Automated Acid Hydrolysis with Abbreviated Soxhlet Extraction for Multiple Matrices

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Introduction

Over the last several years some focus has been placed on improving efficiency for the determination of polychlorinated dibenzo-p- dioxins and polychlorinated dibenzo furans (D/Fs), as well as polychlorinated biphenyl (PCBs) and the polybrominated diphenyl ethers (PBDEs). Most efforts in this area have been focused on instrumentation; moving from expensive magnetic sector mass spectrometers[1] to triple quadrupoles[2,3] and orbital trapping mass spectrometers[4]. Lesser efforts have been placed on sample preparation stages, which can be divided into extraction, cleanup and concentration techniques. Although the manual preparation stage is inherently inexpensive, compared to instrumentation costs, low level contamination or poor recoveries can result in rework which negatively impacts the method, making this stage costly. Additionally, the extraction method varies depending upon the matrix category, which adds to the complexity, thus multiple extraction methods may be necessary depending upon the moisture and fat content of a matrix. The extraction of milk fat by an automated acid hydrolysis and abbreviated Soxhlet extraction for determination of POPs have been previously described[5]. By broadening the extraction method to include egg yolks, prepared cakes, prepared meats and raw tissues, we are moving toward a single, matrix-independent, extraction method.

Materials and methods

All fats were extracted by one of two automated acid hydrolysis methods using 2M sulfuric acid in a Hydrotherm™ (C. Gerhardt GmbH & Co. KG,Oberdollendorf, Germany) system followed by an abbreviated Soxhlet extraction technique using hexane in a Soxtherm™ (C. Gerhardt GmbH & Co. KG). Milk and egg matrices were considered high moisture while tissues and other solid prepared foods were low moisture. All samples were spiked with ^{13}C-Labeled Internal Standards at least 1 hr prior to extraction. Methylene chloride was used to sonicate and rinse the 240mm diameter pleated filters, M-N 715 −1/4, (Macherey-Nagel Duren,Germany) prior to fat collection via the Hydrotherm™. Sulfuric acid (Acros Reagent ACS − NJ USA), 2M solution, was used for the automated acid hydrolysis. High moisture sample preparations start with addition of 100mL of 2M sulfuric acid and low moisture samples begin with 200mL of 2M sulfuric acid, while the remaining process is the same. A total of 100mL of water is added prior to the 5-minute heat up phase, followed by a 20-minute phase I boil, 30-minute phase II boil and a 15-minute cool down phase. Three filter moisture cycles of 25mL each are completed prior to the filter phase. A 15-second filter wait time is introduced during the 18-rinse cycle process. The rinse pipe is open for 200ms each time while the sample rinse time is 120 seconds. A 22mL DI water sample shower and cooling shower are rinsed into the Hydrotherm™ beaker followed by a 25mL per rinse filter shower. The fat was collected on the filters and rinsed with DI water during the approximate 2-hr process. After no liquid remained in the filters, they were dried at 105°C for 1 hour.

The dry filters were then placed into glass thimbles and loaded into Soxtherm™ receivers. The receivers, containing boiling chips, were accurately weighed prior to addition of the thimbles as a tare weight for fat determination. Approximately 100 mL of hexane was added to each receiver prior to the automated Soxhlet extraction. The Soxtherm™ method consists of an extraction temperature of 145°C, a reduction interval of 3 minutes, reduction pulse of 1 s, hot extraction of 30 minutes, Evaporation A of a 6 x interval, Extraction Time of 1 hr, Evaporation B of a 5.8 x interval for a 2 hour and 5-minute extraction procedure. Upon the completion of the extraction, the thimble and filter were moved from the receiver. The receiver was then placed in the oven at 105°C for approximately 1.25 hours to remove the remaining hexane for fat determination.

For routine production work, samples have been adjusted to collect between 2-3 g of fat. These extracts were then subjected to GPC for fat removal, followed by a solvent exchange from DCM to hexane. Further cleanups were accomplished via an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA) with a

pyrenylethylgroup bonded (5-PYE) 4.6 I.D. x 250 mm column (Cosmosil, Nacalai USA, San Diego, CA) for the separation of D/Fs and PCBs or a Fluid Management Systems PowerPrep multicolumn cleanup.

The determination of all D/F extracts, including four non-ortho PCBs, were completed via GC/HRMS using AutoSpec Premier high resolution magnetic sector instrument (Waters, Milford, MA) or APGC MS/MS instrument, XEVO (Waters, Milford, MA). The gas chromatograph used was an Agilent 7890 Series (Agilent Technologies) with a 60 m DB5-MS UI (Agilent, J&W, Santa Clara, CA) GC column, a 0.25 mm id deactivated pre-column (Agilent Technologies), and a split split-less 4 mm × 78.5 mm liner (Thermo Scientific, Waltham, MA). The column and guard column were connected with a deactivated press-fit (Restek Universal). Direct isotope dilution was used for reporting of all analytes. Additionally, a ^{13}C-labeled Recovery standard containing 1,2,3,4-TCDD and 2,3,4,6,7,8-HxCDD was added to each extract for a final volume of 10 uL with a 1.5 uL injection to determine internal standard recoveries.

The determination of the additional dioxin-like (DL) and marker PCBs were accomplished via direct isotope dilution on a Pegasus 4D-GCxGC-TOFMS (LECO, St. Joseph, MI) using an internal U.S. FDA method, described elsewhere[6]. The injection liner, guard column and press-fits used are identical to the above mentioned. A 10 m PCB HT-8 primary column (SGE Analytical Science, Austin, TX) with an additional press-fit connection to a 1 m DB-17MS secondary column (Agilent,J&W) was used.

Results and discussion:
D/Fs, PCBs and PBDEs have successfully been extracted with fat from various matrices using the Hydrotherm™ and Soxtherm™ (HT/ST) technology. Regardless of the matrix, so long as fat exists, the automated acid hydrolysis system works well. The acquired fat from the Hydrotherm™ is subjected to the abbreviated Soxhlet extraction technique that allows a batch of 6 samples to be extracted within a given workday - to an extract cleanup stage, while an additional 6 samples are processed by the HT/ST and ready for extract cleanup the following morning. This process results in up to 1.5 times production increase over manual extraction techniques. Multiple matrices are being evaluated with HT/ST; however, only high fat / high moisture matrices are validated.

Table 1 includes D/F, PCB and PBDE determinations from a Herring obtained from the 2017 Interlaboratory Comparison of Persistent Organic Pollutants in food purchased from the Norwegian Institute of Public Health[7]. The ”Consensus Mean” values are the culmination of 69 participants for the D/Fs and 60 participants for Dioxin-Like PCBs. The “HT/ST- Reportable Amount” listed in Table 1 are results obtained from our HT/ST extraction while “HT/ST-Deviation from Consensus” column indicates the deviation of the HT/ST results from the consensus mean. Three values, OCDD, 1,2,3,7,8,9-HxCDF and PCB-123 were considered as outliers, defined as being greater than 2 times the median of all participant values; for example, 15 of the 69 (21.74%) OCDD reported values were outliers.

Table 2 shows a summary of calculated fat percentages using the HT/ST for various matrices, which were prepared for persistent organic pollutants (POPs) determinations. The determined percent fats were compared to the USDA Food Composition Database[8]. Sixteen of the 18 matrices showed less than a 5% percent difference. The matrices that had the two high percent differences were samples with low fat content, turkey and roasted chicken breast, with skin removed. Target sample weights were adjusted to maintain a resulting fat of 2-3 grams.

The tissue results agree closely with consensus values (Table 1), 84% of the congener results are within two standard deviations of the consensus means. The obtained fat content from each prepared food, as shown in Table 2, agrees closely with USDA results when reported fat content was greater than 5%. If fat is present, the POPs are extracted with the matrix and then prepared for fat removal and cleanup. Demonstrating the congener agreements with the tissue sample and fat content on various prepared foods, these results are reassuring for reaching a single extraction method.

Table 1. HT/ST Summary Comparison to 2017 Herring Results Interlaboratory Calibration Study

Congener	HT/ST Reportable Amount	Consensus Mean	HT/ST Deviation from Consensus	Consenesus Outliers (%)
2,3,7,8-TCDD	**0.09**	0.087	1SD	1.45%
1,2,3,7,8-PeCDD	**0.19**	0.19	1SD	0.00%
1,2,3,4,7,8-HxCDD	**0.04**	0.035	1SD	7.25%
1,2,3,6,7,8-HxCDD	**0.13**	0.12	1SD	1.45%
1,2,3,7,8,9-HxCDD	**0.02**	0.027	1SD	13.04%
1,2,3,4,6,7,8-HpCDD	**0.06**	0.067	1SD	7.25%
1,2,3,4,6,7,8,9-OCDD	**0.16**	0.066	**Outlier**	21.74%
2,3,7,8-TCDF	**2.00**	1.8	1SD	0.00%
1,2,3,7,8-PeCDF	**0.27**	0.25	1SD	0.00%
2,3,4,7,8-PeCDF	**0.96**	0.84	1SD	0.00%
1,2,3,4,7,8-HxCDF	**0.07**	0.076	1SD	2.90%
1,2,3,6,7,8-HxCDF	**0.08**	0.076	1SD	2.90%
2,3,4,6,7,8-HxCDF	**0.08**	0.09	1SD	2.90%
1,2,3,7,8,9-HxCDF	**0.02**	0.0085	**Outlier**	31.88%
1,2,3,4,6,7,8-HpCDF	**0.07**	0.058	1SD	5.80%
1,2,3,4,7,8,9-HpCDF	**0.01**	0.01	1SD	28.99%
1,2,3,4,6,7,8,9-OCDF	**0.03**	0.02	1SD	26.09%
PCB 77	**29.00**	31	1SD	0.00%
PCB 126	**7.20**	7.3	1SD	0.00%
PCB 169	**1.60**	1.8	1SD	2.90%
PCB 81	**0.80**	0.85	1SD	10.14%
PCB 105	**433.00**	328	2SD	0.00%
PCB 114	**11.40**	14	1SD	2.90%
PCB 118	**1430.0**	1123	2SD	0.00%
PCB 123	**85.90**	14	**Outlier**	23.19%
PCB 156	**147.00**	110	2SD	0.00%
PCB 157	**34.40**	31	1SD	0.00%
PCB 167	**106.80**	75	2SD	2.90%
PCB 189	**12.90**	11	1SD	0.00%
PCB-28	**463**	381	1SD	5.00%
PCB-52	**851**	649	2SD	0.00%
PCB-101	**2,110**	1584	2SD	3.33%
PCB-138	**4,100**	2261	3SD	1.67%
PCB-153	**4,660**	3411	2SD	0.00%
PCB-180	**768**	504	3SD	1.67%
BDE-28	**30**	24	2SD	10.81%
BDE-47	**655**	355		5.41%
BDE-99	**143**	77		5.41%
BDE-100	**126**	88	2SD	2.70%
BDE-153	**16.6**	12	2SD	5.41%
BDE-154	**36.3**	43	1SD	0.00%
BDE-183	**1.2**	1.5	1SD	18.18%

Table 2. Fat Content Determination and Comparison to USDA Database

Category	Food	HT/ST - Sample Percent Fat	USDA Database Percent Fat[8]	Fat Percent Difference	Target Weight (g)
Bread / Bakery	Chocolate Cake with icing	17.53	16.5	1.52%	15
Dairy	Cheddar Cheese	35.71	35.7	0.01%	7
Dairy	Boiled Eggs	10.26	10.6	-0.82%	25
Fast Food	Fried Chicken Breast	11.11	13.2	-4.30%	20
Fast Food	French Fries	14.57	14.7	-0.22%	18
Meat	Ground Beef	14.47	15.2	-1.22%	18
Bread	White Cake with Icing	18.95	20.25	-1.65%	15
Dairy	Swiss Cheese	32.00	28.6	2.81%	8
Fast Food	Chicken Breast, skin removed,roasted	2.99	4.5	-10.10%	60
Fast Food	Pizza, cheese + pepperoni	14.47	14.65	-0.30%	18
Meat	Pork Bacon	48.33	44.64	1.99%	5
Meat	Pork Sausage	27.45	27.3	0.14%	10
Bread	Blueberry Muffin	17.22	16.1	1.68%	15
Dairy	American Cheese	24.75	23.7	1.09%	10
Fast Food	Cheeseburger	15.89	14.7	1.95%	15
Meat	Turkey	0.79	1.79	-19.28%	50
Seafood	Catfish	14.74	13.3	2.57%	15

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