Comparison of Accelerated Solvent Extraction and Standard Shaking Extraction for Determination of Dioxins in Foods

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Introduction

We previously developed a highly sensitive method for determining dioxin content in food using a solvent cut large volume (SCLV) injection system coupled to a cyanopropyl phase capillary column[1]. The SCLV injection system coupled to a 40m-length Rtx-2330 column showed sufficient separation of 2,3,7,8-chlorine substituted isomers, and had at least five-times higher sensitivity than the conventional injection technique [2]. In the current method, a large volume of sample (generally 100g) must be treated collectively in order to attain the desirable limit of detection (LODs) at low ppt levels, namely 0.01pg/g for tetra-CDD and -CDF. The present method allowed the reduction of sample volume from 100g to 20g when such usual LODs are demanded. The SCLV injection technique is expected to improve the efficiency of laboratory performance, especially when it is coupled to an automated extraction method, such as accelerated solvent extraction (ASE).

In order to examine the applicability of ASE for the determination of dioxins in food samples, it is important to verify its extraction efficacy against that of the conventional technique. In the present study we examine the applicability of an ASE for the determination of dioxins in food samples, and the method's performance was compared with that of standard conventional shaking extraction (separatory funnel extraction) regarding recovery rates and quantitative determination. It is considered that homogeneous tissue, such as dried seaweed powder or dried milk powder, is suitable for the method's quantitative validation.

Methods and Materials

For the examination of recovery rate, extracts were prepared from homogenates of various fresh vegetables purchased at a market in Japan. The recovery rates for 17 kinds of ^{13}C-labeled 2,3,7,8-substituted PCDD/Fs and ^{13}C-labeled 12 kinds of dioxin-like PCBs were evaluated. For the comparison of quantitative determination, about 1 kg of domestic dried seaweed ('*Nori*') was purchased and ground in a mill, giving a homogeneous powder.

The analytical procedures used in this study are summarized in Table 1. Automated extraction was performed using an ASE-300 (Dionex, USA) under the conditions of 1500psi, 150°C. Shaking extraction was twice carried out using a 1L separatory funnel for one hour each time. Four individual experiments and four simultaneous blank tests were performed for each extraction method. Dioxins were analysed using a model 6890 gas chromatograph (Agilent Technologies, USA) coupled to a model Autospec-Ultima mass spectrometer (Micromass, UK). We employed an Rtx-2330 (0.18mm x 40m) capillary column (Restek, USA) on an SCLV injection system (SGE, Australia) in order to determine tetra- and pentaCDD/Fs, and hexaCDFs. The details of the operating conditions for the SCLV injection system are described in another paper[2]. The LOD for each congener was determined according to the provisional guidelines for analysis of dioxins in foods issued by the Ministry of Health and Welfare of Japan in 1999 ('Guideline'): An absolute quantity corresponding to S/N = 3 is evaluated on HRGC/HRMS chromatograms using verification standards.

Table 1. Analytical procedures for determination of dioxins in food.

		Method 1	Method 2
Extraction		Shaking extraction* Sample size: 20g Time: 60min x 2 (120min) Solvent: acetone/n-hexane (1:1, v/v) , 600mL (300mL x 2)	Accelerated solvent extraction (ASE) Sample size: 20g Time: 25min Solvent: acetone/n-hexane (1:1, v/v) 200mL
Cleanup		Sulfuric acid treatment ↓ Multi-layer silica gel column ↓ Active carbon-dispersed silica gel column	
HRGC/ HRMS analysis	PCDD/DFs and non-ortho PCBs	SCLV injection Injection volume: 4 µL / 20µL Pre-column:BPX-5 (0.25mm x 5m) Analytical columns: a) Rtx-2330 (0.18mm x 40m) b) BPX-5 (0.15mm x 30m)	
	Mono-ortho PCBs	Splitless injection Injection volume: 1µL/20 µL Analytical column: HT-8 (0.22mm x 50m)	

* Method recommended for plant food samples in 'Guideline'.

Results and Discussion

As shown in Table 2, our analysis of 20 g of various plant food items according to Method 2, including the ASE and SCLV injection technique, showed recovery rates for ^{13}C-labeled 29 kinds of isomers ranging from 40.4 to 117%, within the range recommended by the Guideline (40-120%). Data regarding the quantification of principal isomers in dried seaweed are shown in Table 3. Generally, it was found that concentrations from ASE were higher than those from shaking extraction. The greatest difference between the methods was observed regarding OCDD. The ratios of estimated concentrations from ASE to those from shaking extraction ranged from 1.1 for 2,3,7,8-

TCDD, PCB#77 and PCB#123 to 3.2 for OCDD. In contrast, the average concentration of PCB#118 on ASE, that was nearly the same as that of OCDD, showed only a slight difference against shaking extraction. The averaged recovery rates for ^{13}C-labeled OCDD were 85% for ASE, which was similar to that for shaking extraction (89%). On the other hand, the results of method blank showed that the contribution of contamination was negligible on the quantification data, and chromatograms of seaweed extract also showed little interference near the retention time of OCDD. The above results suggested that ASE exhibited a superior extraction efficacy, while the extractions were incomplete on shaking extraction. However, ASE's significant predominance against shaking extraction was not observed in another examination using fresh vegetable samples (data not shown). It could be said that higher chlorinated PCDD/F isomer in the seaweed was more strongly adsorbed on the plant's structure than in the other plant foods. Actually, the solid residue after the shaking extraction process was enclosed in an ASE vessel and then re-extracted, with the result that peaks representing OCDD and other dioxin-isomers were observed on their chromatograms (Fig. 1).

Table 2. Recoveries of added 29 kinds of ^{13}C-labeled compounds on various plant food samples using ASE.

No.	Sample	Range (%)
1	*Komatsuna*	42 - 81
2	*Komatsuna*	47 - 82
3	*Komatsuna*	46 - 77
4	*Komatsuna*	43 - 94
5	*Shungiku*	44 - 87
6	*Shungiku*	44 - 72
7	*Shungiku*	48 - 91
8	Celery	42 - 93
9	Celery	43 - 94
10	Celery	44 - 94
11	Seaweed (dry)	42 - 85
12	Seaweed (dry)	55 - 88
13	Pear	45 - 90
14	Pear	51 - 84
15	Japanese parsley	50 - 93
16	*Shimeji*	52 - 90
17	Broccoli	45 - 120
18	Lotus root	40 - 93
19	Tomato	48 - 98
20	Bamboo shoot	42 - 70

Table 3. Concentrations (pg/g) of dioxins in seaweed. Comparison of ASE versus Shaking extraction.

Congeners	ASE (n=4)		Shaking extraction (n=4)		a / b
	Mean[a]	Range	Mean[b]	Range	
2,3,7,8-TCDD	0.016	0.010 - 0.021	0.015	0.013 - 0.017	1.1
1,2,3,7,8-PeCDD	0.014	0.011 - 0.017	0.011	(0.009) - 0.013	1.3
1,2,3,7,8,9-HxCDD	0.028	0.026 - 0.031	0.013	0.013 - 0.015	2.1
1,2,3,4,6,7,8-HpCDD	0.441	0.398 - 0.474	0.185	0.183 - 0.189	2.4
OCDD	3.200	3.053 - 3.470	1.008	0.946 - 1.105	3.2
2,3,7,8-TCDF	0.033	0.029 - 0.038	0.023	0.020 - 0.026	1.4
1,2,3,7,8-PeCDF	0.027	0.023 - 0.029	0.019	0.017 - 0.023	1.4
2,3,4,7,8-PeCDF	0.017	0.016 - 0.018	0.011	(0.009) - 0.012	1.6
1,2,3,4,7,8-HxCDF	0.026	0.023 - 0.033	0.014	(0.011) - 0.015	1.9
1,2,3,4,6,7,8-HpCDF	0.075	0.075 - 0.076	0.037	0.033 - 0.041	2.1
OCDF	0.051	0.047 - 0.057	0.023	0.021 - 0.026	2.2
3,3',4,4'-TCB(#77)	1.003	0.987 - 1.036	0.881	0.871 - 0.896	1.1
3,4,4',5-TCB(#81)	0.157	0.147 - 0.166	0.128	0.121 - 0.138	1.2
2,3,3',4,4'-PeCB(#105)	1.795	1.741 - 1.873	1.552	1.461 - 1.611	1.2
2,3,4,4',5-PeCB(#114)	0.377	0.360 - 0.425	0.305	0.282 - 0.323	1.2
2,3',4,4',5-PeCB(#118)	4.352	4.222 - 4.550	3.755	3.637 - 3.910	1.2
2',3,4,4',5-PeCB(#123)	0.153	0.139 - 0.166	0.134	(0.100) - 0.179	1.1
2,3,3',4,4',5-HxCB(#156)	0.299	0.256 - 0.352	0.252	0.232 - 0.291	1.2
2,3',4,4',5,5'-HxCB(#167)	0.108	0.089 - 0.126	0.084	(0.057) - 0.100	1.3

Trace data are shown in parentheses and counted in the mean value.

In conclusion, ASE could extract dioxins at high efficiency using a low-volume solvent and could provide a high level of performance for various plant matrices, especially regarding those from which dioxins are difficult to extract using conventional shaking extraction. The applicability of combined SCLV injection and ASE methodology is continuously verified for use regarding animal products.

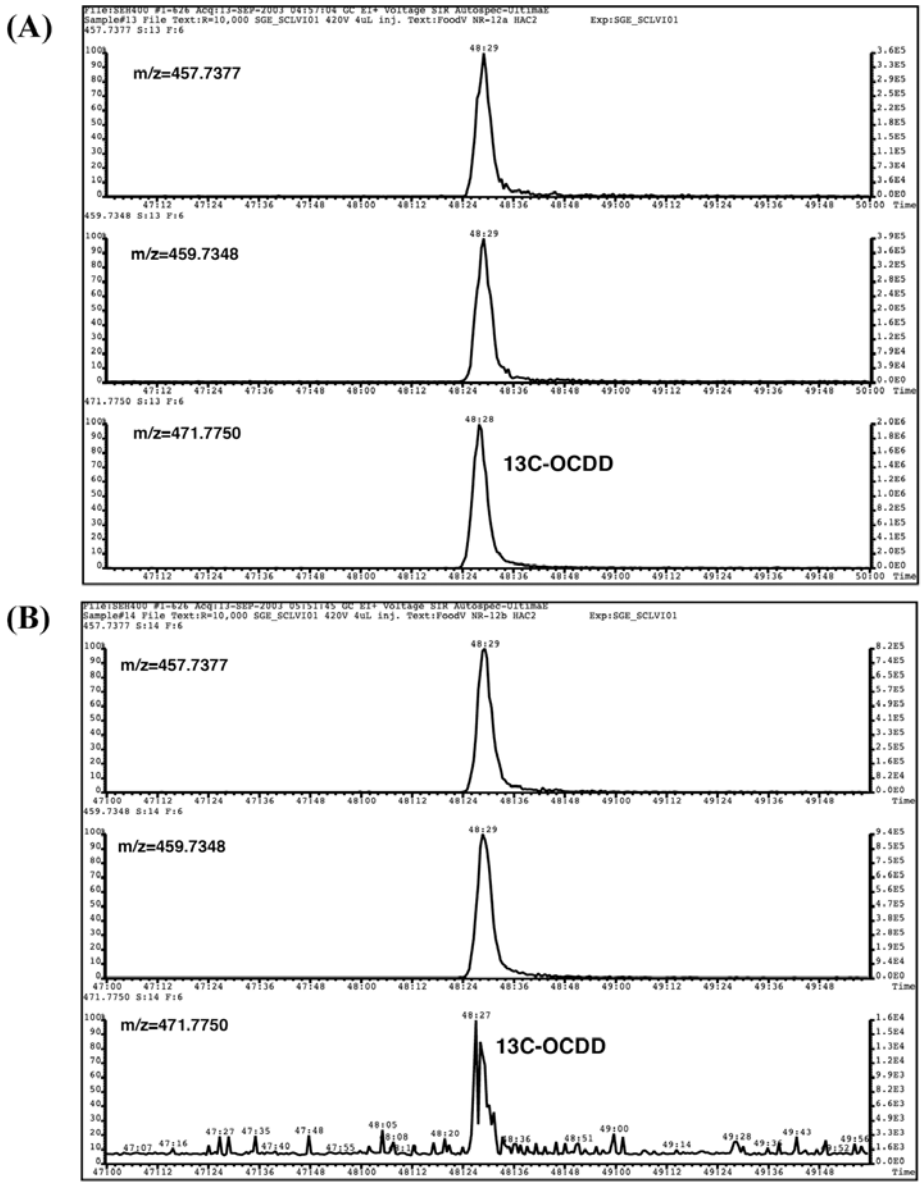

Fig. 1 HRGC/HRMS chromatograms of OCDD (A) obtained from seaweed sample using shaking extraction (B) obtained from the solid residue of shaking extraction process subsequently extracted by ASE.

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References

1 Hori, T., Tobiishi, K., Ashizuka, Y., Nakagawa, R., and Iida, T. Organohalogen Compounds, 55, 95-98 (2002)

2 Tobiishi, K., Hori, T., Kurokawa, Y., Ishiguro, Y., and Iida, T. Organohalogen Compounds, 55, 179-182 (2002)