Comparison and Evaluation of a Method for the Extraction of Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans from Chicken Eggs Using Accelerated Solvent Extraction

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Introduction

A method was developed for the extraction of dioxins and furans from chicken egg yolks using the Accelerated Solvent Extractor (ASE) from Dionex. The purpose of this study was to evaluate the ASE method and compare it to other previously used extraction techniques. ASE uses elevated temperature and pressure to mimic a Soxhlet extraction using significantly less solvent and a shorter extraction time. ASE has been shown by Richter[1] to extract, with comparable efficiency to a soxhlet, dioxins/furans from solid samples including soil/sediment, fly ash, and chimney dust. The ASE method developed involves hard-boiling the egg, separating the yolk from the white, and extracting the yolk by ASE. It was determined that only the yolk should be extracted because it contains all the fat of an egg[2]; therefore, the concentration in the yolk should present the total dioxin/furan content of the egg. The other methods chosen for the study were a soxhlet extraction following USEPA method 1613B for tissue samples[3] and a liquid/liquid rotary extraction technique first developed for human milk samples by Fürst[4]. A similar rotary method was used in a comparison of egg methods by Schmid[5]. The results indicate that all three methods provide similar extraction efficiencies. The results also show that boiling the egg would not alter the final results.

Materials and Methods

For each method, seven samples were prepared including four unspiked samples, one duplicate pair of matrix spikes and a method blank.

ASE Extraction

Eggs were hard-boiled15 minutes, shelled, and the yolks were separated from the white portions. The yolks were homogenized and, a 10 gram sample was taken for analysis and mixed with 10 grams pre-extracted silica 80/100 mesh. This made the sample into a fine powder as the yolk was evenly distributed over the entire surface area of the silica. The method blank was 10 grams of the same silica. For the matrix spike pair, a composite of three yolks was made and a 10 gram aliquot was taken for each sample. Samples were fortified with $^{13}C_{12}$-labeled internal quantitation standards, mixed thoroughly, and allowed to equilibrate for four hours prior to being transferred to pre-cleaned 33mL extraction cells. The extraction was the carried out with 1:1 hexane:dichlormethane at a temperature of 110°C, 1500psi pressure, 5 minute static time for three static cycles, an 80% flush volume, and 60 sec purge time.

A second set of samples was prepared by fortifying three eggs with five $^{13}C_{12}$-labeled analytes shown in Table 3. This was done while the eggs were still raw in the shell. After equilibrating overnight, the eggs were boiled and extracted using the ASE extraction technique. Instead of the 10 gram sample, the entire yolk and the entire white portion were extracted separately. This required using multiple extraction cells per sample. A different set of 12 $^{13}C_{12}$-labeled analytes were added prior to extraction.

Soxhlet Extraction

The yolk portion of the egg was separated from the white. Five composites of three yolks were prepared and used for this method and the rotary method. A 10-gram aliquot of the composite was taken and mixed with 30 grams of pre-extracted sodium sulfate. Samples were allowed to dry for two hours and mixed occasionally to prevent clumping. The method blank for this method was 30 grams of the same sodium sulfate. The dried samples were then transferred to a soxhlet in a cellulose extraction thimble and fortified with the $^{13}C_{12}$-labeled internal quantitation standards. Samples were extracted for 16 hours in 1:1 hexane:dichloromethane.

Rotary Extraction

Composites that were produced for the previous extraction were also used for this method. A 10-gram sample was measured into a Teflon centrifuge tube and fortified with the $^{13}C_{12}$-labeled internal quantitation standards. The method blank for this method was 10mL of pre-extracted Milli-Q water. The samples were then mixed with ethanol and saturated boiling sodium oxalate and shaken vigorously. Hexane was then added to the mix. Samples were extracted on a rotary tumbler for 30 minutes at high speed. This extraction was repeated twice, each time removing the hexane layer.

All extracts were evaporated to dryness for a percent lipid determination, redissolved in hexane and cleaned-up using procedures described in USEPA Method 1613B including silica slurry (40% acid by weight), acid/neutral silica column, neutral silica column, and 18% carbopack C/ Celite 545 carbon column. Extracts were reduced to a final volume of 10µL and fortified with $^{13}C_{12}$-labeled recovery standards.

HRGC separation was accomplished using a Hewlett Packard 5890 gas chromatograph with a DB-5ms fused silica capillary column (60 meter, 0.25mm id, 0.25µm film thickness) at settings specific for the resolution of 2378 TCDD. Mass spectrometry analysis was performed with a VG70-250S in SIM mode operating at a resolving power of 10,000. All quantitation was based on Method 1613B.

Results and Discussion

Data Quality of Methods

The results show that either of the three methods could be used to obtain good extractions of chicken eggs. Shown in Table 1, the ASE, soxhlet, and rotary methods were comparable with percent recovery of labeled analytes ranging from 60.1% to 98.5% (ASE), 64.4% to 98.2 (soxhlet), and 61.3% to 103% (rotary). The %RSD of labeled analyte recovery shows similar precision for all methods with ranges of 5.4% to 18% (ASE), 6.5% to 16% (soxhlet), and 11% to 22% (rotary). This precision was reflected in the percent lipid determination for each method. The two matrix spike samples and the corresponding unspiked sample were all taken from the same composite. The %RSD of the percent lipid was 0.44% (ASE), 2.0% (soxhlet), and 6.2% (rotary). These results

indicate all three methods yield an exhaustive lipid extraction. All three methods also showed good precision for native analytes in the duplicate matrix spike samples shown in Table 2 with %RPD ranging from 0.0% to 20% (ASE), 0.0% to 63% (soxhlet), and 0.8% to 14% (rotary). The 20% for ASE and 63% for soxhlet, obtained for the OCDF congener, can be considered outliers by the Dixon test at the 99% level with the next lowest numbers at 5.3% for ASE and 9.3% for soxhlet.

Effects of Boiling the Egg

The second set of samples was run to determine if boiling the egg would alter the final results. The results for the analytes added before boiling the egg are shown in Table 3 with average percent recovery ranging from 69.6% to 106% for the two eggs. The entire egg, white and yolk, was extracted because the spike could not be directed once inside the egg. The variation observed in the recovery is more likely due to spiking inefficiency rather than the boiling process. The overall recovery of these analytes shows the native dioxins and furans should not be lost, and the final results would not be altered by the boiling.

A density determination was performed on the first set of composites to examine possible differences in the sample size for the unboiled and boiled egg yolks. The average density of the unboiled egg yolks was 0.988g/mL and 1.07g/mL for the boiled egg yolk. These sets of data were determined to be not statistically different by the t-test at the 95% level and F-test at 0.025. Also, the average weight of boiled and unboiled yolks was similar at 16.93 grams and 17.11 grams respectively. Therefore, a 10-gram sample of unboiled egg yolk should be similar to 10 of boiled egg yolk.

Method Considerations

These methods differ from each other when considering the labor hours and volume of solvent required per method are considered. The rotary extraction was extremely labor intensive requiring constant attention while extracting. The time required for extraction of seven samples was approximately 8 hours. The soxhlet and ASE extractions are much less labor intensive to set up (about 1-2 hours for seven samples) and do not require constant monitoring while the extraction is taking place. After setup, the ASE extraction takes approximately 20 minutes per sample, and the samples are extracted in series, while the soxhlet runs for 16 hours. These methods also differ by the volume of solvent required per sample. The ASE only requires 60mL, the rotary requires 150mL, and the soxhlet requires 350mL. The larger volumes are hazardous to the analyst, take longer to evaporate, and create excessive waste. Based on these considerations and the similarity in data quality of the three methods, the ASE extraction technique would be favored.

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Table 1. IQS Recoveries (%) n=7

	ASE		Soxhlet		Rotary	
Labeled Analog	Average	%RSD	Average	%RSD	Average	%RSD
13C2378TCDF	74.4	8.0	82.3	9.4	77.7	16
13C2378TCDD	76.4	8.3	83.2	11	78.0	14
13C12378PeCDF	76.0	5.4	80.6	14	76.9	11
13C23478PeCDF	80.5	8.8	86.2	11	83.3	12
13C12378PeCDD	60.2	7.9	66.5	14	61.3	12
13C123478HxCDF	86.4	11	91.2	8.0	91.4	13
13C123678HxCDF	93.5	6.4	91.0	9.5	92.8	17
13C234678HxCDF	93.1	7.3	98.2	7.5	99.3	14
13C123789HxCDF	76.0	8.5	94.9	7.5	95.6	15
13C123478HxCDD	98.5	8.5	102	6.5	103	11
13C123678HxCDD	81.1	6.6	87.8	7.6	84.4	14
13C1234678HpCDF	74.5	7.4	77.2	7.1	86.5	16
13C1234789HpCDF	60.1	18	76.2	16	88.8	12
13C1234678HpCDD	84.5	9.6	83.5	9.9	92.6	12
13C12346789OCDD	70.1	7.0	64.4	7.5	84.6	22
Average		8.5		9.7		14.1

Table 2. Matrix Spike Recoveries (%) n=2

	ASE		Soxhlet		Rotary	
Isomer	Average	%RPD	Average	%RPD	Average	%RPD
2378TCDF	101	1.0	98.0	0.0	104	1.0
2378TCDD	95.0	4.2	93.0	0.0	98.5	1.0
12378PECDF	115	2.6	116	6.9	123	5.7
23478PECDF	93.0	2.2	91.5	5.5	98.5	9.1
12378PECDD	137	0.7	131	6.1	143	2.1
123478HXCDF	110	1.8	109	4.6	118	2.6
123678HXCDF	116	0.9	120	5.9	126	0.80
234678HXCDF	87.0	0.0	88.0	4.5	93.0	4.3
123789HXCDF	94.5	3.2	97.0	4.1	103	3.9
123478HXCDD	101	2.0	100	8.0	106	3.8
123678HXCDD	104	1.9	104	4.8	112	0.9
123789HXCDD	113	1.8	113	2.7	120	14.2
1234678HPCDF	132	5.3	115	2.6	124	1.6
1234789HPCDF	95.5	1.0	96.5	9.3	102	3.9
1234678HPCDD	86.0	4.7	86.0	0.0	96.5	5.2
12346789OCDF	50.0	20	69.0	63	106	1.9
12346789OCDD	111	1.8	109	5.5	121	2.5
Average		3.2		7.9		3.8

Table 3. Boiling Surrogate Recovery (%)

Labeled Analog	Egg 1	Egg 2	Average	%RSD
37Cl 2378TCDD	89.9	91.1	90.5	1.3
13C23478PeCDF	67.0	72.2	69.6	7.5
13C123478HxCDF	104	107	106	2.6
13C123478HxCDD	87.8	94.5	91.2	7.4
13C1234789HpCDF	75.6	85.7	80.7	12.5
Average				6.3