Nondestructive steps for the lipid reduction in PCDD and PCDF analyses.

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ABSTRACT

An evaluation of different nondestructive steps for reducing the lipid content of a sample in a cleanup procedure shows several possibilities. Gel permeation chromatography and dialysis membrane techniques gives 90-98% reduction of lipids. The recovery of PCDDs and PCDFs is better than 80%. Supercritical fluid extraction indicate a slight increase of PCDDs and PCDFs in the first fraction.

INTRODUCTION

One of the key steps in the cleanup of fatty biological tissue samples is the removal of fat. Several different methods have been used. Treatment with H_2SO_4 or NaOH, followed by extraction using a non polar solvent such as hexane, is commonly used in the determination of organochlorine compounds. In the case of polychlorinated dibenzo-p-dioxins and dibenzofurans the treatment using H_2SO_4 or selective adsorption on activated carbon has been the most accepted methods.

Developing a new cleanup method it is favourable to be able to analyze the majority of xenobiotica and pollutants from the same sample. For that reason nondestructive steps are requested in the early stages. Many different compounds can then be analyzed from the same sample, even if the amount of sample is limited.

Dialysis and supercritical fluid extraction (SFE) can be used to reduce the lipid content in a sample. In our study we have used a homogenized salmon (Salmo salar) sample and oil from Baltic Herring (Clupea harengus) for the comparison of different extraction and concentration methods.

MATERIALS AND METHODS

All PCDD and PCDF analyses have been performed on an HRGC/HRMS VG70-250S instrument equipped with a 60m Restek Rt- 2330 capillary column. The instrumental resolution has been 8000 mass units. The Baltic herring oil was prepared from 1700kg of fresh herring collected in the Gulf of Bothnia during June, 1988. First the whole fishes were heated to 60°C and then compressed. The liquid was heated to 90°C and filtrated excluding particles over 100um. The fish oil was separated from the rest of the liquid. During the last process a temperature of 95°C was maintained. The preparation of the herring oil was performed at Norwegian Herring Oil and Meal Industry Research Institute, Bergen, Norway. The main part of the oil have been used to a salmon feeding study.

The Gel permeation chromatography (GPC) was performed on an ABC Laboratories Autovap^{R} 602 with the sample input module and equipped with a 43 cm long column with 60g Bio Beads S-X3 (ABC Laboratories Inc., Columbia, Mo, USA). The samples were eluted with a 50:50(v/v) mixture of methylenechloride (MC) and cyclohexane (CH) and the sampling fraction was 150mL to 300mL.

Supercritical fluid extraction (SFE) was performed at Department of Food Technology, Chemical Center, University of Lund by Björn Sivik and Maja Jakobsson. 100g of cod liver oil from the Baltic proper was extracted with CO_2 as the eluent. The pressure was 150psi and 250psi. Several fractions were collected and analyzed in Umeå (1).

The use of dialysis membrane (DM) for extraction of xenobiotica as a cleanup method have been discussed by J. Huckins et al.(2). We appreciate the donation of DM from J. Huckins. The DM was prewashed in cyclopentane and used as described (2). Each fraction was evaporated with rotary evaporation until the solvent was gone and then dissolved in 50:50 MC/CH and applied to the analytical procedure described below. DM extraction was also used on wet fish muscle. The tissue was digested with Pancreon powder (a mixture of several different digesting enzymes) in a tris(hydroxymethyl)aminomethane buffer (pH8, 0.02M, 7.0mL)(3).

The analytical procedure is based on a cleanup method discussed in detail by Smith et al. (4). This procedure involves mixing with sodium sulfate, passing the sample through several chromatographic columns in sequence with methylene chloride:cyclohexane 50:50(v/v), and the key step is a column with carbon (PX-21) dispersed on glass fibers. All samples were applied to the first set of columns in 50 mL 50:50 MC/CH and then eluted with 400 mL of the same solvent. PCDDs and PCDFs were removed from the carbon column with 200mL toluene reverse elution. The second set of columns was used as explained in the original method by Smith et al. (1). Before the sample is extracted in the first column, 6 or 8 ^{13}C-labeled PCDDs and PCDFs is added. They are used for calculating the total amount of native isomers in the sample including adjustment for losses during the whole cleanup. All solvents used have been of glass distilled quality (Burdick and Jackson, USA).

RESULTS AND CONCLUSIONS

Gel permeation chromatography

With the sample input module the loading of the oil on the GPC column is 88% to 93% with a total loading of approx. 1 gram oil in 5 mL 50:50 solvent mixture. A subsequent analysis of PCDDs and PCDFs in the second fraction from the Autovap^{R} gave comparable results to earlier studies on the same herring oil.

Supercritical fluid extraction

The first SFE were made at 150 and 250psi. All identified native isomers found in the cod liver oil were extracted with SFE using CO_2. The amounts extracted were independent from the original levels of PCDDs and PCDFs and both fraction 1 (0-6g oil) and fraction 3 (13-18g oil) contained similar levels, 10% to 14% of total level calculated from a separate analyses of the original oil. This indicates a slight increase in the concentration of PCDDs and PCDFs in the first fraction, however additional work is necessary to make this technique useful as an analytical tool.

Dialyses membrane

Extraction of the herring oil in dialysis membrane showed good recoveries (70% - 80%) for all ^{13}C-labeled PCDDs and PCDFs added prior to dialysis. The extraction was performed in glass beakers at 37°C on shaking bath or stationary in the 37°C room. For the native compounds identified in the herring oil about 80% were extracted after 24h and additional 10% after a second extraction during 24h. Less than 10% remained in the oil inside the tube after 48h extraction (se diagram 1). Consequently the membrane technique can be used for the cleanup of fish oil samples.

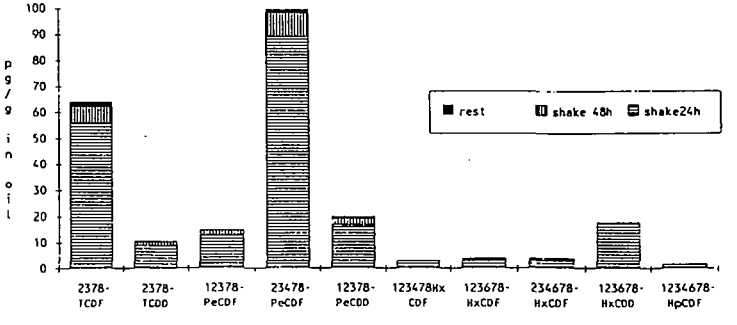

Diagram 1. Extraction of 2.5g herring oil in dialysis membrane (shake, 37°C) followed by the normal cleanup.

A similar experiment was carried out with fresh, blended salmon muscle. The muscle was mixed with Pancreon in a water buffer. At this time the samples were spiked with the ^{13}C-labeled PCDDs and PCDFs dissolved in toluene. A dialysis tube was filled with this slurry and extracted as previously described. After 24h 20% of the naturally occurring isomers and 70% of the added ^{13}C-labeled PCDDs and PCDFs were extracted. After a second 24h extraction, the total extracted native compounds reach 60% and ^{13}-labeled compounds nearly 90% (see diagram 2). Presently the technique starting with fish muscle is not yet completely explored and additional work is being performed.

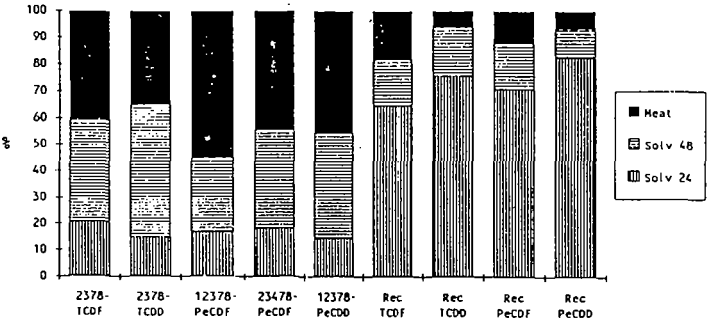

Diagram 2. Extraction of salmon muscle with stationary dialysis membrane (37°C, pancreon in the tube) followed by the normal cleanup.

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