

# APPLICATION OF MICROWAVE ASSISTED EXTRACTION TO THE ANALYSIS OF DIOXINS FROM SOIL AND SEDIMENT CERTIFIED REFERENCE MATERIALS

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## Abstract

Microwave assisted extraction (MAE) was applied to the extraction of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) from soil and sediment certified reference materials. Six grams of the samples were extracted with mixed solvents composed of 6 ml of toluene, 2.4 ml of water, and 4.8 ml of ethanol at a temperature of 125°C for 30 min. The average concentrations of PCDD/DFs and DL-PCBs corresponded to the results of the certified value, and the reproducibility of this MAE method was below 19% of the relative standard deviation for the certified reference materials. These results indicated that a rapid analysis of dioxins from soil and sediment could be performed with accuracy and precision by our MAE method.

## Introduction

In Japan, the legal testing of dioxins in soils and sediments is carried out in accordance with the methods specified by the manuals for the survey and measurement of dioxins published by Japan Environment Ministry<sup>1,2</sup>. These methods have a common problem in that the analysis cycle time is relatively long because the analytical procedures are complicated. Thus, we have studied on the rapid analysis by MAE. In our previous studies, we found that the extraction efficiency of dioxin increased with the addition of water and ethanol to the extraction solvent<sup>3</sup>. Furthermore, we confirmed that the extracting solvent was composed of 6 ml of toluene, 2.4 ml of water, and 4.8 ml of ethanol for 6 g of soil and/or sediment, and that the extraction efficiency under conditions of 125°C for 30 min was comparable to that of Soxhlet extraction using test sample<sup>4</sup>. In this study, we performed the extraction of dioxins from soil and sediment certified reference materials in order to evaluate the validation of our MAE method. We analyzed the concentrations of dioxins and compared the values obtained with the certified values presented by The Japan Society for Analytical Chemistry.

## Methods and Materials

### Samples

Soil and sediment certified reference materials were purchased from The Japan Society for Analytical Chemistry; the serial numbers of these samples were JSAC 0421 and JSAC 0431, respectively. The samples were stored in a desiccator at room temperature until chemical analysis.

### Microwave assisted extraction

The experimental procedure of this study is summarized in Fig. 1. Six grams of soil and sediment certified materials were weighed into quartz glass extraction cells (45 ml, Q-20; Milestone General, Italy). After the addition of an internal standard as a cleanup spike (400 pg of both <sup>13</sup>C-labeled PCDD/DFs chlorinated at positions 2, 3, 7, 8, and <sup>13</sup>C-labeled DL-PCBs: #77, #81, #105, #114, #118, #123, #126, #156, #157, #167, #169, #189), the sample was extracted with mixed solvent composed of 6 ml of toluene, 2.4 ml of water, and 4.8 ml of ethanol at a temperature of 125°C for 30 min using a microwave extraction system (ETHOS TC; Milestone General). After cooling, the ethanol contained in the extract was removed using a rotary evaporator, and then the

water was removed with anhydrous sodium sulfate. The remaining extract was refined by multilayer silica gel and activated carbon dispersed silica gel column chromatography. The refined solution was finally concentrated to 20  $\mu\text{l}$  by nitrogen flow following the addition of an internal standard as a syringe spike (400 pg of  $^{13}\text{C}$ -labeled 1,2,7,8-TCDF, 1,2,3,4,6-PeCDF, 1,2,3,4,6,9-HxCDF, and 1,2,3,4,6,8,9-HpCDF). The total time required for these analytical procedures was approximately 5 h.

### **Analysis**

The identification and quantification of PCDD/DFs and DL-PCBs were performed by high-resolution gas chromatography coupled with a high-resolution mass spectrometric detector (HRGC/HRMSD) (Agilent 6890/JEOL JMS-700D, Agilent 6890/JEOL JMS-800). BPX-DXN capillary column (60 m  $\times$  0.25 mm i.d.; SGE, USA) and RH-12ms capillary column (60 m  $\times$  0.25 mm i.d.; Inventx, USA) were used for the separation of PCDD/DFs and DL-PCBs. The column oven temperature of the BPX-DXN was programmed at a rate of 20°C min<sup>-1</sup> from an initial temperature of 150°C (1 min hold) to a temperature of 220°C, then at a rate of 2°C min<sup>-1</sup> to a temperature of 260°C, and subsequently at a rate of 5°C min<sup>-1</sup> to a final temperature of 320°C (3.5 min hold). The column oven temperature of the RH-12ms was programmed at a rate of 10°C min<sup>-1</sup> from an initial temperature of 150°C (1 min hold) to a temperature of 210°C, then at a rate of 3°C min<sup>-1</sup> to temperature of 280°C, and at a rate of 20°C min<sup>-1</sup> to a final temperature of 320°C (11.7 min hold). The injector temperature was retained at 250°C and each sample (2  $\mu\text{l}$ ) was injected in the splitless mode. Analysis was performed using EI ionization and selected ion monitoring mode with mass resolution greater than 10000.

## **Results and Discussion**

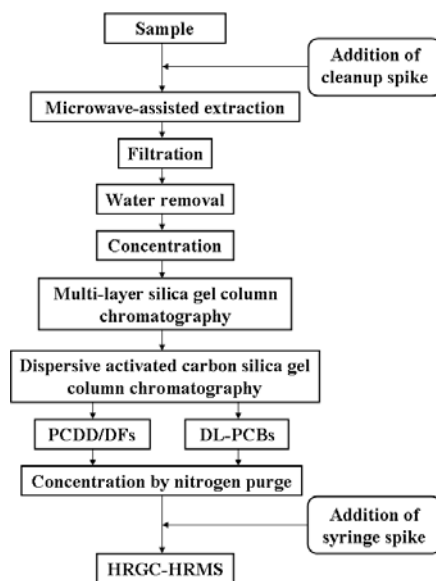
### **Recovery of spiked $^{13}\text{C}$ -labeled PCDD/DFs and DL-PCBs**

Analysis of the soil and sediment certified reference materials were performed three times, respectively. The recoveries of  $^{13}\text{C}$ -labeled PCDD/DFs from the soil were between 76 and 120%, whereas those of  $^{13}\text{C}$ -labeled DL-PCBs were between 74 and 97%. In addition, the recoveries of  $^{13}\text{C}$ -labeled PCDD/DFs from the sediment were between 73 and 113%, whereas those of  $^{13}\text{C}$ -labeled DL-PCBs were between 68 and 114%. All data were within the permissible range of 50 to 120%, as defined by the official manual<sup>1,2</sup>. These results demonstrate that most of the PCDD/DFs and DL-PCBs could be recovered efficiently from the sample matrices of the soil and sediment by our MAE method.

### **Comparison with the concentration of dioxins from soil certified reference material**

A comparison of the PCDD/DF and DL-PCB concentrations obtained by our MAE method with those of the certified values of JSAC 0421 is shown in Table 1. The average concentration and reproducibility were based on the repeated analysis (n = 3). The values for many of the isomers of the PCDD/DFs and DL-PCBs were within the permissible concentration range of the certified value; however, the values for some isomers were outside the range. Thus, we evaluated the validation of these data using the Z-score calculated with an interlaboratory standard deviation certified by The Japan Society for Analytical Chemistry. As the result, the Z-score of all isomers of PCDD/DFs and DL-PCBs were within  $\pm 2.00$ , it was shown that the concentrations in soil were corresponded satisfactorily to the certified value by statistical method based on robust.

As a reason for the consistency our results with respect to the certified value, it was considered that the extracting power of our MAE method was sufficient for the extraction of PCDD/DFs and DL-PCBs from the soil. Our MAE method is characterized by the addition of water and ethanol to toluene. Previous studies on MAE



**Fig.1** Flow chart of the experimental design

**Table 1** Comparison of dioxins concentration in soil certified material by microwave-assisted extraction with those of the certified value

Compound	MAE			R.S.D. %	Certified value			
	Concentration (pg/g dry wt.)	Average (pg/g dry wt.)	S.D.		Concentration (pg/g dry wt.)	S.D.	Z-score	
2,3,7,8-TeCDD	1.6	1.4	1.3	1.4	11	1.46 ± 0.23	0.40	-0.05
1,2,3,7,8-PeCDD	10	9.4	8.7	10	9.3	9.0 ± 1.4	2.5	0.20
1,2,3,4,7,8-HxCDD	10	8.9	8.9	9.3	7.6	8.43 ± 0.96	1.73	0.51
1,2,3,6,7,8-HxCDD	23	21	20	21	7.1	19.4 ± 1.6	2.9	0.59
1,2,3,7,8,9-HxCDD	28	25	24	26	8.0	22.2 ± 2.4	4.1	0.82
1,2,3,4,6,7,8-HpCDD	169	138	139	149	12	135 ± 16	30	0.46
OCDD	807	644	583	678	17	682 ± 60	108	-0.04
2,3,7,8-TeCDF	16	13	12	13	16	11.3 ± 1.4	2.5	0.79
1,2,3,7,8-PeCDF	15	14	14	15	4.4	16.8 ± 2.5	4.5	-0.48
2,3,4,7,8-PeCDF	23	23	21	22	5.4	18.8 ± 2.1	3.8	0.93
1,2,3,4,7,8-HxCDF	28	24	22	24	12	22.5 ± 2.3	4.1	0.45
1,2,3,6,7,8-HxCDF	30	26	24	27	10	23.2 ± 2.3	4.2	0.79
1,2,3,7,8,9-HxCDF	2.1	2.0	1.8	1.9	8.3	2.12 ± 0.73	1.16	-0.16
2,3,4,6,7,8-HxCDF	46	38	37	40	11	32.2 ± 3.5	6.0	1.34
1,2,3,4,6,7,8-HpCDF	118	104	99	107	9.2	96 ± 1.0	19	0.58
1,2,3,4,7,8,9-HpCDF	16	13	12	14	14	12.9 ± 1.0	1.8	0.44
OCDF	85	75	68	76	11	75.0 ± 9.5	17.1	0.05
#81 3,4,4',5'-TeCB	10	8.7	8.4	9.0	8.7	9.5 ± 1.3	2.3	-0.22
#77 3,3',4,4'-TeCB	109	96	87	97	11	100 ± 13	23	-0.11
#126 3,3',4,4',5'-PeCB	46	43	40	43	6.6	38.1 ± 5.5	9.5	0.55
#169 3,3',4,4',5,5'-HxCB	14	13	12	13	7.9	12.00 ± 0.96	1.52	0.77
#123 2',3',4,4',5'-PeCB	26	18	20	21	19	20.0 ± 3.4	5.6	0.23
#118 2,3',4,4',5'-PeCB	639	526	498	554	14	543 ± 51	88.4	0.13
#105 2,3,3',4,4'-PeCB	265	213	198	226	16	205 ± 24	41	0.50
#114 2,3,4,4',5'-PeCB	13	9.4	9.2	10	18	9.4 ± 1.8	3.0	0.33
#167 2,3',4,4',5,5'-HxCB	52	53	57	54	5.4	56.7 ± 4.9	8.2	-0.35
#156 2,3,3',4,4',5'-HxCB	132	109	104	115	13	104.0 ± 9.2	15.9	0.69
#157 2,3,3',4,4',5'-HxCB	49	40	37	42	15	39.3 ± 3.4	5.5	0.51
#189 2,3,3',4,4',5,5'-HpCB	25	22	22	23	7.1	21.6 ± 2.4	4.1	0.35

have reported that the addition of water to the extraction solvent was effective for the extraction of organic pollutants<sup>5,6</sup>. Water molecules have a high dipole moment and absorb microwaves strongly; consequently, the efficient heating of a sample is obtained. Thus, it is considered that high yield extraction was performed. However, Budzinski et al. reported that the efficiency of PAH extraction from sediment, in which the water content was greater than 50%, was decreased by the high water content, although they confirmed an improvement in extraction when water was added to dichloromethane<sup>6</sup>. These authors indicated that an excessive amount of water possibility acts as a barrier between the extraction solvent and the matrix. In our previous study, we used ethanol, which dissolves in toluene and water, and optimized the volume ratio of the extraction solvent. Using several soils and sediments with different pollution patterns, we confirmed that the efficiency of PCDD/DF and DL-PCB extraction by our MAE method was comparable to that of Soxhlet extraction<sup>4</sup>. This optimization was assumed to have reduced the barrier between toluene and the matrix, thereby allowing the toluene to diffuse in the matrix and extract the PCDD/DFs and DL-PCBs.

In this study, the relative standard deviations of the concentrations of PCDD/DFs and DL-PCBs obtained by our MAE method were below 19%. Therefore, it was found that our MAE method could be performed with not only rapidness but also accuracy and precision.

#### **Comparison with the concentration of dioxins from sediment certified reference material**

A comparison of the PCDD/DF and DL-PCB concentrations obtained by our MAE method with those of the certified values of JSAC 0431 is shown in Table 2. The average concentrations are based on repeated analysis (n = 3). As described above, we evaluated the validation of the concentrations of PCDD/DFs and DL-PCBs using

**Table 2** Comparison of dioxins concentration in sediment certified material by microwave-assisted extraction with those of the certified value

Compound	MAE				R.S.D. %	Certified value		
	Concentration (pg/g dry wt.)			Average (pg/g dry wt.)		Concentration (pg/g dry wt.)	S.D.	Z-score
2,3,7,8-TeCDD	1.2	1.3	1.4	1.3	8.4	1.36 ± 0.11	0.19	-0.25
1,2,3,7,8-PeCDD	7.7	7.3	7.9	7.6	3.6	7.71 ± 0.45	0.81	-0.13
1,2,3,4,7,8-HxCDD	11	11	13	12	7.4	12.31 ± 0.48	0.83	-0.95
1,2,3,6,7,8-HxCDD	25	26	29	27	7.5	28.9 ± 1.2	2.2	-0.91
1,2,3,7,8,9-HxCDD	22	23	24	23	4.8	23.9 ± 1.3	2.4	-0.40
1,2,3,4,6,7,8-HpCDD	611	649	698	653	6.7	702 ± 41	73	-0.68
OCDD	11558	12304	12217	12026	3.4	12010 ± 480	835	0.02
2,3,7,8-TeCDF	11	13	14	13	10	12.01 ± 0.92	1.60	0.37
1,2,3,7,8-PeCDF	16	17	19	17	8.2	15.6 ± 1.5	2.8	0.60
2,3,4,7,8-PeCDF	15	16	19	17	13	17.2 ± 1.3	2.5	-0.17
1,2,3,4,7,8-HxCDF	25	26	28	27	6.2	27.4 ± 1.3	2.5	-0.33
1,2,3,6,7,8-HxCDF	22	22	25	23	7.6	24.4 ± 1.0	1.9	-0.73
1,2,3,7,8,9-HxCDF	2.1	2.3	2.4	2.3	6.9	2.27 ± 0.30	0.50	-0.03
2,3,4,6,7,8-HxCDF	37	37	39	37	4.4	36.7 ± 3.4	6.3	0.13
1,2,3,4,6,7,8-HpCDF	142	130	146	139	6.0	142 ± 11	21	-0.14
1,2,3,4,7,8,9-HpCDF	21	20	24	22	9.5	22 ± 2.0	3.7	-0.05
OCDF	234	235	259	243	5.8	254 ± 12	23	-0.49
#81 3,4,4',5'-TeCB	120	133	142	132	8.6	149 ± 12	19	-0.91
#77 3,3',4,4'-TeCB	4865	5295	5823	5328	9.0	6020 ± 430	780	-0.89
#126 3,3',4,4',5'-PeCB	62	61	65	63	3.1	64.4 ± 6.2	11.3	-0.14
#169 3,3',4,4',5,5'-HxCB	5.2	5.7	6.4	5.8	11	6.52 ± 0.91	1.58	-0.46
#123 2',3,4,4',5'-PeCB	219	194	221	211	7.1	220 ± 36	65	-0.13
#118 2,3',4,4',5'-PeCB	9204	8845	9506	9185	3.6	9600 ± 1100	1700	-0.24
#105 2,3,3',4,4'-PeCB	4070	3706	3911	3896	4.7	3850 ± 300	530	0.09
#114 2,3,4,4',5'-PeCB	264	197	282	248	18	311 ± 47	81	-0.78
#167 2,3',4,4',5,5'-HxCB	273	286	308	289	6.1	328 ± 33	60	-0.65
#156 2,3,3',4,4',5'-HxCB	809	780	815	801	2.3	812 ± 70	127	-0.08
#157 2,3,3',4,4',5'-HxCB	186	195	215	199	7.5	212 ± 23	39	-0.35
#189 2,3,3',4,4',5,5'-HpCB	55	54	59	56	4.7	61.3 ± 6.9	10.8	-0.49

the Z-score calculated with the interlaboratory standard deviation certified by The Japan Society for Analytical Chemistry. As a result, the Z-score of all the isomers of PCDD/DFs and DL-PCBs were within ± 2.00. It was also shown that the concentrations in the sediment corresponded statistically to the certified value. In addition, the relative standard deviations of the concentrations of PCDD/DFs and DL-PCBs obtained by our MAE method were below 18%.

## References

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