REMOVAL OF DIOXINS FROM RETAIL FISH BY HIGH-SPEED SOLVENT EXTRACTION

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Introduction

Studies of the Japanese diet have identified fish and shellfish as the main sources of PCDD/Fs and dioxin-like PCBs (dioxins).[1,2] Assessing the risk posed by dioxins in retail fish requires the development of rapid quantitative methods. HRGC/HRMS is the standard technique for dioxin analysis; however, the lengthy extraction process makes it time consuming. The most widely used method for extracting fish dioxins is Soxhlet extraction, although alkaline digestion followed by solvent extraction is also often used in Japan. Both conventional methods take over 16 h. Some new techniques, such as pressurised liquid extraction (or accelerated solvent extraction) have been applied to dioxins, but rarely to those in fish samples. We recently developed a high-speed method based on extraction in heated liquid solvents under near-atmospheric pressure. This technique has been used for dioxin extraction from contaminated soil and fly ash, yielding similar concentrations to Soxhlet extraction but much more rapidly.[3] Here, we report the first data from the application of this method to the extraction of dioxins from retail fish.

Materials and Methods

Samples: Retail fish samples were purchased during the years 2004 and 2005 from supermarkets in Tokyo, Japan. The muscular parts of the samples were homogenised using a food cutter and stored at –20°C until required for analysis.

High-speed solvent extraction: A model SE-100 (Dia Instruments Co., Ltd., Japan) high-speed solvent extractor was employed. Homogenised fish samples (20 g) and sodium anhydrous sulphate (80 g) were ground into powder using a mortar and pestle. The samples were then packed into 160-ml stainless-steel extraction cells. The dead volume was filled with extraction solvents and the top of the cell was sealed with a cap. $^{13}C_{12}$-labelled internal standards were used to spike samples before extraction, and also to spike extracts in order to determine the following optimal extraction conditions: 30°C and 80°C when using acetone/*n*-hexane (1:1) and toluene, respectively, as extraction solvents. The flow rate was set at 6 ml/min. A schematic diagram of the extractor is shown in Figure 1.

Alkaline digestion followed by hexane extraction: The extracts were prepared as described previously.[4] Homogenised fish samples (20 g) spiked with $^{13}C_{12}$-labelled internal standards were incubated in aqueous KOH for 16 h at room temperature. The alkaline hydrolysates were added to methanol and extracted three times by mechanically shaking with *n*-hexane.

Cleanup and HRGC/HRMS analysis: The cleanup and analysis of dioxins generally followed the methods reported previously.[4] Briefly, the extracts were treated with concentrated sulphuric acid and then purified on a silver nitrate/silica gel column. The elute obtained with *n*-hexane was loaded onto an alumina column. After washing with *n*-hexane, the first fraction (containing mono-*ortho* PCBs) was eluted with 2% dichloromethane/*n*-hexane, while the second fraction (containing non-*ortho* PCBs and PCDD/Fs) was eluted with 60% dichloromethane/*n*-hexane. The second fraction was then loaded onto an activated carbon column and eluted with toluene. Both fractions were spiked with $^{13}C_{12}$-labelled recovery standards. The quantification of dioxins was conducted using an HP6890-plus gas chromatograph coupled to a JEOL JMS-700 mass spectrometer. The determination of 2,3,7,8-chlorine-substituted PCDD/Fs was performed in DB-5MS and DB-17 columns. The determination of dioxin-like PCBs was performed in an HT-8 column. The limits of quantification were 0.01–0.2 pg/g for PCDD/Fs and non-*ortho* PCBs, and 0.5–3.0 pg/g for mono-*ortho* PCBs. The TEQ concentrations were calculated using the WHO-TEFs.

Results and Discussion

We initially determined the extraction conditions for the fish dioxins using the high-speed extractor with various extraction times and solvents. Two types of fish, sea bass and yellowtail, were treated with acetone/*n*-hexane for up to 4 h, followed by toluene for 1 h (Figure 2). The cumulative concentrations of 2,3,7,8-chlorine-substituted PCDD/Fs and dioxin-like PCBs reached a plateau after 1 h of extraction with acetone/*n*-hexane in both samples. Although the sea bass samples contained relatively high amounts of dioxin-like PCBs, the 1-h extraction period was sufficient to extract them fully. This was therefore selected as the recommended extraction condition for the practical analysis of fish dioxins.

The suitability of the high-speed solvent extraction method for analysing fish dioxins was compared with that of the conventional alkaline digestion extraction. Table 1 shows the concentrations and relative standard deviations (RSDs) for the two methods when applied to yellowtail samples. The concentration ratios of the two methods were 0.9–1.1, indicating that the concentrations of each isomer were similar for both extractions. The RSDs of the quantified isomers using the novel method were acceptable (0.0–17.4%), and were similar to those obtained using the conventional method (0.0–24.2%). The recoveries of the internal quantification standards using the new method were 72.8–109%, and were similar to those obtained using the conventional method (67.5–105%). The selected ion-mode chromatograms obtained from both extractions were visually inspected, but showed no differences in the homologous groups of dioxins present (data not shown). These results suggest that the methods tested achieved similar extraction efficiencies for dioxins to the conventional extraction method.

Finally, we used the high-speed extraction method to determine the TEQ concentrations of samples of 12 popular retail fish from Japan compared with those obtained by the conventional extraction. As shown in Figure 3, the TEQ concentrations produced by both extractions showed excellent correlations for both PCDD/Fs (r = 0.99) and dioxin-like PCBs (r = 0.99), with the slopes and y-intercepts of the linear regression equations being close to 1 and 0, respectively. This confirmed that the TEQ concentrations obtained using the present method were comparable to those obtained with the conventional extraction method.

Overall, our results indicate that high-speed solvent extraction is a useful method for extracting dioxins from retail fish. The main advantage of this method is the short extraction time (~1 h) compared with the alkaline digestion extraction method (~20 h). This method allows the rapid determination of dioxins and will therefore be a valuable tool for monitoring dioxin levels in retail fish.

Acknowledgements

This work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan.

References

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Table 1 Comparison of dioxin concentrations in yellowtail using two extractions (n=3)

Dioxins		High-speed solvent extraction (A)		Alkaline digestion-extraction (B)		Ratio
		Mean±SD, pg/g	RSD, %	Mean±SD, pg/g	RSD, %	(A/B)
PCDDs	2378-TCDD	0.13 ± 0.010	7.7	0.13 ± 0.010	7.7	1.0
	12378-PeCDD	0.27 ± 0.010	3.7	0.27 ± 0.020	7.4	1.0
	123478-HxCDD	tr [1)]	-	tr	-	-
	123678-HxCDD	0.11 ± 0.0058	5.2	0.12 ± 0.010	8.3	0.9
	123789-HxCDD	tr	-	tr	-	-
	1234678-HpCDD	0.079 ± 0.0031	3.9	0.077 ± 0.0066	8.5	1.0
	OCDD	0.15 ± 0.023	15.4	0.14 ± 0	0.0	1.1
PCDFs	2378-TCDF	2.1 ± 0.12	5.5	1.9 ± 0	0.0	1.1
	12378-PeCDF	0.28 ± 0.010	3.6	0.27 ± 0.012	4.3	1.0
	23478-PeCDF	0.91 ± 0.035	3.9	0.96 ± 0.010	1.0	0.9
	123478-HxCDF	0.052 ± 0.0091	17.4	0.050 ± 0.012	24.2	1.0
	123678-HxCDF	0.058 ± 0.0035	6.0	0.057 ± 0.0081	14.2	1.0
	123789-HxCDF	nd [2)]	-	nd	-	-
	234678-HxCDF	0.060 ± 0.0012	1.9	0.055 ± 0.0070	12.7	1.1
	1234678-HpCDF	tr	-	tr	-	-
	1234789-HpCDF	nd	-	nd	-	-
	OCDF	nd	-	nd	-	-
Non-*ortho* PCBs	33'44'-TCB	84 ± 2.1	2.5	83 ± 2.1	2.5	1.0
	344'5-TCB	4.5 ± 0.058	1.3	4.4 ± 0.20	4.5	1.0
	33'44'5-PeCB	22 ± 0.58	2.6	21 ± 0.58	2.7	1.0
	33'44'55'-HxCB	3.0 ± 0.058	1.9	3.0 ± 0.058	1.9	1.0
Mono-*ortho* PCBs	233'44'-PeCB	910 ± 25	2.8	920 ± 12	1.3	1.0
	2344'5-PeCB	62 ± 4.7	7.6	61 ± 2.6	4.3	1.0
	23'44'5-PeCB	2800 ± 0	0.0	2800 ± 58	2.1	1.0
	2'344'5-PeCB	45 ± 0.58	1.3	44 ± 2.1	4.7	1.0
	233'44'5-HxCB	290 ± 5.8	2.0	290 ± 5.8	2.0	1.0
	233'44'5'-HxCB	84 ± 1.7	2.1	84 ± 2.0	2.4	1.0
	23'44'55'-HxCB	190 ± 0	0.0	180 ± 5.8	3.2	1.1
	233'44'55'-HpCB	31 ± 1.2	3.7	29 ± 1.5	5.3	1.1

1) tr: trace (detection limits ≦tr＜quantification limits)

2) nd: not detected

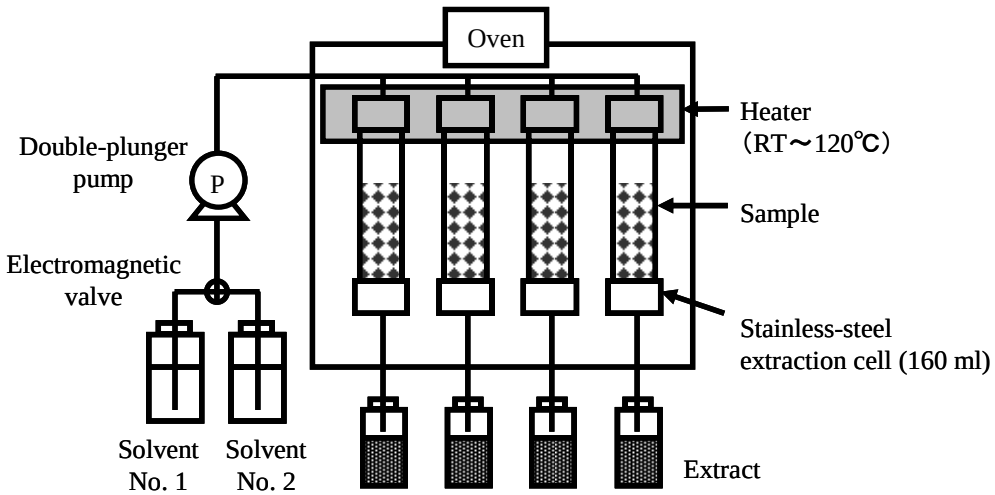

Figure 1 Schematic diagram of the extractor (SE-100)

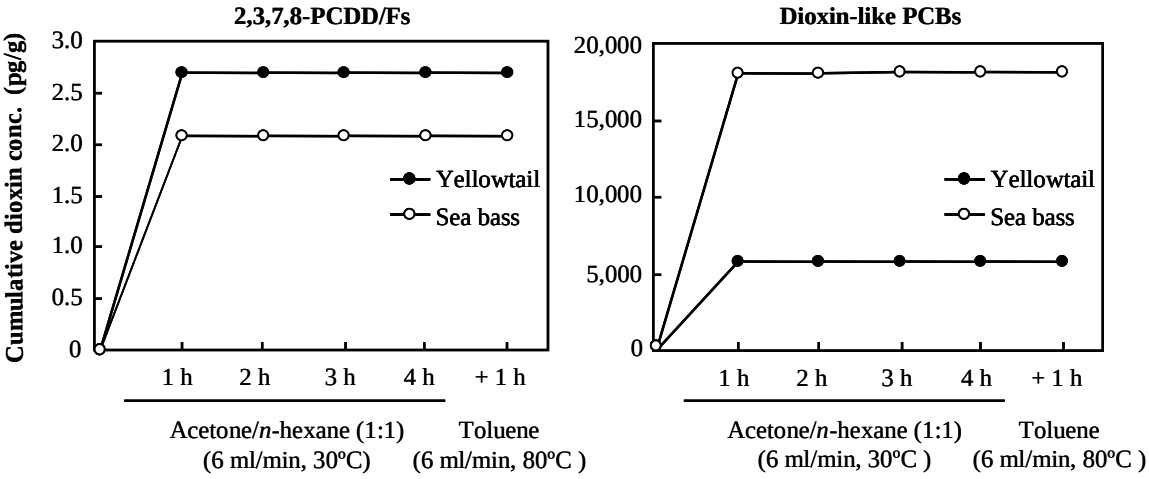

Figure 2 Dioxin concentrations in the high-speed solvent extraction under various extraction conditions. Two popular fish samples were serially extracted by the high-speed solvent extraction with acetone/*n*-hexane for up to 4 h under 30°C and then extracted with toluene for 1 h under 80°C. The hourly extracts were spiked with $^{13}C_{12}$-labelled internal standards and cleaned up for HRGC/HRMS analysis.

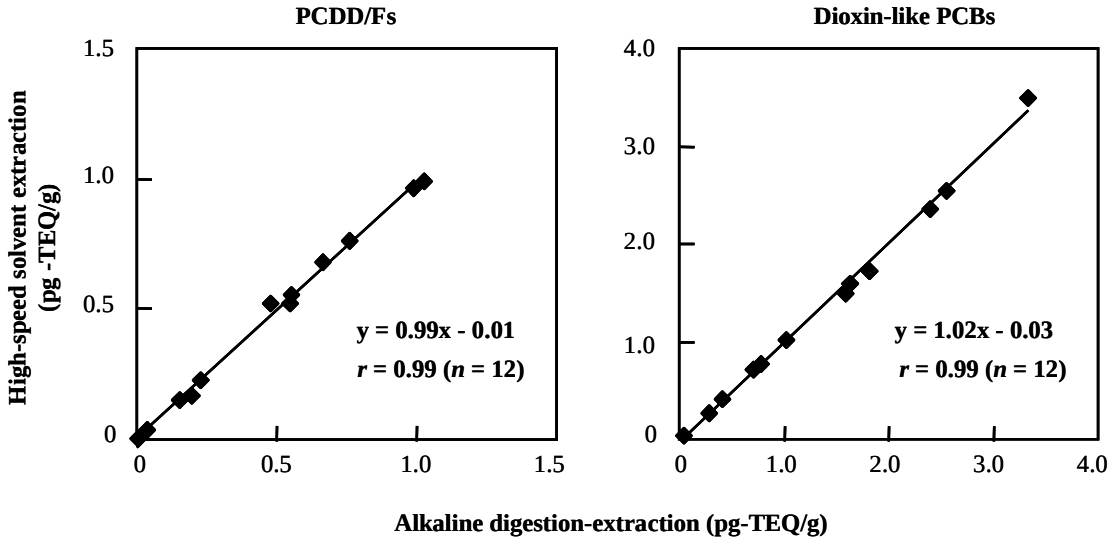

Figure 3 Comparison of TEQ concentrations of dioxins in retail fish determined by the two extraction methods. Twelve retail samples (bonito, conger eel, horse mackerel, marlin, two salmon, sardine, tuna, four yellowtail) were extracted by the two extraction methods and analyzed by HRGC/HRMS analysis.