A ROBUST METHOD FOR SIMULTANEOUS EXTRACTION AND CLEAN-UP OF DIOXINS AND DIOXIN-LIKE COMPOUNDS IN FOODSTUFF

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Introduction

The Government Laboratory first adopted a method based on a combination of gel permeation chromatography and column chromatography including acidic silica gel, basic silica gel, alumina and/or florisil for cleanup, and high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS) for confirmation and quantitation of dioxins. Subsequently, the method was modified into a simplified Smith's method[1] that employs only silica gel and activated carbon for column chromatographic cleanup of food samples with high lipid contents. The latter method was, however, found ineffective in retaining the coplanar polychlorinated biphenyl (PCB) congeners in the carbon column and has critical dependence on the amount of fat in the sample matrix. To overcome the inadequacies, a robust method that combined the extraction and column chromatographic cleanup steps was developed for testing of food samples with high lipid contents. The method was validated by analysis of certified reference materials, reference materials and was found to have good recoveries of carbon-13 labelled internal standards and native congeners. The validated method was employed for the analysis of proficiency test samples issued under the "Interlaboratory Comparison on Dioxins in Food 2003" programme organised by the Norwegian Institute of Public Health. Amongst which, 77 laboratories have reported their results. The reported results of dioxins and dioxin-like PCB congeners obtained z-score below 2 for all three food samples and standard solutions. Furthermore, none of the 206 congener data is "outlier". Thus, the developed method is considered a robust method applicable for the simultaneous analysis of dioxins and dioxin-like compounds for foodstuff.

Methods and Materials

About 40 grams of sample was cut into small pieces, blended, freeze dried or dried with anhydrous sodium sulphate, then homogenized with 200 mL of hexanes and spiked with carbon-13 labelled internal standards of dioxins and dioxin-like compounds. The slurry was transferred to a clean-up column (a 60 cm i.d. Kontes extraction column loaded with 10 g anhydrous sodium sulphate, 20 g alumina, 10 g anhydrous sodium sulphate, 30 g basic silica gel, 10 g silica gel, 50 - 100 g acidic silica gel [~10 g acidic silica gel for 1 g fat], 10 g silica gel and 60 g anhydrous sodium sulphate, pre-washed with 150 mL hexanes). The loaded column was extracted and cleaned up with 50% dichloromethane/hexanes mixture; the eluant was collected and evaporated to near dryness under a gentle stream of nitrogen. The extract was dissolved in 5 mL of hexanes for fractionation on a 5% Amoco PX21 activated carbon on glass fiber column. The di-ortho PCBs, mono-ortho PCBs, and the non-ortho PCBs was eluted with 2% dichloromethane:hexanes mixture, 50% dichloromethane:hexanes mixture and 50% ethylacetate:toluene mixture and collected into fraction 1, 2 and 3 respectively. The dioxins were back eluted with toluene. Fraction 2 and 3 were combined and the excess solvent was removed under a gentle stream of nitrogen and the sample was spiked with injection standards and reconstituted in 20 µL of nonane for dioxin-like PCB congeners analysis. Similarly, the excess solvent of the dioxins fraction was removed under a gentle stream of nitrogen and the sample was spiked with injection standards and reconstituted in 20 µL of nonane. HRGC/HRMS was carried out using SIM with a MicroMass AutoSpec Ultima HRMS equipped with a Hewlett-Packard 6890 GC and a 0.25 mm i.d. 60 m DB-5 column (0.25 µm stationary phase). The resolution of the mass spectrometer was set at 10,000 for determination of both the dioxins and dioxin-like PCB congeners.

Results and Discussion

Recoveries of dioxins in four different food matrix (40 g of each was fortified with 5 µL of 10 times diluted PAR standard of USEPA Method 1613) that were processed using the developed method, ranged from 84 to 120%, with means greater than 95% for most of the 17 WHO listed dioxins and 12 WHO listed PCB congeners (Table 1). The method gave satisfactory spike recovery of ^{13}C labelled dioxin and dioxin-like PCB congeners in many different real samples (chickens, fish, milk, etc.) and some examples are presented in Table 2. Mean recoveries of the ^{13}C labelled dioxins and dioxin-like PCB congeners were between 85 and 90%. The results demonstrated that the method is suitable for the analysis of trace levels of dioxins and dioxin-like PCB congeners in foodstuffs.

As certified reference material covering all 17 dioxins and 12 dioxin-like PCB congeners is not available commercially, the developed method was evaluated by the use of a certified reference fish material, (CARP-1) from the National Research Council of Canada, that has certified values for 7 PCDD, 2 PCDF and 2 dioxin-like PCB congeners. The reference material was analyzed in triplicate and the data obtained was tabulated and compared with the certified values as illustrated in Table 3. In general, the measurement uncertainties of our method are lower than those determined through the interlaboratory study performed on the certified fish material.

The limits of detection (LODs) is defined as the level at which the signal to noise ratio of detected peak is greater than 3. The limits of quantitation (LOQs) are defined as 4 times of LODs. Thus, the LOQs for dioxins on whole weight basis were 0.02 pg/g for the tetra to penta congeners, 0.05 pg/g for the hexa through hepta congeners, and 0.1 pg/g for the octa congeners.
Furthermore, the developed method was also found to be applicable to the analysis of other dioxin-like compounds such as polybrominated biphenyls (PBDEs) that were present in the combined fractions 1 and 2. Since there is no certified reference material exists for the PBDE congeners, a fish reference material (WMF-01) was analyzed to evaluate the performance of the method. The data obtained for the 17 dioxins, 12 dioxin-like PCB and 7 PBDE congeners, and the reference values are summarized in Table 4. The results indicated that the developed method is versatile and is suitable for the analysis of dioxins and other dioxin-like compounds of interest.

Table 1. Recoveries of fortified native dioxins and dioxin-like PCB congeners in different matrices.

Matrix / % Recovery / Analyte	Chicken	Fish	Milk	Shellfish
2,3,7,8-TCDD	103	97	105	100
1,2,3,7,8-PeCDD	111	104	109	98
1,2,3,4,7,8-HxCDD	96	101	109	93
1,2,3,6,7,8-HxCDD	96	109	111	95
1,2,3,7,8,9-HxCDD	93	100	107	94
1,2,3,4,6,7,8-HpCDD	91	101	106	98
OCDD	84	113	102	98
2,3,7,8-TCDF	89	93	110	94
1,2,3,7,8-PeCDF	99	111	117	96
2,3,4,7,8-PeCDF	100	101	110	97
1,2,3,4,7,8-HxCDF	105	102	110	99
1,2,3,6,7,8-HxCDF	95	106	109	95
1,2,3,7,8,9-HxCDF	99	103	107	96
2,3,4,6,7,8-HxCDF	99	99	107	97
1,2,3,4,6,7,8-HpCDF	94	104	111	93
1,2,3,4,7,8,9-HpCDF	91	102	108	93
OCDF	106	120	110	93
PCB 77	91	99	100	93
PCB 81	98	99	96	93
PCB 126	89	102	99	92
PCB 169	93	108	85	91
PCB 105	97	105	95	98
PCB 114	103	97	95	95
PCB 118	102	117	108	106
PCB 123	102	98	98	96
PCB 156	95	102	90	95
PCB 157	94	87	94	93
PCB 167	94	94	93	94
PCB 189	96	91	95	95

Table 2. Mean surrogate recoveries and standard deviations for fortified carbon-13 labelled standards in chicken, fish, milk and shellfish.

Matrix	Chicken (n = 20)		Fish (n = 18)		Milk (n = 9)		Shellfish (n = 12)	
% Recovery / ^{13}C labelled std.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
2,3,7,8-TCDD	81	10	88	10	93	8	84	12
1,2,3,7,8-PeCDD	85	8	90	10	94	12	89	13
1,2,3,4,7,8-HxCDD	81	11	96	11	80	8	94	10
1,2,3,6,7,8-HxCDD	86	16	100	12	92	14	95	12
1,2,3,7,8,9-HxCDD								
1,2,3,4,6,7,8-HpCDD	80	11	94	19	83	7	90	16
OCDD	77	21	87	25	75	8	80	20
2,3,7,8-TCDF	76	10	80	9	78	10	81	9
1,2,3,7,8-PeCDF	91	6	94	14	95	14	94	14
2,3,4,7,8-PeCDF	89	10	92	11	99	18	94	11
1,2,3,4,7,8-HxCDF	78	8	96	14	85	7	98	14
1,2,3,6,7,8-HxCDF	92	15	101	12	100	12	102	13
1,2,3,7,8,9-HxCDF	78	10	94	15	85	8	95	14
2,3,4,6,7,8-HxCDF	83	11	100	17	86	8	96	14
1,2,3,4,6,7,8-HpCDF	82	13	96	16	87	11	95	12
1,2,3,4,7,8,9-HpCDF	83	18	98	20	82	7	94	16
OCDF								
PCB 77	93	6	96	10	87	11	92	7
PCB 81	87	7	93	10	88	11	90	7
PCB 126	85	13	84	18	92	18	81	14
PCB 169	78	11	78	12	87	13	73	7
PCB 105	89	10	87	8	91	11	88	9
PCB 114	85	12	83	7	89	10	86	7
PCB 118	86	11	85	5	91	12	86	8
PCB 123	86	11	84	5	90	10	86	7
PCB 156	93	9	93	7	99	11	96	14
PCB 157	93	10	95	8	99	12	97	14
PCB 167	93	10	92	9	96	11	95	10
PCB 189	94	9	90	7	93	12	93	12

Table 3. The concentrations of dioxins in certified reference material (CARP-1) determined by the combined extraction and cleanup method.

Analytes	Assigned Values (μg/kg)		Measured Values (μg/kg)			
	Conc.	Uncertainty	Mean	SD	RSD %	Trueness %
2,3,7,8-TCDD	6.6	± 1.7	5.9	0.047	0.8	90.1
1,2,3,7,8-PeCDD	4.4	± 1.1	3.6	0.044	1.2	82.5
1,2,3,4,7,8-HxCDD	1.9	± 0.7	1.4	0.035	2.5	74.6
1,2,3,6,7,8-HxCDD	5.6	± 1.3	4.7	0.081	1.7	83.5
1,2,3,7,8,9-HxCDD	0.7	± 0.4	0.6	0.087	15	84.4
1,2,3,4,6,7,8-HpCDD	6.5	± 1.8	6.6	0.224	3.4	102
OCDD	6.3	± 1.9	6.3	0.306	4.9	100
2,3,7,8-TCDF	11.9	± 2.7	10.9	0.262	2.4	91.4
1,2,3,7,8-PeCDF	5	± 2	4.5	0.110	2.4	90.3
PCB-105	54	± 24	51	4.42	9.3	94.9
PCB-118	132	± 62	135	6.28	12.6	100

Table 4. The concentrations of dioxins and dioxin-like compounds in fish reference material (WMF-01) determined by the combined extraction and cleanup method.

Analyte	Assigned value	Acceptable range	Measured value
2,3,7,8-TCDD	13.1	8.7 – 17.5	12.2
1,2,3,7,8-PeCDD	2.72	1.42 – 4.02	2.38
1,2,3,4,7,8-HxCDD	0.22	0.00 – 0.52	0.09
1,2,3,6,7,8-HxCDD	0.88	0.48 – 1.28	0.81
1,2,3,7,8,9-HxCDD	0.27	0.00 – 0.67	0.07
1,2,3,4,6,7,8-HpCDD	0.59	0.00 – 1.29	0.19
OCDD	3.91	0.00 – 10.11	1.61
2,3,7,8-TCDF	13.1	8.2 – 18.0	14.5
1,2,3,7,8-PeCDF	1.53	0.13 - 2.93	1.24
2,3,4,7,8-PeCDF	7.15	4.95 – 9.35	8.16
1,2,3,4,7,8-HxCDF	0.86	0.00 – 1.86	0.50
1,2,3,6,7,8-HxCDF	0.51	0.00 – 1.21	0.54
1,2,3,7,8,9-HxCDF	0.25	0.00 – 0.65	0.05
2,3,4,6,7,8-HxCDF	0.68	0.28 – 1.88	1.02
1,2,3,4,6,7,8-HpCDF	1.01	0.00 – 2.91	0.50
1,2,3,4,7,8,9-HpCDF	0.30	0.00 – 0.80	0.18
OCDF	1.38	0.00 – 3.48	0.64
PCB 77	2233	1513 - 2953	2188
PCB 81	201	143 – 259	185
PCB 126	739	479 – 999	785
PCB 169	76	46 – 106	80
PCB 105	49050	34850 – 63250	48820
PCB 114	3523	1853 – 5193	3671
PCB 118	130100	97600 – 162600	127900
PCB 123	4233	1613 – 6853	3370
PCB 156	14890	9870 – 19910	16070
PCB 157	3488	2618 – 4358	3620
PCB 167	9750	6660 – 12840	10210
PCB 189	2016	1405 - 2627	2048
PBDE 28	3124	2834 – 3414	3177
PBDE 47	123200	98400 – 148000	119600
PBDE 99	37500	33280 – 41720	36200
PBDE 100	35870	21370 – 50370	38130
PBDE 153	17040	9040 – 25040	16310
PBDE 154	19790	16910 – 33670	21270
PBDE 183	532	132 - 932	415

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References

1 Chung W.C., Ng C.S. (2003) Organohalogen Compounds 60, 346.