IMPROVEMENT OF THE METHODS FOR ANALYZING MONO-ORTHO PCBS IN FOOD

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Introduction

PCBs are ubiquitous environmental contaminants. Due to the distribution of Co-PCBs in food, drinking water, and air, we need to pay attention to their hazardous, dioxin-like effects on human health. At present, the tolerable daily intake of dioxins in Japan has been determined to be 4pg TEQ/kg b.w./day, totaling 7 dioxins, 10 furans, 4 non-ortho PCBs, and 8 mono-ortho PCBs, following the recommendations by the World Health Organization (WHO)[1]. In our investigation of dioxin in foods, we experienced difficulties in analyzing mono-ortho PCBs, which might be caused from co-elution of the sample matrix, probably, like paraffin and highly fatty acids. Therefore, as one more purification step, we adopted a traditional treatment, liquid-liquid partition with acetonitrile and n-hexane, which is commonly used in the analysis of pesticides[2], following active carbon column chromatography. In this study, we show the results on interference removing effects by the treatment in the analysis of dioxin-like mono-ortho PCBs in fish and spinach.

Materials and Method

Chemicals

Native coplanar PCB as authentic standards and [$^{13}C_{12}$]-PCB as internal standards were purchased from Cambridge Isotope Laboratories, Massachusetts, USA. An active carbon column was prepared as follows: Active carbon was purchased from Nacalai Tesque, Kyoto, Japan, refluxed three times with toluene for 1hr, and dried *in vacuo* ; then 500mg of the active carbon was mixed with 500g of anhydrous sodium sulfate (Wako Pure Chemicals Ind. Co. Ltd., Tokyo, Japan). Silver nitrate / silica gel was purchased from Wako Pure Chemicals Ind. Co. Ltd. Acetonitrile was PR grade (guaranteed for purity by gas chromatography [GC] with electron capture detection after 300-fold concentration), and was purchased from Wako Pure Chemicals Ind. Co. Ltd. Acetone, n-hexane and dichloromethan were used at the analytical grade of dioxin and were purchased from Kanto Chemicals.

Sample preparation

Spinach and fish were used as samples in this study, because these foods contain a lot of matrix. One hundred grams of the spinach and 50g of the fish were homogenized. After internal standards were added to the homogenized samples, the spinach was extracted with acetone/n-hexane (1:1), and the fish was digested by KOH / ethanol, and then extracted with n-hexane. Each extract was treated with sulfuric acid and applied to a Silver nitrate / silica gel column. The column was prewashed with 100ml n-hexane, and the dioxins were eluted with 100ml n-hexane. The extract was loaded to an active carbon column, and the mono-ortho PCB fraction was eluted with 10%

(v/v) dichloromethan /n-hexane. The eluate was evaporated and concentrated to 2ml. Half (1ml) of each concentrate was used for partitioning with acetonitrile/n-hexane to remove the matrix. As a comparison, the rest of the non-partitioned concentrate (1ml) was used. Both were concentrated to a final volume of approximately 50µl. These samples were analyzed by HRGC/HRMS.

Partition with acetonitrile/n-hexane

2ml of acetonitrile (n-hexane saturated) was added to 1ml of active carbon first fraction, and shaken vigorously. After the hexane layer was separated from the acetonitrile layer, the acetonitrile layer was collected to another tube. Thus, the first fraction was extracted with 2ml of acetonitrile three times. Twenty ml of 2% NaCl solution was added to 6ml of the acetonitrile layer, and extracted with 5ml of hexane three times. After 15ml of the collected hexane layer was washed with 20ml of water, it was dried on anhydrous sodium sulfate. The dried hexane layer was concentrated to a final volume of approximately 50µl, and analyzed by HRGC/HRMS.

Instrument

High resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS) analysis was performed on an Autospec Ultima connected to a HP6890 GC.

Samples were introduced through a splitless injector connected to a 60m x 0.25mm, 0.25µm film thickness, BPX-5 column with helium as the carrier gas. The temperature program was 130°C (held for 1min), increased at 20°C/min to 220°C, increased at 3°C /min to 280°C, and increased at 20°C /min to 320°C (held for 2.5min). Monitorised masses were $[M]^+$ and $[M+2]^+$ for penta-CB and $[M+2]^+$ and $[M+4]^+$ for hexa-CB and hepta-CB.

Results and Discussion

The samples of spinach and fish prepared by the two methods (with and without partitioning with acetonitrile/n-hexane) were analyzed by GC/MS. Figure 1 shows SIM chromatograms of hepta-CB. When the samples were not treated with an acetonitrile/n-hexane partition, the matrix influence was shown on the peak at #180, #170 and #189 after a retention time of 22 min (chromatogram of the fish), and on the peak of #189 after a retention time of 26 min (chromatogram of the spinach). In such cases, accurate quantification was difficult. However, when partitioned with acetonitrile/n-hexane, the matrix influence was almost negligible on the chromatograms of both spinach and fish. In order to identify the matrix in these samples, we analyzed these samples with TIC by GC/MS. As a result of our measurements, aliphatic hydrocarbons were detected in the samples without partitioning on the retention time of hexa-CB and hepta-CB (data not shown). It is thought that these compounds were almost removed to the hexane layer by partitioning with acetonitrile and n-hexane.

Table 1 shows the recoveries on this analysis. With partitioning, the recoveries decreased compared with the recoveries of the sample without partitioning. But the recoveries of all of the congeners were in the range of 40%-120%; thus it is acceptable to determine the concentration of mono-ortho PCBs by this improved method.

These results show that partitioning with acetonitrile/n-hexane is an effective purification method for removing matrix from food.

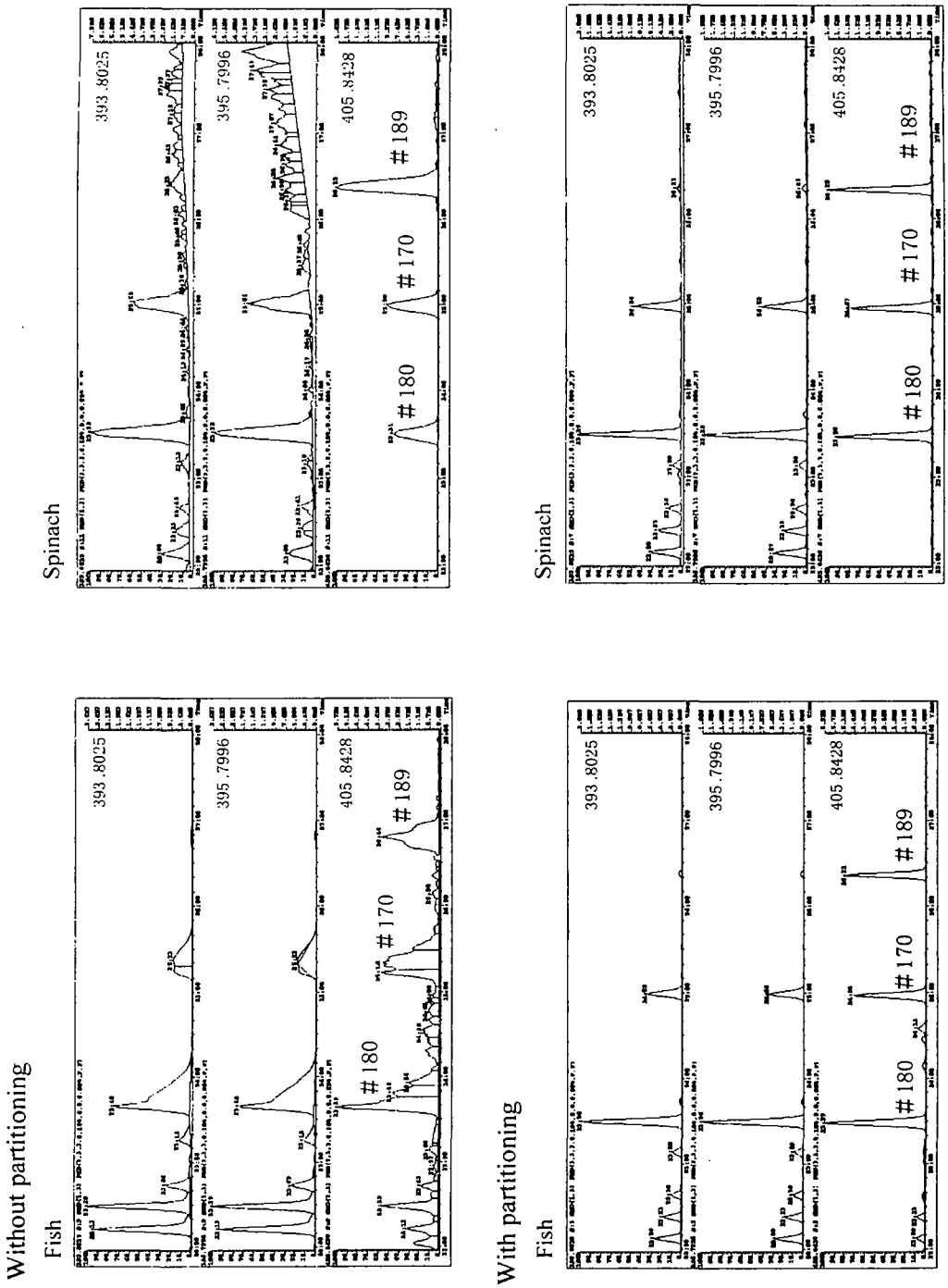

Figure 1. SIM chromatograms of hepta-CB

Table 1. Recoveries of PCB congeners on the analysis of fish and spinach

(A) without partitioning (B) with partitioning

PCB congeners	IUPAC No.	fish		Spinach	
		(A)	(B)	(A)	(B)
2',3,4,4',5-PeCB	# 123	89.9	67.1	75.7	51.9
2,3',4,4',5-PeCB	# 118	128.6	97.6	105.0	54.0
2,3,4,4',5-PeCB	# 114	97.7	77.1	91.8	66.0
2,3,3',4,4'-PeCB	# 105	103.9	78.9	64.3	54.7
2,3',4,4',5,5'-HexCB	# 167	148.1	60.8	65.6	44.8
2,3,3',4,4',5-HexCB	# 156	77.9	69.7	74.6	53.4
2,3,3',4,4',5'-HexCB	# 157	28.8	48.7	40.1	41.4
2,2',3,4,4',5,5'-HpCB	# 180	113.3	106.2	96.8	55.3
2,2',3,3',4,4',5-HpCB	# 170	121.5	92.6	161.7	61.1
2,3,3',4,4',5,5'-HpCB	# 189	69.1	66.8	268.2	51.7

* # 180 and # 170 are diortho-PCB

References

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