g		Fish Oil 2 g		Fish Oil 2 g				
	PPT	% Recovery	PPT	% Recovery	PPT	% Recovery	PPT	% Recovery	Mean	SD	CV
2378D	0.48	53.0	0.46	70.0	0.39	66.5	0.35	70.8	0.42	0.062	14.8
12378D	1.5	50.0	1.1	68.7	1.1	65.4	1.3	69.1	1.25	0.193	15.4
123478D	0.24	56.3	0.19	77.2	0.20	72.6	0.21	84.2	0.21	0.020	9.4
123678D	0.67	54.9	0.61	74.8	0.73	66.8	0.67	84.3	0.67	0.049	7.3
123789D	0.29	55.6	0.27	76.2	0.23	70.4	0.21	82.3	0.25	0.039	15.6
1234678D	0.86	46.0	0.52	73.1	0.56	63.3	0.53	67.3	0.62	0.164	26.4
1234679D	0.03	46.0	0.02	73.1	0.01	63.3	0.01	67.3	0.02	0.008	45.2
OCDD	.	51.5	1.5	63.0	1.8	46.2	1.1	55.4	1.46	0.323	22.2
2378F	8.9	50.6	8.2	68.9	9.6	66.8	8.4	76.1	8.77	0.636	7.3
12378F	1.1	51.8	1.0	71.7	1.1	66.9	1.0	78.0	1.08	0.059	5.5
23478F	4.4	49.5	4.1	68.3	4.4	66.4	4.0	71.4	4.22	0.187	4.4
123478F	0.45	58.2	0.40	74.6	0.37	71.2	0.37	81.0	0.40	0.037	9.3
123678F	0.31	55.7	0.32	73.6	0.30	69.3	0.27	79.6	0.30	0.024	8.1
123789F	0.000	54.1	0.00	73.4	0.00	69.9	0.00	80.9	0.00	0.000	.
234678F	0.63	51.6	0.49	71.5	0.59	66.4	0.41	62.1	0.53	0.098	18.5
1234678F	0.45	65.2	0.42	68.7	0.41	66.9	0.37	68.8	0.41	0.032	7.8
1234789F	0.00	65.2	0.00	68.7	0.04	66.9	0.00	68.8	0.01	0.019	.
OCDF	0.00	53.8	0.23	62.0	0.00	52.8	0.12	60.8	0.09	0.112	.
3344P	215.3	42.0	190.00	50.4	203.2	42.0	188.0	44.1	199.13	12.708	6.4
3445P	19.6	38.6	17.6	42.1	15.6	35.1	14.7	34.4	16.88	2.198	13.0
33445P	37.6	50.7	35.3	55.0	40.4	46.9	37.5	45.5	37.68	2.098	5.6
334455P	8.1	52.6	7.2	59.2	8.3	52.4	7.3	50.6	7.74	0.562	7.3

Table 3. PLE Sample Extraction, Power-Prep Sample Clean-up, and Automated Extract Evaporation of Fish Meal with ID-HRGC/HRMS							
Analyte	Fish Meal 5 g		Fish Meal 5 g				
	PPT	% Recovery	PPT	% Recovery	Mean	SD	CV
2378D	0.11	92.6	0.11	77.2	0.11	0.001	0.5
12378D	0.00	87.0	0.00	79.4	.	.	.
123478D	0.06	96.9	0.06	90.4	0.06	0.001	2.4
123678D	0.00	95.5	0.00	91.4	.	.	.
123789D	0.08	94.2	0.09	90.0	0.08	0.005	6.6
1234678D	0.00	99.8	0.00	84.6	0.00	0.000	8.9
1234679D	0.51	99.8	0.51	84.6	0.51	0.002	0.3
OCDD	0.67	76.8	0.74	68.5	0.70	0.045	6.3
2378F	0.09	89.9	0.09	75.3	0.09	0.000	0.2
12378F	0.33	90.3	0.34	81.9	0.33	0.011	3.4
23478F	0.04	87.3	0.04	79.4	0.04	0.002	6.4
123478F	0.03	92.9	0.03	86.8	0.03	0.001	4.6
123678F	0.00	90.7	0.00	86.5	.	.	.
123789F	0.05	92.8	0.05	87.4	0.05	0.004	9.0
234678F	0.04	88.4	0.06	84.6	0.05	0.007	14.5
1234678F	0.00	104.6	0.00	85.3	.	.	.
1234789F	0.00	104.6	0.00	85.3	.	.	.
OCDF	18.0	83.2	18.7	70.1	18.35	0.538	2.9
3344P	1.7	59.1	1.8	44.1	1.75	0.006	0.3
3445P	2.5	48.0	2.7	36.6	2.60	0.081	3.1
33445P	0.56	76.2	0.56	59.4	0.56	0.000	0.1
334455P	0.00	81.7	0.00	68.2	.	.	.

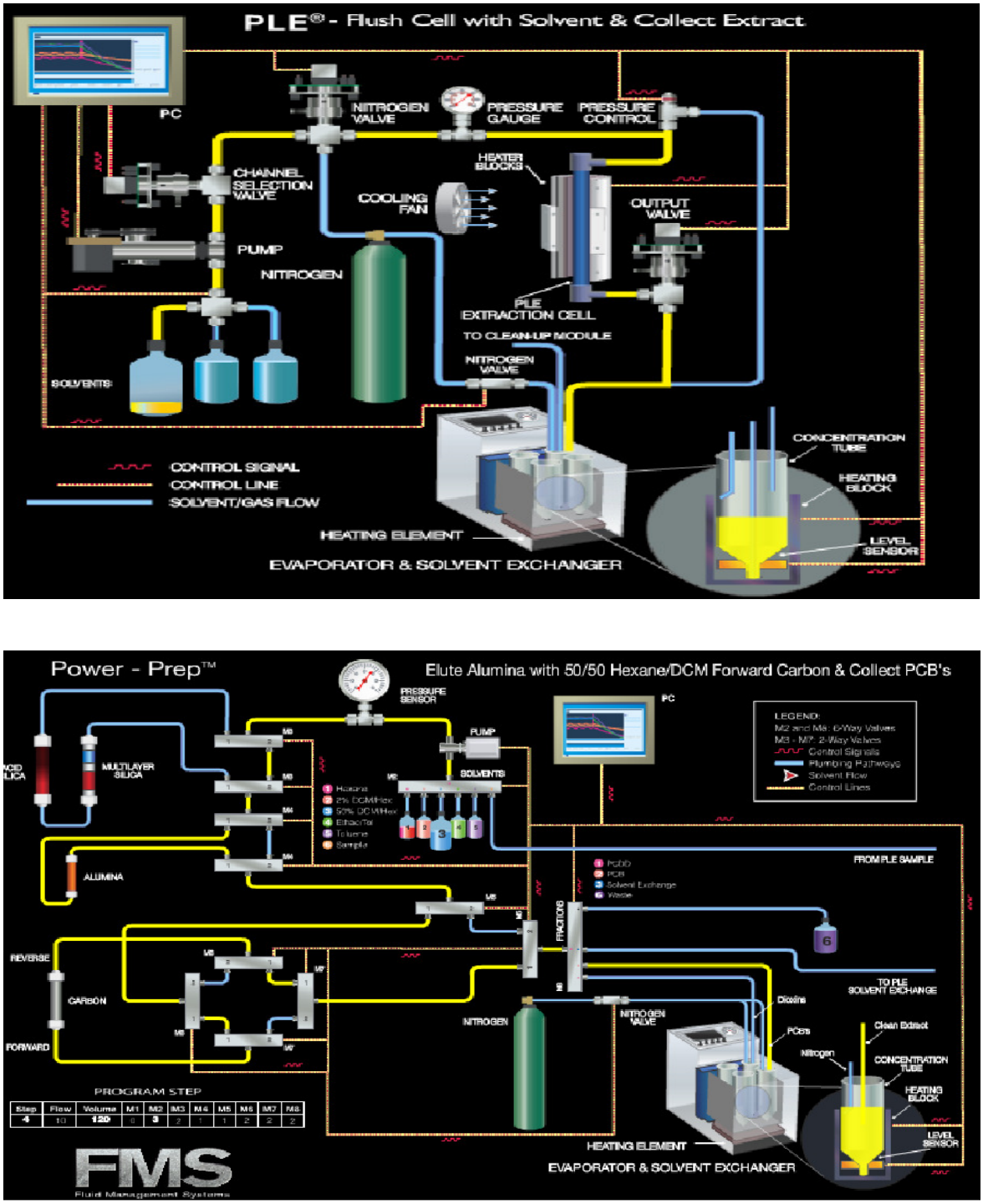

Figure 1. Schematic of the automated extraction (PLE), clean-up (Power-Prep), and evaporation systems (see Reference 1 for details)

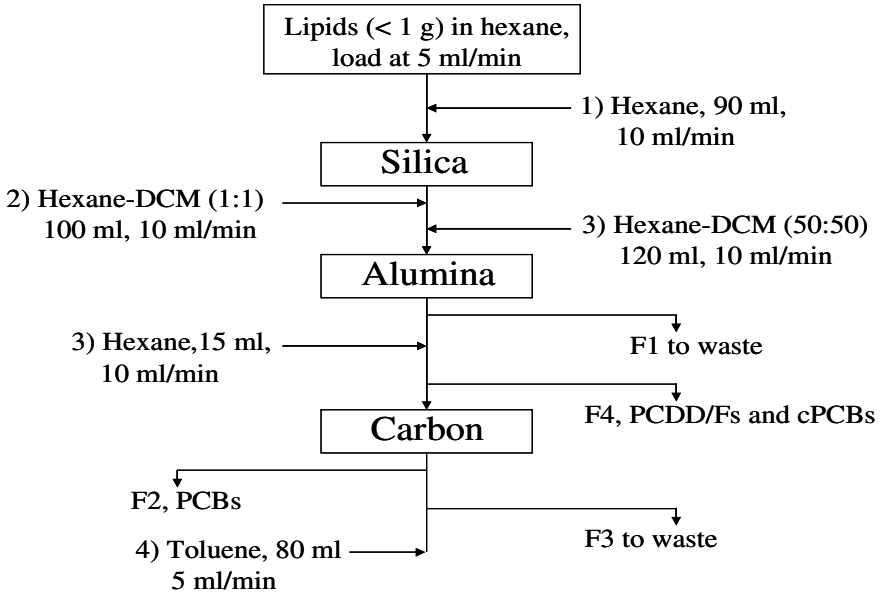

Figure 2. Flow chart for the automated clean-up system (See Reference 1 for details)

Conclusion

The integrated system described in Reference 1and briefly outlined here performs remarkably well for real world samples of fish oil and fish meal. The analytes recoveries for PCDDs, PCDFs, and cPCBs are very good and the analytical CVs for the analytes are excellent considering the very low levels in the fish samples. The goal using the integrated system is to be able to attain 'same day testing' for a large series of samples.

References

1. J.- F. Focant, H. Shirkhan, D. G. Patterson Jr., One step closer to a "cook book' method for dioxin analysis Part 1: The procedure, Dioxin-2009, August 23-28, Beijing, China 2009.