CLEAN-UP OF INTERFERING SPECIES FOR DIOXINS ANALYSIS

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Introduction

In general, the analysis of PCDD/Fs and PCBs requires labor intensive multi-step clean-up procedures in combination with expensive and time consuming. Therefore, a rapid and reliable analyse method is needed. Recently, we reported the efficiency of PCDD/Fs based on a variety of extraction techniquessuch as Supercritical Fluid Extraction (SFE), Accelerated Solvent Extraction (ASE) and Automated Soxhlet techniques[1]. In Japan, the dioxins analysis in foods has been essentially evaluated according to provisional guideline for analytical method of dioxins and Co-PCBs in food (the ministry of health, Japan, 1999).However, since the vegetables contain various interfering compounds, such as oils, pigments or wax, an additional clean-up procedure is required high clean-up efficiencies for the dioxins analysis. In order to complement the guideline for analytical method, we have been verified the characteristics of an interfering compound for the analysis of PCDD/Fs and PCBs in leafy vegetables[2]. It was found that the major interfering compound was n-hentriacontane (major) from long chain aliphatic compounds (alkanes) from leaf cuticular wax, and suggested that the method using by gel permeation chromatography (GPC) or liquid chromatography (LC) was the effective clean-up procedures of the removal of interfering compound (alkanes) from the mono-*ortho* PCBs fraction.

In this paper, to remove interfering compounds (alkanes) in leafy vegetables, we attempted the application of conveniently available clean-up procedures, using Carboxen 1016 short column.

Methods and Materials

Standards and reagents: All PCDD/Fs and co-PCBs used as internal, recovery and calibration standards were purchased from Wellington Laboratories. Hentriacontane was purchased from Fluka. Nonacosane and Triacontane were obtained from SIGMA. All other chemical used were for organic trace analysis and were obtained from Kanto Kagaku. The spinach samples were purchased from supermarket in Tsukuba, Japan.

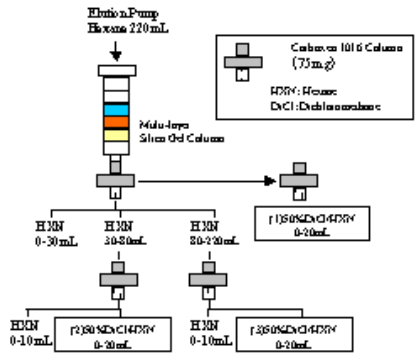

Figure 1. Clean-up procedure of a mono-*ortho* PCBs fraction

Extraction and clean-up procedures: The spinach samples extraction were based on the provisional guideline for analytical method of dioxins and Co-PCBs in food (the ministry of health, Japan, 1999). The extracted hexane layer was repeatedly treated with concentrated sulfuric acid until it became colorless. This was repeatedly washed with water until pH 7.0. In order to remove long-chain alkanes from the mono-*ortho* PCBs elution, the concentrated hexane layer was subjected to homemade multi-layer silica gel column combined with Carboxen 1016 (75mg) short column (Fig.1).

HRGC-HRMS: Detection of dioxins was determined by of GC (6890 plus, Agilent) with a DB-5MS column (J&W Scientific) and an SP 2331 column (Supelco), coupled to a HRMS (AutoSpec-Ultima, Micromass).

Results and Discussion

Fig. 2 shows the plant species in the mono-*ortho* PCBs fraction after the typical column chromatography clean-up procedure for leafy vegetable samples based on the present dioxins

analysis method in Japan. The obtained our previous result[2] of ^{1}H, ^{13}C NMRand GC/MS indicated that those species were mainly due to hentriacontane ($C_{31}H_{64}$) accompanying nonacosane ($C_{29}H_{60}$). These species consist of the pathway for synthesis of the principal components of wax. In order to remove these species in the mono-*ortho* PCBs fraction, we investigated the several clean-up procedures using Carboxen 1016 short column.

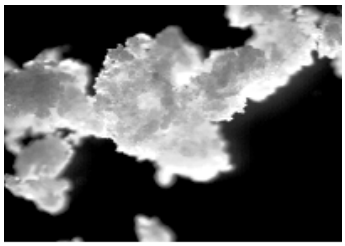

Figure 2. Interfering compound crystals at the clean-up procedures

Fig.3 shows the recovery rates of each fractionation test using the ^{13}C mono-*ortho* and diortho PCBs standards. In the elution with hexane, it was revealed that the major fractionation of mono-*ortho* PCBs was 30-80mL of hexane eluate. Thus, shown in fig. 4, the 0-30mL of hexane elution steps allows to remove the hentriacontane ($C_{31}H_{64}$) and nonacosane ($C_{29}H_{60}$) in the spinach samples. The interfering compounds (alkanes) were mainly found in the hexane eluate. It was confirmed that hentriacontane ($C_{31}H_{64}$) and nonacosane($C_{29}H_{60}$) can be removed by washing with hexane using Carboxen 1016 short column.

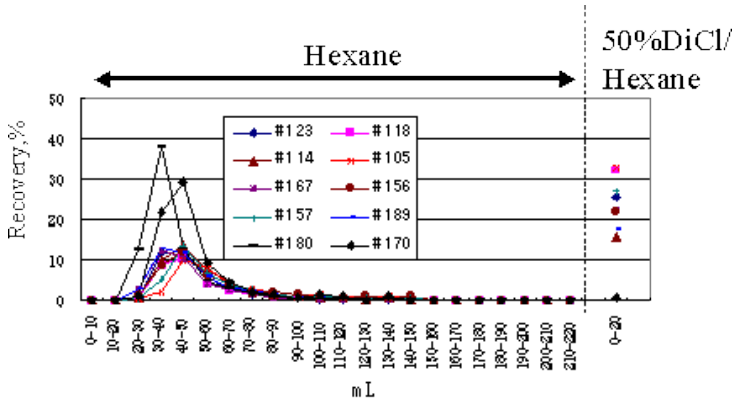

Figure 3. The recovery percentage of mono-*ortho* and diortho PCBs using homemade multi-layer silica gel column combined with Carboxen 1016

Thus, the interfering compound (alkanes) almost nothing remained in the 50vol% dichloromethane/hexane elution (fig1. (1), (2), (3)). The sum of the recovery percentages of mono-*ortho*-Co-PCBs in the spinach samples was over 80% (Fig. 5). We conclude that the proposed methods using Carboxen 1016 short column can effectively be applied to remove the long-chain alkanes from the leafy vegetables.

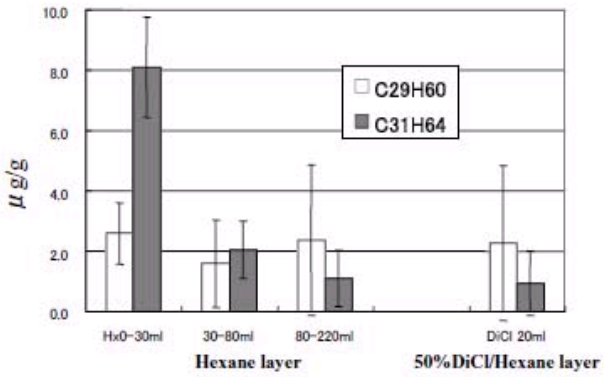

Figure 4. The concentration of hentriacontane ($C_{31}H_{64}$) and nonacosane ($C_{29}H_{60}$) in each fractionation

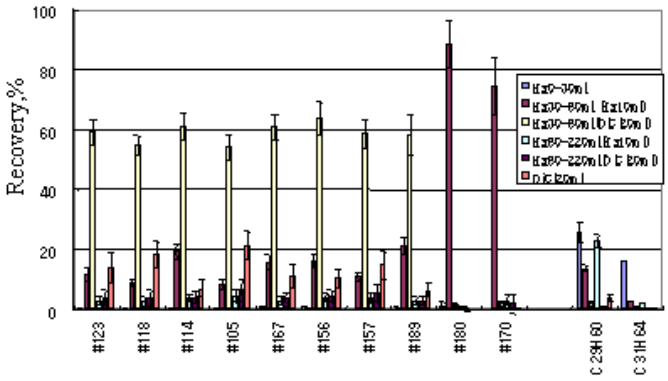

Figure 5. The each fraction recovery percentage of mono-*ortho* PCBs in the spinach samples

References

1. Eun H., Seike N., Baba K., Uegaki R., Ishii Y., Kuwahara M, Uezi M. (2002). Organohalogen Compounds, 55: 163-166

2. Eun H., Watanabe E., Baba K., Hiradate S., Uezi M. (2003). Organohalogen Compounds, 60: 25-28