Simultaneous determination of PCDD/Fs, Dioxin-like PCBs and Indicator PCBs in food.

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzo-furans(PCDD/Fs) as well as dioxin-like polychlorinated biphenyls(DL-PCBs) are ubiquitous highly toxic environmental pollutants which exhibit a potential risk for human health.[1] Also, Non dioxin-like PCBs are focused with health risk at recent days. Additionally, PCBs including with dioxin-like PCBs were classified to 1 group of IARC in 2016.[2] Indicator-PCBs are 7 congeners of PCBs covered approximately 50% of amount of total PCBs in food.[3] So, 7 congeners of indicator-PCBs were regulated in Korea and 6 congeners except for DL-PCBs 118 in EU.[4] Because of physicochemical properties, these compounds tend to concentrate and magnify in the food chain. Consumption of food is considered as the major source of non-occupational human exposure to these compounds with foodstuffs from animal origin accounting for more than 90% of the human body burden. The purpose of this study was to establish the simultaneous analytic method of PCDD/Fs, DL-PCBs and Indicator-PCBs for food in order to survey contaminations of these compounds in food and level of exposure to human by eating food. The best ways of extraction, purification and analysis were established based on USEPA 1613 and 1668 Method. It was verified to reliability and reproducibility by being applied to CRM(Certified reference material).

Materials and methods

(1) Preparation of samples

All organic solvents were ultra-residue grade for dioxin analysis (Wako, Japan). Calibration standard solutions, ^{13}C-labeled surrogate standards, cleanup standards and injection standards specified in USEPA Method 1613 for PCDD/Fs 17 congeners and USEPA Method 1668C for DL-PCBs 12 congeners and Indicator-PCBs 7 congeners analysis were purchased from Wellington Laboratories Inc.[5,6,7]

(2) Extraction of fat from samples

The methodology used for PCDD/Fs analysis based on the USEPA method 1613 has been described in detail elsewhere. There are few ways to extract the fat depending on the phase of the sample. It uses soxhlet or ASE in case of a solid phase. The methodology was examined to extract fat after comparing between soxhlet and ASE on the phase of the solid.

Soxhlet extraction : About 20 g of the analytical samples were mixed with anhydrous sodium sulfate and extracted using n-hexane : dichloromethane(1:3,v/v) as solvents in soxhlet extractor during 18-24h.

ASE(Accelerated Solvent Extraction) : About 10 g for each sample that was mixed with anhydrous sodium sulfate extracted in 100 ml stainless steel extraction cell with an ASE 350 Accelerated Solvent Extractor (Dionex Sunnyvale, California). The extraction solvent was hexane : dichloromethane(1:1, v/v) and 2 × 5 min extraction cycles, 100 ℃ temperature, 1500 psi pressure, and 60% flush volume were used.

For identification and quantification, appropriate ^{13}C -labeled internal standard were added to sample prior to extraction. The extracts were concentrated to determine the fat contents.

(3) Purification

Each extract was then purified in a sequence that comprises purification on column with sodium sulphate and sulfuric acid impregnated silica gel. The obtained extract was then transferred to multilayer chromatography clean-up column in order to further remove the interference. Clean-up colums were composed with silica, alumina and chacoal. All colums were activated and then extract was flowed through clean-up colums. And then we received an effluent eluted by flowing n-hexane : dichloromethane(98:2,v/v) solvent to alumina colum(fraction 2) and then by flowing n-hexane : dichloromethane(50:50,v/v) solvent to alumina and chacoal colums(fraction 3). We combined to fraction 2 with fraction 3 solution. DL-PCBs 8 congeners and Indicator-PCBs 7 congeners in this effluent were analysed by HRGC/HRMS. And then we recieved effluent eluted by flowing opposite direction with toluene to chacoal colum. PCDD/Fs 17 congeners

and DL-PCBs 4 congeners in this effluent(fraction 5) were also analysed by HRGC/HRMS.
The quantification of PCDD/Fs, DL-PCBs and Indicator-PCBs was carried out by the isotopic dilution method and methodology was validated according to US EPA Method by performing an initial, ongoing precision and recovery studies.

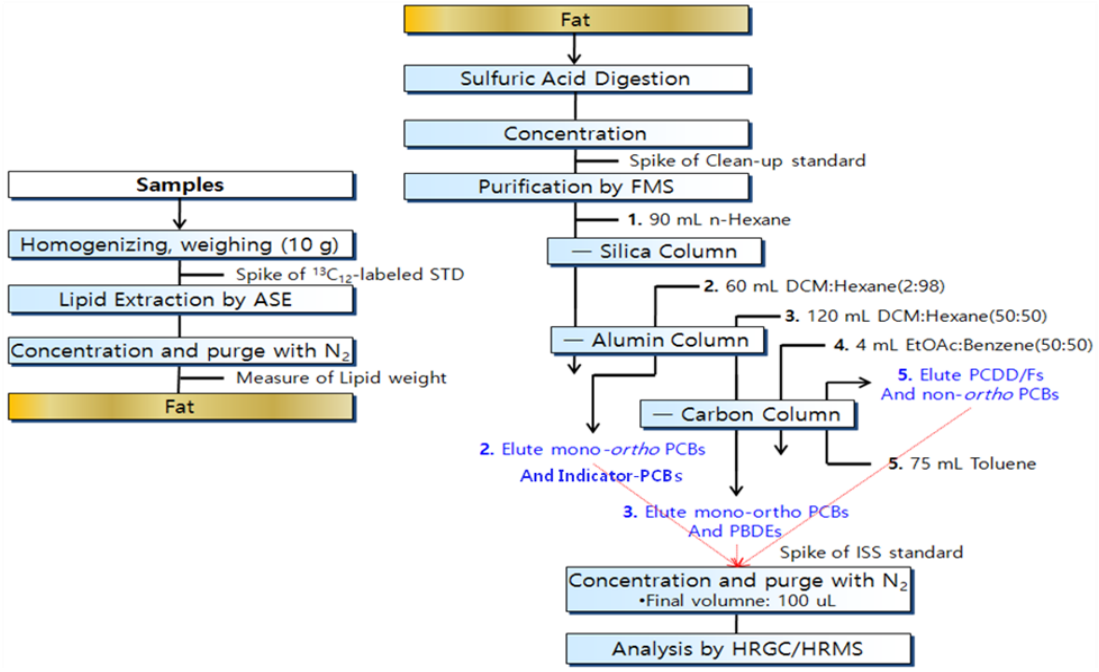

Fig. 1. Flow chart to analysis PCDD/Fs, DL-PCBs and Indicator-PCBs in Food samples.

(4) Instrumental analysis
Qualitative and quantitative determination of PCDD/Fs, DL-PCBs and Indicator-PCBs was done by HRGC/HRMS. HRGC/HRMS analysis were performed with Thermo trace Ultra gas chromatography interfaced to a Finnigan DFS mass spectrometer which were in MID mode operating positive electron ionization at a resolving power of >10,000 at *m/z* 314 of FC43. As for PCDD/Fs and DL-PCBs global concentrations, toxic equivalents (TEQ) were calculated using the toxic equivalent factors (TEFs) reported by the World Health Organization in 2005.[8]

Table 1. The parameters of HRGC/HRMS to analyze PCDD/Fs, DL-PCBs and Indicator-PCBs.

Parameter	PCDD/Fs	Dioxin-like PCBs	Indicator-PCBs
Column	DB-5MS(60 m × 0.25 mm × 0.25 ㎛)		DB-1(60 m × 0.25 mm × 0.25 ㎛)
Oven temperature	Initial 160℃(4 min) 220℃(15 min) / 5℃/min - 290℃(10 min) / 5℃/min - 300℃(7 min)	Initial 150℃(1 min) 185℃(3 min) / 20℃/min - 245℃(10 min) / 2℃/min - 300℃(4 min)	Initial 100℃(1 min) 160℃(2 min) / 10℃/min - 200℃(2 min) / 5℃/min - 210℃(5 min) / 5℃/min – 290℃(5 min)
Carrier gas	He, 1.0㎖/min		
Injector/transferline	280℃/280℃		
Type of Inj., volume	Splitless mode, 1 μL		
Ionization type	EI (positive)		

Resolution	>> 10,000 at m/z 314 (FC43)
Ion Source	260℃

(5) Validation of analytical method

To validate this method, evaluated parameters were the selectivity, linearity, accuracy, precision and recovery. It was verified to reliability and reproducibility by being applied to CRM. To assess the reliability of our results, we have participated in international inter-laboratory studies related to PCDD/Fs, DL-PCBs and Indicator-PCBs (Interlaboratory Comparison on Dioxins in Food, 2014, Division of Environmental Medicine, Norwegian Institute of Public Health, Folkehelse, Norway).

Results and discussion

(1) Establishment of analytic method

This paper compares the extraction effectiveness of two different commonly applied extraction techniques for the determination of Dioxin, DL-PCBs and Indicator-PCBs in food. ASE was initially performed at 100℃ using n-hexane/dichloromethane (1:1,v/v) with a single 5 min extraction step.

This resulted in extraction rate of fat, which were close to Soxhlet, or in some cases even below extraction rate of Soxhlet. But, two cycle extraction of ASE could get more rate of fat than Soxhlet. However Soxhlet usually requires large amounts of solvent and is often carried out for 18 h or more. As the demands for minimizing solvent consumption and time has decreased, extraction conditions of ASE were modified. When ASE was performed at higher temperature than 100℃, it was often loosing the gas of solvent from jointer of cell of ASE. The fat content of Extraction using ASE with two times(2 cycles) was higher than soxhlet. Thus, extraction conditions of ASE by comparing temperature and cycle were determined 100℃/ 2cycle (Fig.1).

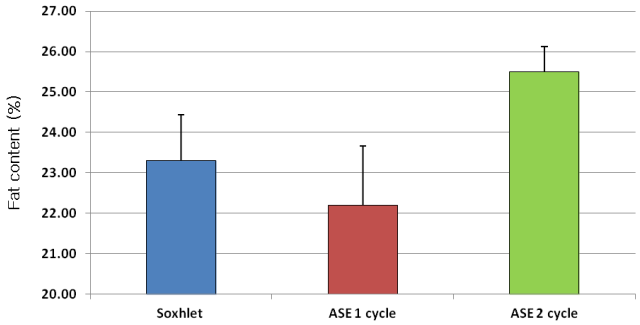

Fig.1. Comparison of Soxhlet and ASE and cycle-specific extraction

(2) Validation of analytical method

(2)-1 Selectivity

The each peaks of PCDD/Fs, DL-PCBs and Indicator-PCBs congeners in chromatogram were well separated in instrumental conditions of HRGC/HRMS. We could confirm good selectivity in which chromatogram of standard solution compare retention times and area of peaks with it of standard spiked sample.

(a) **(b)** **(c)**

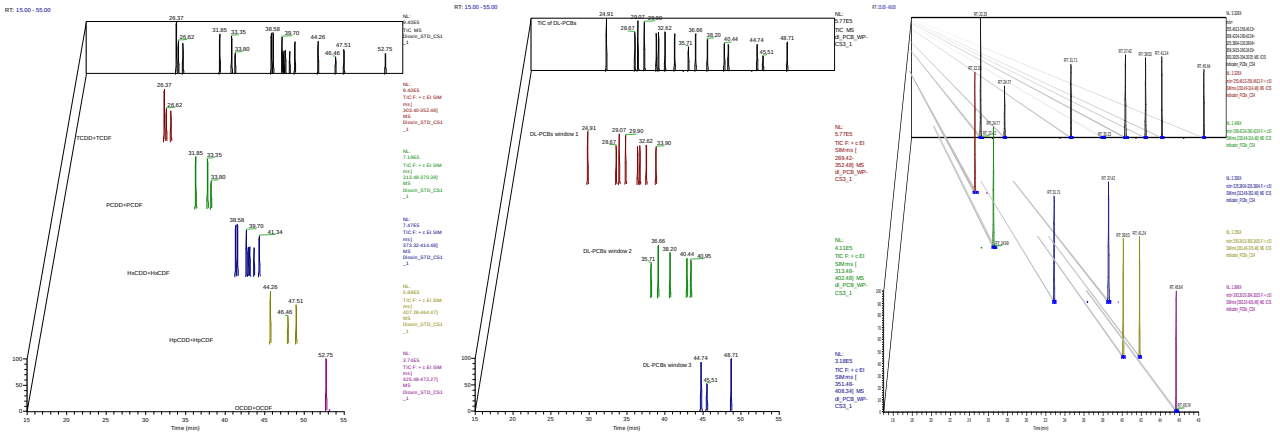

Fig. 2. GC/MS chromatogram of (a) PCDD/Fs, (b) DL-PCBs and (c) Indicator-PCBs

(2)-2 Linearity of calibration curves and sensitivity

We made calibration curves using 5 concentrations of calibration standard(CSL, CS0.5, CS1, CS2 and CS3, wellington) for PCDD/Fs and calibration standard(CS1, CS2, CS3, CS4 and CS5, wellington) for DL-PCBs. The linearity of all calibration curves were good values as over 0.999. The limits of detection were 0.01 ~ 0.14 pg/g, and limits of quantification were 0.03 ~ 0.42 pg/g. we could confirm enough sensitivity for analyzing PCDD/Fs, DL-PCBs and Indicator-PCBs in food.

Table 1. The linearity of calibration curves and limits of detection and quantification.

Group		Congeners	Y=aX+b	r^2	LOD	LOQ
					(pg/g)	
Dioxins	PCDDs	2378-TCDD	$0.010 \times +6.2618E^{-4}$	0.999727	0.01	0.03
		12378-PeCDD	$9.920 E^{-3} \times +3.098E^{-4}$	0.999959	0.01	0.03
		123478-HxCDD	$9.897E^{-3} \times +1.149E^{-3}$	0.999975	0.004	0.012
		123678-HxCDD	$9.087E^{-3} \times +2.348 E^{-3}$	0.999773	0.01	0.03
		123789-HxCDD	$9.221E^{-3} \times +2.120 E^{-3}$	0.999850	0.01	0.03
		1234678-HpCDD	$9.784 E^{-3} \times +1.382E^{-3}$	0.999983	0.03	0.09
		OCDD	$4.852E^{-3} \times +4.506E^{-4}$	0.999993	0.01	0.03
	PCDFs	2378-TCDF	$9.9189 E^{-3} \times +7.610E^{-5}$	0.999984	0.01	0.03
		12378-PeCDF	$9.241E^{-3} \times +1.102E^{-3}$	0.999994	0.01	0.03
		23478-PeCDF	$9.511E^{-3} \times +7.042E^{-4}$	0.999996	0.01	0.03
		123478-HxCDF	$0.011 \times +1.471E^{-3}$	0.999973	0.01	0.03
		123678-HxCDF	$0.011 \times +7.171E^{-4}$	0.999996	0.01	0.03
		234678-HxCDF	$0.012 \times +1.103E^{-3}$	0.999990	0.01	0.03
		123789-HxCDF	$0.010 \times +2.421E^{-3}$	0.999839	0.02	0.06
		1234678-HpCDF	$0.013 \times +9.670E^{-4}$	0.999998	0.002	0.006
		1234789-HpCDF	$0.013 \times +1.210E^{-3}$	0.999985	0.002	0.006
		OCDF	$5.634E^{-3} \times +5.682E^{-4}$	0.999979	0.01	0.03
DL-PCBs	Non-ortho PCBs	PCB 81	$0.023 \times +1.115E^{-4}$	0.999994	0.02	0.06
		PCB 77	$0.023 \times +7.457E^{-4}$	0.999996	0.03	0.09

		PCB 126	0.023 x +2.800E^{-4}	0.999959	0.01	0.03
		PCB 169	0.022 x +4.813E^{-4}	0.999948	0.02	0.06
	Mono-ortho PCBs	PCB 123	0.022 x +3.882E^{-4}	0.999997	0.3	0.9
		PCB 118	0.022 x +7.824E^{-4}	0.999992	0.3	0.9
		PCB 114	0.023 x +2.836E^{-4}	0.999999	0.3	0.9
		PCB 105	0.021 x +5.059E^{-4}	0.999998	0.3	0.9
		PCB 167	0.022 x +5.565E^{-4}	0.999994	0.2	0.6
		PCB 156	0.022 x +3.929E^{-4}	0.999997	0.3	0.9
		PCB 157	0.022 x +1.049E^{-3}	0.999974	0.2	0.6
		PCB 189	0.021 x +5.498E^{-4}	0.999991	0.02	0.06
Indicator-PCBs		PCB 28	0.021 x +0.019	0.999481	0.08	0.2
		PCB 52	0.022 x +0.014	0.999770	0.6	1.8
		PCB 101	0.020 x +0.011	0.999808	0.5	1.5
		PCB 138	0.020 x +8.858E^{-3}	0.999915	0.3	0.9
		PCB 153	0.020 x +0.012	0.999694	0.3	0.9
		PCB 180	0.020 x +0.011	0.999760	0.2	0.6

(2)-4 Reproducibility and Precision

The recoveries of each internal standard of PCDD/Fs, DL-PCBs and Indicator-PCBs congeners were suitable in criteria of EPA methods.

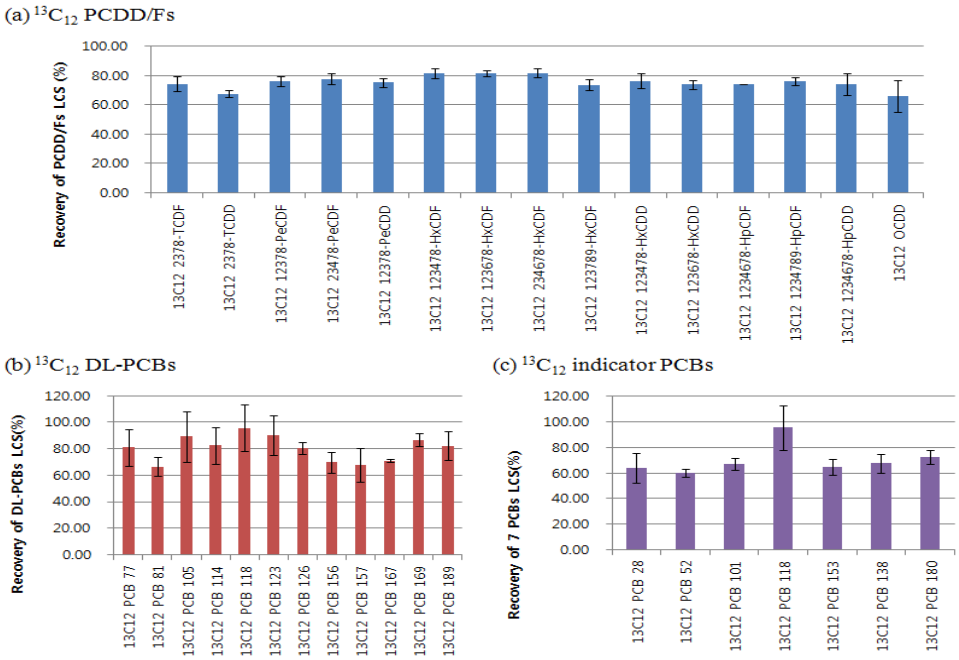

Fig. 1. Recovery ranges of internal standards of (a) PCDD/Fs, (b) DL-PCBs in WMF-01 and (c) Indicator-PCBs in CARP-2.

(2)-5 Accuracy and Proficiency

The values of PCDD/Fs, DL-PCBs analyzed in CRM(WMF-01, wellington) and Indicator-PCBs in CRM(CARP-2, wellington) by this method were in range of certified values of CRM. The best ways of extraction, purification and analysis were established based on USEPA 1613 and 1668 methods. It were applied to CRM WMF-01 and CARP-2 to verify reliability and reproducibility (Fig.2.).

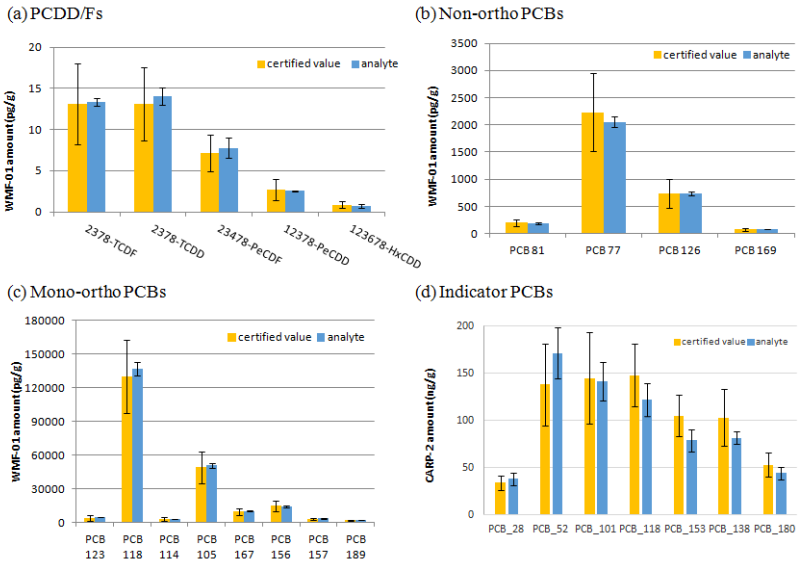

Fig. 2. Comparison between analytical values and certified values of CRM(WMF-01, CARP-2, wellington).

To assess the reliability of our results, we have participated in international inter-comparison program related to PCDD/Fs, DL-PCBs and Indicator-PCBs (Interlaboratory Comparison on Dioxins in Food, 2017, Division of Environmental Medicine, Norwegian Institute of Public Health, Folkehelse, Oslo, Norway) and our result was submitted to NIPH(table 3), The result of inter-comparison will be discussed in 37th international symposium on halogenated POPs.

Table 3. The results of analysis PCDD/Fs, DL-PCBs and Indicator-PCBs in samples from NIPH.

(unit : pg/g w.w.)

PCDD/Fs	Herring	Cod liver	DL-PCBs	Herring	Cod liver	Indicator-PCBs	Herring	Cod liver
2378-TCDD	0.07	0.51	PCB 77	28.14	127.41	PCB 28	259.93	2705.95
12378-PeCDD	0.19	0.04	PCB 126	6.74	88.57	PCB 52	472.29	6766.08
123478-HxCDD	0.04	0.02	PCB 169	1.40	16.64	PCB 101	1139.53	9066.57
123678-HxCDD	0.09	0.25	PCB 81	0.49	5.82	PCB 138	2289.90	20457.65
123789-HxCDD	0.01	0.03	PCB 105	324.22	4816.62	PCB 153	2452.10	21705.18
1234678-HpCDD	0.05	0.12	PCB 114	11.82	291.07	PCB 180	318.57	4242.79
OCDD	0.04	0.11	PCB 118	1067.15	13311.09			
2378-TCDF	1.89	11.16	PCB 123	52.40	250.43			
12378-PeCDF	0.30	1.42	PCB 156	103.54	1189.66			
23478-PeCDF	0.79	0.64	PCB 157	29.05	392.29			
123478-HxCDF	0.11	0.19	PCB 167	76.99	827.18			

123678-HxCDF	0.11	0.39	PCB 189	10.86	85.36
234678-HxCDF	0.12	0.33			
123789-HxCDF	0.14	0.09			
1234678-HpCDF	0.04	0.14			
1234789-HpCDF	0.01	0.03			
OCDF	0.08	0.05			

Conclusion

The methodology presented above enables the fractionation of a range of toxic chlorinated pollutants present in a single food sample. This simultaneous determination has advantages in terms of analytical efficiency and the integrity implicit in a single representative sample. Confience is provided by the analysis of reference materials and the participation in international inter-comparison exercises provided from NIPH. This method will be used to survey the level of contamination and exposure of PCDD/Fs, DL-PCBs and Indicator-PCBs in food.

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