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SIMULTANEOUS ANALYSIS OF DIOXINS, PCBS, AND PBDES WITH A FULLY AUTOMATED SAMPLE PREPARATION SYSTEM (II: VALIDATION)

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Introduction

Unique automated sample preparation technologies for dioxins and PCBs analysis have been developed at our R&D site since 2000[1, 2]. Systems (GO-HT series, Miura Co., Ltd., Japan) equipped with the developed technologies have been on the European market since 2015. The systems are authorized as validated methods by some European accreditation laboratories. In the same way, the EU commission has adopted legislation to reduce or halt the sale and use of certain brominated flame retardants (BFRs) in order to protect human health and the environment. The EFSA recommended the monitoring of BFRs in foodstuffs based on commission recommendation 2014/118[3]. Therefore, the demand for viable technologies for the simultaneous analysis of PCDDs/PCDFs, PCBs, and PBDEs in foodstuffs has increased in European laboratories. We previously reported a procedure for the simultaneous analysis for PCDDs/PCDFs, PCBs, and PBDEs using the developed system[4]. This method is much more effective than manual purification steps, which involve complicated operations. The advantages are as follows:

1. Reduced solvents as compared to conventional methods (as little as one-tenth of the required amount)
2. Negligible cross contamination (lower blank level)
3. High purification efficiency by heated purification columns

Notably, cross contamination presents a significant issue in manual analysis with numerous glass apparatuses and complicated instruments. On the other hand, the systems we developed eliminate the need for various valves, operations, and the use of large amounts of solvents, which are a considerable source of cross contamination. In this presentation, we report the simultaneous analysis of PCDDs / PCDFs / PCBs / PBDEs in feed and food samples as an application of the developed system. Based on the results of the quality control samples, real samples (unknowns), procedure blanks, and standard reference materials (SRMs), the fractionations of PCDDs / PCDFs / PCBs / PBDEs, limit of quantification (LOQ), accuracy, and recoveries are discussed.

Materials and methods

1. Preparation of samples and standards

Hen eggs and pork meat were purchased from a supermarket in Matsuyama, Japan. The shells from 50 hen eggs were removed, and homogenized; they were kept at -20 ˚C in the freezer for 2 d, and subsequently freeze-dried for 1 d. Two kilograms of pork meat were directly freeze-dried, then homogenized. Fish oil, palm oil, sunflower seed oil, and fish liver oil were obtained from Sigma-Aldrich.

Standard solutions of PCDDs/PCDFs, PCBs, and PBDEs were purchased from Wellington Laboratories. Standard reference materials (EDF-5462, EDF-5463) were obtained from Cambridge Isotope Laboratories Inc.

2. Extraction

Fat solutions of freeze-dried hen egg and pork meat were prepared by Soxhlet extraction with a mixture of (7+3) toluene and acetone. Next, the solvent was completely evaporated.

3. Purification

Aliquots of fat spiked with $^{13}C_{12}$-labeled PCDD/PCDF, PCB, and PBDE internal standard mixtures (0.5 g ~ 3 g), were dissolved in about 2 ~ 5 mL of n-hexane and were directly applied to the top of the purification column using a disposable syringe. The assembled columns were attached to the system. In about 94 min, two fractions, dioxin and PCB fractions, were obtained automatically. The dioxin fraction included PCDD/DFs and non-ortho PCBs in about 1.3 mL of toluene. Mono-ortho PCBs, 6-isomer PCBs, and PBDEs comprised the PCB fraction in about 1.0 mL of toluene. Elution solvents included 90 mL of n-hexane and 5 mL of toluene. Ethyl acetate, 2-propanol, THF, acetone, dichloromethane, and toluene were used as additive eluents for PBDEs. Dioxin and PCB fractions spiked with $^{13}C_{12}$-labeled recovery standards were concentrated until the final volume was 10 or 20 μL and 50 μL, respectively, using a CentriVap Centrifugal Vacuum Concentrator (LABCONCO).

4. GC-MS measurement

PCDD/PCDFs, DL-PCBs, 6-isomer PCBs, and PBDEs were determined by GC-HRMS (JMS-800D-JEOL). All sample solutions (1 or 2 μL) were injected in splitless mode. High resolution mass spectrometry was carried out in EI mode, using selected ion monitoring. The resolution of the MS was routinely more than 10,000 (10% valley). The gas chromatograph capillary columns for each compound were as follows:

Gas chromatograph program for PCDD/DFs and DL-PCBs: GC equipped with VF-Xms (60 m x 0.25 mm, 0.25 μm film thickness, Agilent Technologies); total run time was 45 min.

Gas chromatograph program for NDL-PCBs: GC equipped with HT8-PCB (60 m x 0.25 mm, 0.2 μm film thickness, SGE USA); total run time was 42 min.

Gas chromatograph program for PBDEs: GC equipped with DB-5HT (15 m x 0.25 mm, 0.10 μm film thickness, Agilent Technologies); total run time was 30 min.

Results and discussion

1. Additive eluents and recovery

PCDDs/PCDFs, DL-PCBs, and 6 indicator-PCBs could be eluted with only 90 mL of n-hexane from the purification column. However, it was difficult to elute PBDEs from the purification column with only n-hexane, perhaps because PBDEs are strongly adsorbed on the silica gel because PBDEs have higher polarities than PCDDs/PCDFs and PCBs, as noted from the log Pow of each compound. To promote the elution of PBDEs from the silica gel column (Table 1), polar eluents were added to the blank sample solutions. In addition, another mobile phase (n-heptane and cyclohexane) with a slightly higher polarity was also assessed. As evidenced by the recoveries shown in Table 1, the addition of polar eluents into the blank sample solution promoted the elution of PBDEs from silica gel effectively. Notably, ethyl acetate resulted in good recoveries. With respect to other eluents, it would be difficult to consider that they are useful as the additive eluents for PBDEs elution. Dichloromethane and toluene affected the fractionation of PCDDs/PCDFs and PCBs. 2-propanol, THF, and acetone reacted with silver nitrate and sulfuric acid impregnated silica gel and discolored the sulfuric acid silica gel. As far as the eluents shown in Table 1 are concerned, ethyl acetate was the best additive eluent, and was the most user-friendly because this solvent is generally used as a food additive. On the other hand, the elution patterns of all target compounds with n-heptane were basically the same as those with n-hexane, whereas some mono-ortho PCBs were included in the dioxin fraction when cyclohexane was utilized.

Figure 1 shows the recoveries of $^{13}C_{12}$-labeled PBDE compounds in 1 g of fish oil (A) and 3 g of sunflower seed oil (B) using the GO-HT system. These results indicate that the optimum volume of ethyl acetate was less than 1 mL, but it depended on the sample species (e.g., fatty acid oil and fat composition) and/or sample volume and weight. In the case of sunflower seed oil, it was not necessary to add ethyl acetate. Before routine analysis, it might be more appropriate to validate the additive amount of ethyl acetate. Additional research is needed on this application data with many species of samples.

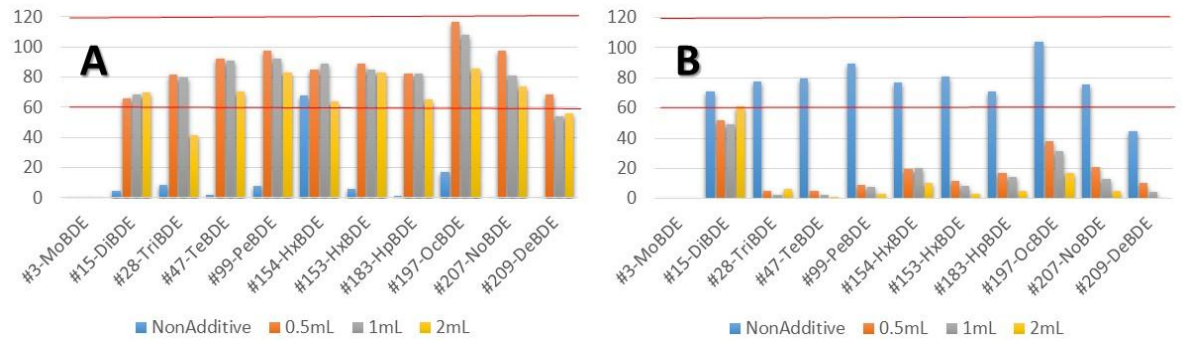

Figure 1 Recoveries of $^{13}C_{12}$-labeled PBDE compounds in 1 g of fish oil (A) and 3 g of sunflower seed oil (B) using the GO-HT system.

Table 1 Recoveries of PBDEs with different mobile phases and amounts of additive eluents.

Mobile Phase	Additive	Polarity	bp	Recovery [%]		
		ε	dC	DD/DF&nPCB	m&6-PCBs	PBDEs
n-Hexane	-	1.88	69	94-111	85-105	0
	Ethyl acetate	6.02	76	1 mL/ 97-110 2 mL/ 95-117 3 mL/ 97-120	1 mL/ 80-103 2 mL/ 83-104 3 mL/ 83-105	1 mL/ 63-114 2 mL/ 79-111 3 mL/ 75-120
	2-propanol	20.33	98	1 mL/ 92-112 2 mL/ 102-119 3 mL/ 97-117	1 mL/ 81-102 2 mL/ 79-99 3 mL/ 86-104	1 mL / 6.6-107 2 mL/ 84-113 3 mL/ 79-114
	THF	7.58	65	0.5 mL/ 97-107 1 mL/ 79-91 2 mL/ 89-104	0.5 mL/ 79-94 1 mL/ 84-97 2 mL/ 69-82	0.5 mL/ 0-110 1 mL/ 69-115 2 mL/ 67-98
	Acetone	20.7	56	1 mL/ 89-105 2 mL/ 89-101 3 mL/ 87-113	1 mL/ 81-102 2 mL/ 76-99 3 mL/ 80-98	1 mL/ 61-114 2 mL/ 63-113 3 mL/ 46-110
	DCM	8.93	40	0.5 mL/ 74-105 1 mL/ 72-90 2 mL/ 85-105	0.5 mL/ 0-75 1 mL/ 0-33 2 mL/ 0-10	0.5 mL/ 0-51 1 mL/ 22-82 2 mL/ 16-60
	Toluene	2.38	110	0.1 mL/ 63-100 0.2 mL/ 34-103 0.4 mL/ 10-105	0.1 mL/ 84-105 0.2 mL/ 88-101 0.4 mL/ 77-100	0.1 mL/ 0-53 0.2 mL/ 0-65 0.4 mL/ 2.5-63
n-Heptane	-	1.92	98	77-98	83-102	0
Cyclohexane	-	2.02	80	94-107	30-103	0

ε: Dielectric constant indicates dipole moment. bp: boiling point [dC: degrees centigrade °C]. nPCB: non-ortho PCBs. m&6-PCB: mono-ortho PCBs and 6 indicator PCBs. PBDEs: #15, #28, #47, #99, #154, #153, #183, #197, #207, #209.

2. Background level

LOD and LOQ are typically calculated in accordance with Japanese Industrial Standard (JIS K0311 and 0312). Based on JIS and EU regulations, we used the specific definition of LOQ as follows: first, the lowest concentration point on a calibration curve was injected 5 or 6 times into the GC-MS. The determined amount of each target isomer was calculated using the average relative response factor of each isomer. The instrument LOD and LOQ of GC-MS (iLOD and iLOQ) were defined as 3 and 10 times the standard deviation (SD) of the injected amount, respectively. Next, to obtain the method LOQ (mLOQ) of the GO-HT system, the spiked amount of target compounds was calculated as iLOQ × (final volume µL / injection volume µL). The target compounds were spiked into the blank sample (n=6) and sunflower seed oil (n=6), and 2 mL of ethyl acetate was added to the blank samples; purification and concentration were carried out using the GO-HT system and CentriVap concentrator. Finally, the mLOQ was defined as the blank level plus 3 times the SD. On the other hand, the target compounds were not detected from the GO-HT and columns, and the blank level was zero. Table 2 shows the mLOQ calculated from blank samples and sunflower seed oil analyzed with six replicates. To facilitate comparison, the LOQ based on a signal to noise ratio of 3:1 is shown. The mLOQ obtained in this study was similar to that calculated from a signal to noise ratio of 3:1. This purification method using the GO-HT system and CentriVap concentrator does not require a large amount of solvent or glass apparatuses, and the system does not contact the sample solutions, resulting in a low background level. However, these mLOQs can only be applied if the samples are expressed on the basis of fat. Therefore, it is necessary to assess the method including the extraction steps of whole weight samples.

Table 2 mLOQ of sum of PCDDs/PCDFs calculated from spiking experiments.

From spiked blank [pg-TEQ]		From spiked sunflower [pg-TEQ/g]	
mLOQ by 3SD	mLOQ by S/N=3	mLOQ by 3SD	mLOQ by S/N=3
0.0688	0.0801	0.137	0.114

3. Accuracy of PBDE analysis

In 2015 and 2016, validation results of PCDDs/PCDFs, DL-PCBs, and NDL-PCBs fulfilled the analytical criteria of the regulations. Accuracy was expressed as trueness (difference between the mean value measured for PBDEs in a reference material EDF-5463 (n=4) and its reference value (assigned value), expressed as a percentage of this value), precision (RSD_R), mLOD (=3 times SD), and mLOQ (=10 times SD) calculated from blank controls and spiking experiments in Table 3. The mean values measured in 2015 and 2016 were highly

compatible with the reference values; the difference between assigned values and measured mean values were within ± 30%, and the RSD_R of each congener were less than 17%. The blank level of #209-DeBDE from GO-HT and consumable columns was zero and #209-iLOQ calculated from the lowest concentration of calibration points was 1.4 pg/μL (=10 times SD). However, its LOD and LOQ were higher than others, which was largely dependent on the GC-MS and/or GC capillary column conditions.

Table 3 Accuracy and reproducibility.

	Reference Value		2015's Validation				2016's Validation					
Compounds	Assigned	SD x2	Mean	SD	RSD_R	Deviation	Mean	SD	RSD_R	Deviation	LOD	LOQ
Isomers	pg/g fat		pg/g fat		%	%	pg/g fat		%	%	pg/g	
#15-DiBDE	-		(2.1)	0.21	10		ND				1.6	5.4
#17-TriBDE	8.83	± 5.76	(6.6)	0.94	14	-25	(8.4)	0.46	5	-5	2.7	9.0
#28-TriBDE	40.1	± 13.9	37.9	1.54	4	-5	38.3	1.36	4	-5	1.1	3.8
#49-TeBDE	-		278.8	8.84	3		260.3	7.09	3		2.1	6.9
#47-TeBDE	1480	± 480	1499.2	51.84	3	1	1497.4	23.07	2	1	2.2	7.2
#66-TeBDE	48.4	± 29.4	50.1	6.70	13	3	49.5	1.66	3	2	2.0	6.8
#77-TeBDE	-		ND				(5.9)	0.43	7		3.4	11.4
#100-PeBDE	357	± 50.6	350.7	18.62	5	-2	372.3	19.22	5	4	2.0	6.6
#119-PeBDE	-		26.8	4.42	16		33.1	2.18	7		5.1	16.9
#99-PeBDE	193	± 70.4	160.6	4.93	3	-17	165.4	4.47	3	-14	3.1	10.3
#154-HxBDE	229	± 88.8	207.2	7.41	4	-10	233.4	9.81	4	2	5.4	18.0
#153-HxBDE	33.9	± 6.64	32.1	2.13	7	-5	35.0	3.16	9	3	2.6	8.7
#183-HpBDE	-		(11.4)	0.84	7		ND				5.9	19.7
#207-NoBDE	-		(24.0)	2.37	10		(17.9)	2.48	14		8.8	29.4
#206-NoBDE	-		(30.6)	3.53	12		(16.7)	2.87	17		12.9	43.0
#209-DeBDE	-		131.3	21.65	16		134.1	13.54	10		35.5	118.3

4. Validation in the range of the maximum level and general quality control measures

For more reliable results, we performed the validation in the range of the maximum level (0.75 pg PCDDs/PCDFs TEQ/g fat) of vegetable oil; sunflower seed oil was used for this experiment. The results shown in Table 4 meet the analytical criteria for WHO-TEQ. DL-PCBs also exhibited good results, which are not shown in Table 4.

Table 4 Deviation from target values of vegetable oil.

PCDDs/PCDFs	Target	Mean	SD	RSD	Deviation
Spiked level	pg-WHO-TEQ/g fat			%	%
0.5 x ML	0.389	0.387	0.020	5	-0.6
1 x ML	0.779	0.768	0.052	7	-1.4
2 x ML	1.56	1.50	0.035	2	-4.2

5. Conclusion

When PBDEs are analyzed with PCDDs/PCDFs and PCBs, it is necessary to add a polar eluent, namely less than 1 mL of ethyl acetate, to the sample solution. However, this method should be tested one step further because there might be samples which do not require the addition of ethyl acetate, as can be seen from the results of 3 g of sunflower seed oil. The validation results met the established regulations for all target compounds. Compared with previous reports, this method with the GO-HT system might serve as a greener alternative because this method can be used to simultaneously analyze dioxins, PCBs, and PBDEs in a short amount of time without the use of large amounts of toxic solvents.

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