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**DEVELOPING A UNIFIED EXTRACTION TECHNIQUE FOR FOODSTUFFS**

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**Introduction:**

The extraction of persistent organic pollutants (POPs) can be a costly process, and varies with matrix type. Lipid determinations are sometimes required so concentrations may be reported on a lipid weight basis. While many matrix types are reported on wet weight basis, this does not simplify the extraction procedure; it only eliminates the gravimetric determination. Our goal is to standardize and automate the sample preparation, extraction, and cleanup procedures regardless of matrix.

Various methodologies are currently being used, as described in a comparison study [1] for the extraction of tissue, egg, and milk. The extraction method dictates the needed sample preparation technique. Milk, whether raw or processed, will be extracted by a liquid-liquid technique or, if lyophilized, can be extracted by a pressurized liquid extraction (PLE) or a Soxhlet method. Tissues, such as fish, can be extracted via liquid-solid techniques, similar to the lyophilized milk, to isolate the lipid material or assisted with a homogenizer. Eggs can be extracted via PLE or Soxhlet, if freeze-dried, or assisted with a homogenizer with no pre-treatment.

A validation study for fat determinations [2] on multiple matrices using an automated acid hydrolysis system suggests a potential use in the POPs field. We have implemented the use of these contained acid hydrolysis and soxhlet systems with method modifications for the extraction of POPs with the lipids.

Materials and Methods:

A simple alternative extraction technique for fat determination uses an Automated Acid Hydrolysis System - HydrothermTM, (C. Gerhardt GmbH & Co. KG, Konigswinter, Germany) followed by an abbreviated Soxhlet extraction System - SoxthermTM (C. Gerhardt). We are using a 2M H2SO4 solution followed by multiple water rinses to hydrolyze the fat, which is deposited on filter paper. The filters are placed into the SoxthermTM receivers and allowed to dry at 100oC for 1 hour. The filters are then transferred to glass thimbles and placed in the receivers to be extracted at 145oC with hexane for 2 hours. To minimize analytical background and potential compound interferences, two major modifications were made to the original hydrolysis techniques. Background PCBs were being identified during blank extractions, so the acid was changed from 4M HCl to 2M H2SO4 thus eliminating potential chlorine source during the heated acid hydrolysis stage. Additionally, 19 different filter papers were tested from three manufacturers to result in minimum interferences. The decision of filter paper was based upon a minimum of 90% fat recovery with no interferences for the analytes of interest (M-N 715, Macherey-Nagel, GmbH & Co. KG, Germany). The filters are pre-cleaned at our facility by sonication with methylene chloride.

**Results and Discussions:**

The sample preparation for milk, only involved the addition of alcohol and sodium oxalate. Fish preparation involved homogenizing the tissue, while eggs were separated using homogenized yolks only. Milk results between 315 historical samples, extracted via a liquid-liquid technique, compared to 6 samples extracted via the automated acid hydrolysis were statistically equivalent. An upper-bound average PCDD/PCDF TEQ for the 315 results is 0.354 pg/g fat with a standard deviation of 0.207 pg/g and a median of 0.321 pg/g fat. The PCDD/PCDF TEQ average for the automated extraction was slightly lower (0.292 pg/g +/- 0.069) than the 315 samples yet are within a single standard deviation, and the median of 0.318 pg/g fat. The “hands-on” time for the extraction has been significantly reduced, by approximately 60%, which is the real impact given similar results for the techniques.

An approximate 3g fish sample was extracted for direct comparison (Table 1) between extraction methods. All congener concentrations “Found” were nearly identical, as well as the lipid determination, which was 0.1% difference. The reported labeled recoveries were lower for the automated procedure, but acceptable since the detection limits were comparable between the methods.

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**References:**

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2. Bench BJ, Whittington W, Kranz M, Guerrera F; Validation Study - Total fat content by automated acid hydrolysis [HYDROTHERM] – ISO8262-1 http://www.gerhardt.de/fileadmin/Redaktion/Validierungsstudie_HYDROTHERM_EN.pdf

Table 1.

| | Traditiona | HT/ST | Traditiona | HT/ST | Traditiona | HT/ST | Traditional | HT/ST |
|---|---|---|---|---|---|---|---|---|
| | Found | Found | TEQ | TEQ | LOD | LOD | 13C-Labeled IS | 13C-Labeled IS |
| Congener | pg/g | pg/g | pg/g | pg/g | pg/g | pg/g | % Recovery | % Recovery |
| 2378-TCDD | 0.28 | 0.28 | 0.28 | 0.28 | 0.017 | 0.021 | 75 | 53 |
| 12378-PeCDD | 0.49 | 0.48 | 0.49 | 0.48 | 0.025 | 0.023 | 79 | 65 |
| 123478-HxCDD | ND | ND | ND | ND | 0.019 | 0.031 | 75 | 64 |
| 123678-HxCDD | ND | 0.2 | ND | 0.02 | 0.18 | 0.019 | 72 | 60 |
| 123789-HxCDD | ND | ND | ND | ND | 0.019 | 0.018 | N/A | N/A |
| 1234678-HpCDD | ND | ND | ND | ND | 0.044 | 0.035 | 90 | 74 |
| OCDD | ND | ND | ND | ND | 0.11 | 0.14 | 75 | 64 |
| 2378-TCDF | 4.3 | 4.4 | 0.43 | 0.44 | 0.016 | 0.023 | 75 | 52 |
| 12378-PeCDF | 0.6 | 0.6 | 0.018 | 0.018 | 0.021 | 0.024 | 79 | 62 |
| 23478-PeCDF | 3.4 | 3.2 | 1 | 0.96 | 0.022 | 0.023 | 76 | 63 |
| 123478-HxCDF | 0.08 | 0.092 | 0.008 | 0.0092 | 0.022 | 0.014 | 79 | 65 |
| 123678-HxCDF | 0.12 | 0.14 | 0.012 | 0.014 | 0.021 | 0.014 | 77 | 64 |
| 234678-HxCDF | 0.11 | 0.095 | 0.011 | 0.0095 | 0.022 | 0.014 | 76 | 63 |
| 123789-HxCDF | ND | ND | ND | ND | 0.026 | 0.016 | 75 | 66 |
| 1234678-HpCDF | ND | ND | ND | ND | 0.023 | 0.036 | 83 | 71 |
| 1234789-HpCDF | ND | ND | ND | ND | 0.029 | 0.024 | 90 | 75 |
| OCDF | ND | ND | ND | ND | 0.033 | 0.026 | N/A | N/A |
| | | | | | | | | |
| 12C-81-PCB | 1.6 | 2 | 0.00049 | 0.00059 | 0.042 | 0.054 | 60 | 37 |
| 12C-77-PCB | 83 | 93 | 0.0083 | 0.0093 | 0.04 | 0.051 | 65 | 41 |
| 12C-126-PCB | 35 | 37 | 3.5 | 3.7 | 0.032 | 0.054 | 69 | 50 |
| 12C-169-PCB | 7.3 | 7 | 0.22 | 0.21 | 0.014 | 0.015 | 83 | 69 |

| | Traditiona | HT/ST | |
|---|---|---|---|
| Total TEQ of PCDDs/PCDFs: | 2.2 | 2.2 | pg/g |
| Total TEQ of coplanar PCBs: | 3.7 | 3.9 | pg/g |
| Sum of total TEQs: | 5.9 | 6.1 | pg/g |
| % Lipid: | 11.9 | 12 | % |