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CHARACTERIZATION AND CLEAN-UP OF INTERFERING SPECIES FOR DIOXIN-LIKE PCBS ANALYSIS IN VEGETABLES

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Introduction

Last decade, a large number of studies have been conducted in order to monitor dioxins and dioxin-like compounds present in the environment[1,2,3]. In general, the analysis of PCDD/Fs and PCBs requires a labor intensive multi-step clean-up procedure that is 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 techniques such as Supercritical Fluid Extraction (SFE), Accelerated Solvent Extraction (ASE) and Automated Soxhlet techniques[4]. It was demonstrated that, by careful consideration of the experimental parameters, ASE and automated Soxhlet techniques are capable of replacing method such as the classical Soxhlet extraction.

Activated carbon columns are generally used to separate PCDD/Fs, non-*ortho* PCBs and mono-*ortho* PCBs in one of the clean-up steps prior to the final analysis. This kind of columns can achieve the separation of planar aromatic compound from non-planar aromatic compounds. Although various types of activated carbon column have been tested in an effort to improve sample preparation efficiency, not many applications have been developed concerning the interfering compounds and the clean-up procedures for the analysis of PCDD/Fs and PCBs in vegetables. The

analysis of dioxins in vegetables requires high clean-up efficiencies due to the presence of various interfering compounds, such as oils, pigments or wax. Thereby, there has been an increasing demand for fast and easy clean-up method.

In this study, we verified the characteristics of an interfering compound for the analysis of PCDD/Fs and PCBs in leafy vegetables. Furthermore, we investigated the possibility of a new clean-up procedures using by gel permeation chromatography (GPC) and liquid chromatography (LC).

Methods and Materials

Standards and reagents: All PCDD/Fs and co-PCBs used as internal, recovery and calibration standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Hentriacontane was purchased from Fluka (Buchs SG, Switzerland). Nonacosane and Triacontane were obtained from SIGMA (St. Louis, USA). All other chemical used were for organic trace analysis and were obtained from Kanto Kagaku (Tokyo, Japan). *Separation experiments*: *LC*: The separation of mono-ortho PCBs and alkanes was performed using an Agilent 1100 series LC system (Agilent technologies, Ville St-Laurent, Canada). Liquid chromatographic separations were achieved using a Wakopak Navi C30-5 column (4.6×250mm) (Wako Pure Chemical Industies, Ltd., Osaka, Japan). The column temperature was kept constant at 20 ºC. The mobile phase consisted of methanol and was delivered at a flow-rate of 1 ml/min. *GPC*: A gel permeation chromatography (GPC) equipped with a HPLC pump (PU614, GL Sciences Inc., Tokyo, Japan), an automatically injector (Midas, GL Sciences Inc., Tokyo, Japan), and a multi-channel UV-Vis detector (Model MD-1510, Jasco Co., Tokyo, Japan) was used for the separation of mono-ortho PCBs and alkanes. GPC separations were achieved using a Shodex EV-2000 column (SHOWA DENKO K.K., Tokyo, Japan) that was preceded by a Shodex EV-G guard column (SHOWA DENKO K.K., Tokyo, Japan). The mobile phase was dichloromethane. The flow rate was maintained at 4 ml/min. *NMR*: 1D (^{1}H, ^{13}C and DEPT) NMR spectra were recorded in Chloroform-d at 303K on a JEOL (Tokyo, Japan) JNM

alpha-600 spectrometer (^{1}H: 600.05 MHz, ^{13}C: 150.80 MHz) using standard JEOL software (Alpha Data System). *HRGC-HRMS*: Detection of dioxins was performed by GC (6890 plus, Hewlett Packard, US) with a DB 5MS column (J&W Scientific, US) and an SP 2331 column (Supelco, Inc., US), coupled to a HRMS (AutoSpec-Ultima, Micromass, UK).

Results and Discussion

Fig 1 shows crystals of interfering compounds from plant species that precipitate in the first fraction of the active-carbon-dispersed silica gel column chromatography procedure for leafy vegetable samples. Mono-*ortho*-Co-PCBs were also included in the first fraction elution. In order to characterize these significant interfering species, ^{1}H, ^{13}C NMR and GC/MS were used. The result of ^{1}H, ^{13}C NMR (Fig. 2) and GC/MS indicated that those species were long-chain alkanes, such as hentriacontane ($C_{31}H_{64}$) and nonacosane ($C_{29}H_{60}$). These species consist of the pathway for synthesis of the principal components of wax. C_{16} or C_{18} precursors for wax are produced by the de novo fatty acid synthesis pathway in the plastids. After export from the plastid, these acyl chains are elongated to C_{26} or C_{32} fatty acyl chains in the endoplasmic reticulum. The fatty acids can be reduced to alcohols or to aldehydes, and the aldehydes can be decarbonylated to form alkanes with an odd number of carbons. In order to remove these long-chain alkanes from the first fraction elution after the activated carbon column procedure, we investigated the possibility of an alternative clean-up procedures using by gel permeation chromatography (GPC) and liquid chromatography (LC).

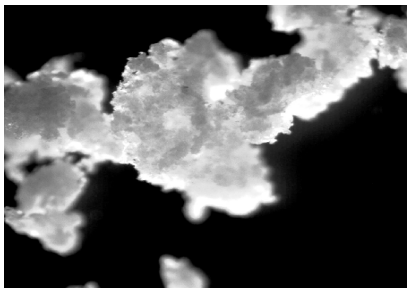
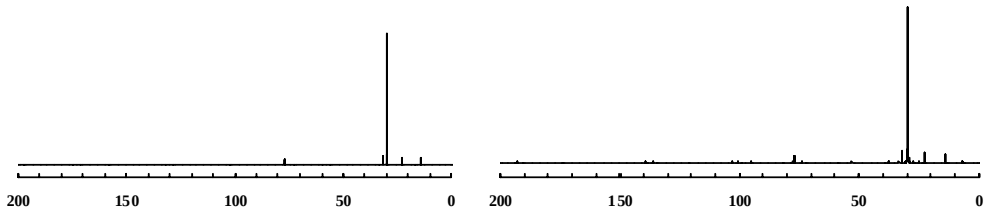


Figure 1. Interfering compound crystals



(a) ^{13}C NMR (b) ^{1}H NMR

Figure 2. NMR Spectrums of interfering compound crystals

Experimental results are presented to illustrate the feasibility of removing alkanes from the first fraction elution (Fig. 3). The separated mono-*ortho*-Co-PCBs were easily achieved by manual fraction collection that is equipped with a LC or a GPC at a specified time. The recovery yields of mono-*ortho*-Co-PCBs were about 90%. We conclude that the proposed methods can effectively be applied to remove these long-chain alkanes.

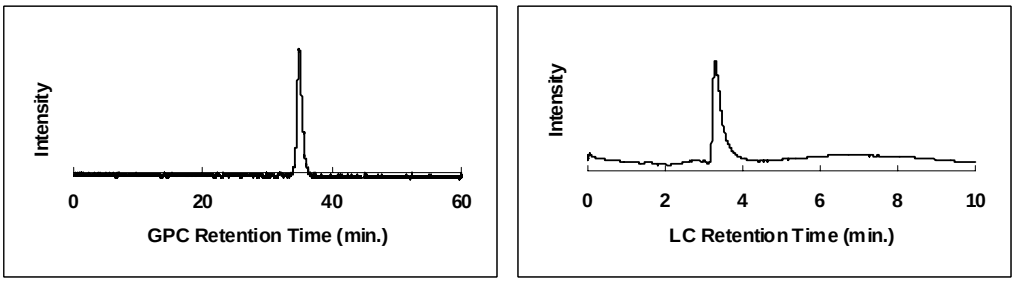


Figure 3. Mono-*ortho* PCBs clean-up using by GPC and LC

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