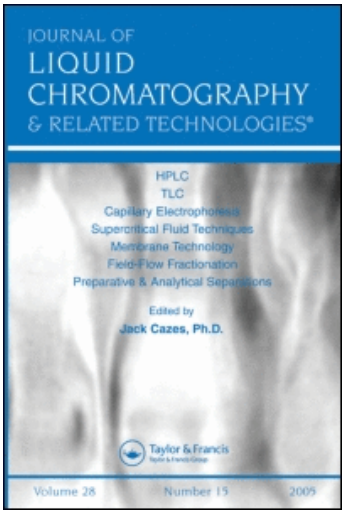

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SIMULTANEOUS DETERMINATION OF PCBs AND TRIGLYCERIDES IN A MODEL FAT SAMPLE USING SELECTIVE SUPERCRITICAL FLUID EXTRACTION

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ABSTRACT

This work presents an extraction method, which selectively extracts PCBs and triglycerides from a model fat sample containing PCBs, triglycerides, and phospholipids. All model fat sample constituents were added to the same cell, and by using alumina, PCBs were quantitatively extracted with little co-extraction of interfering triglycerides. In a subsequent step, triglycerides could be selectively extracted by the addition of 4% methanol as modifier. Phospholipids were not extracted even after the addition of 10% methanol.

INTRODUCTION

Today, many research groups utilise fat retainers in supercritical fluid extraction, in order to generate extracts reduced on interfering fat components. This is especially important when performing trace analysis of minor components such as chlorinated pesticides,[1] PCBs,[2-7] and PCDD,[8] in, for example, fish, chicken fat, and egg. Despite the widespread use of fat retainers, very few publications have been investigated to clarify the relations between the amount fat and fat retainers that will generate a sufficient time window to specifically extract minor components. Recently, however, it was demonstrated that such

time windows can be obtained, and that the length can be varied by using different amounts of adsorbents in relation to the amount of fat in the sample.[9] Until now all publications dealing with fat retainers have been focusing on obtaining fat free extracts, but the possibility of a simultaneous determination of both minor components (e.g. PCBs) and major sample constituents (e.g. triglycerides) will dramatically facilitate many environmental applications. Both trace contaminants and fat content in organisms are of interest, since this information is important when studying bio-uptake of contaminants from the surrounding environment.[10-13] It is of great importance to develop simple, reliable, and cost effective methods, which requires a minimum of sample handling. In this respect, the simple technical set-up presented here for extracting minor components such as PCBs, using basic alumina as fat retainer, will dramatically improve the situation compared to classical methodology. The PCB extraction step is followed by a subsequent selective extraction of triglycerides from the same sample. In the experiments performed, a model fat sample was used consisting of PCBs, lard fat, and a phospholipid mixture obtained from egg yolk.

EXPERIMENTAL

Supercritical Fluid Extraction

A Hewlett-Packard HP7680T Supercritical Fluid Extractor equipped with a modifier pump HP 1050 (Hewlett Packard, DE, USA) was used in all experiments. Carbon dioxide (4.8, AGA Gas AB, Stockholm, Sweden) was used as extraction gas, and the flow rate of the extraction fluid was set to 2 mL/min in all experiments. The density was set to 0.90 g/mL, and the extraction temperature was held at 40°C, throughout. The PCBs were extracted with pure carbon dioxide, while methanol (Kemetyl AB, Sweden) was added to modify the polarity of the extraction fluid when extracting triglycerides. The analytes were collected on a trap packed with octadecyl silica (ODS, 0.6 g). The PCBs were collected at 10°C, while fat was trapped at 90°C. It is important that the trap is sufficiently heated, in order to avoid modifier condensation in the trap as demonstrated by Mathiasson et al.[14]

Standards

A commercially available PCB Isomer Calibration Mix (Accustandard Inc., New Haven, USA), containing 8 different congeners (PCB IUPAC #5, #29, #50, #87, #154, #188, #200, #209), was diluted to between 200 and 1000 ppm with cyclohexane. 50 μL of the diluted standard mixture was added to the extraction thimble. PCB #30 (Accustandard), dissolved in cyclohexane was used as internal standard. The internal standard was added directly to the vials into which the extracted PCBs were automatically eluted by the SFE system.

Lard fat consisting of triglycerides (Swedish Meat Research Institute, Kävlinge, Sweden) was melted on a water bath, and ca 100 mg was added to the extraction thimble. Phospholipids (purified egg yolk lecithin, Pharmacia & Upjohn, Stockholm, Sweden) were dissolved in ethanol and the solution was applied to the extraction thimble with a 50 µL syringe to the desired quantity, 20 mg.

PCB Quantification

After the PCBs were trapped, they were eluted with 1.8 mL cyclohexane (p.a., Merck, Darmstadt, Germany) into vials. The PCBs were analysed on a Hewlett-Packard GC 5890 equipped with a HP 5972 MS. The samples were injected with a HP 7673 GC/STC autoinjector at 250°C, 73 kPa, splitless mode. The injection volume was 2 µL. Helium 5.6 (AGA) was used as carrier gas with the flow set to 1 mL/min. Temperature programming was 100°C isothermal for 2 min, increasing to 200°C at a rate of 40°C/min and held for 5 min, followed by an increase to 230°C at a rate of 10°/min and a subsequent increase to 300°C at a rate of 50°C/min, held for 4 min. The PCBs were analysed in SIM-mode, based on the two most intensive fragments from each congener. The interface temperature was set to 280°C, and the electron multiplier voltage was constant at 2000 V.

Lipid Quantification

Triglycerides and phospholipids were eluted with 1.8 mL cyclohexane and ethanol, respectively. Pre-weighed vials were left uncapped, allowing the eluent to evaporate at in-door temperature for approximately 24h. The vials were then weighed on a Satorius MC1 (Goettingen, Germany). Subtracting the latter from the former weight, gave the quantity of the lipids extracted. This has previously been demonstrated to be a simple and reliable method for quantifying fat.[9]

Packing the Extraction Thimble

The thimble was packed with 6 g of stainless steal beads (100-500 µm, Anval, Torshälla, Sweden) corresponding to a volume of 2 mL. PCBs, triglycerides, and phospholipids were added on top of the beads, in the mentioned order. Basic alumina (Fluka Chemie, Buchs, Germany) was then applied on top of the beads (Figure 1). With the above set-up of the extraction thimble, about 3 mL of dead volume was obtained above the alumina. A stainless steel spring with a metal frit was designed (Chemical Centre, Lund, Sweden), in order to keep the alumina in place.

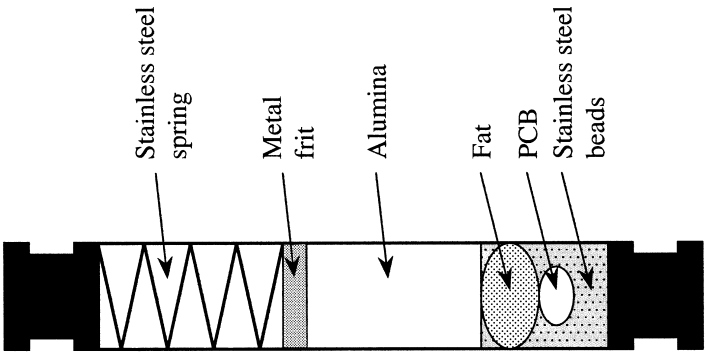

Figure 1. The packed extraction thimble.

An extraction chamber of sapphire was utilised to visually check the process inside the extraction thimble. It was found that the adsorbent was forced towards the outlet of the cell leaving a dead volume above the stainless steel beads, when the extraction cell was fast pressurised at 52 bar, without the spring-loaded frit in place. This might lead to an inefficient retardation of the lipids during the initial PCB extraction step, since channels might be formed with a freely "floating" retainer in a large dead volume. However, with the technical set-up described in Figure 1, the results can be interpreted without consideration of these effects.

RESULTS AND DISCUSSION

Extraction of PCBs from Stainless Steel Beads

In order to get initial information of the behaviour of PCBs extracted through alumina, the PCB mixture was dropped on stainless steel beads and 2 g of alumina was loaded on top. Independent experiments with extraction times of 5, 15, and 30 min, gave average recoveries for eight PCBs of 83, 98, and 102%, respectively (n=2). Individual PCB recoveries for the 15 min extraction ranged from 95 to 103%, demonstrating that PCBs with varying chemical properties could be quantitatively extracted through alumina within a reasonable time.

Extraction of Triglycerides

The fat is most likely retained by hydrogen bonds between triglycerides and the alumina, meaning that the fat will slowly be chromatographed through the alumina. A polar modifier such as methanol will help break these interactions and allow the triglycerides to pass the alumina layer. The speed with

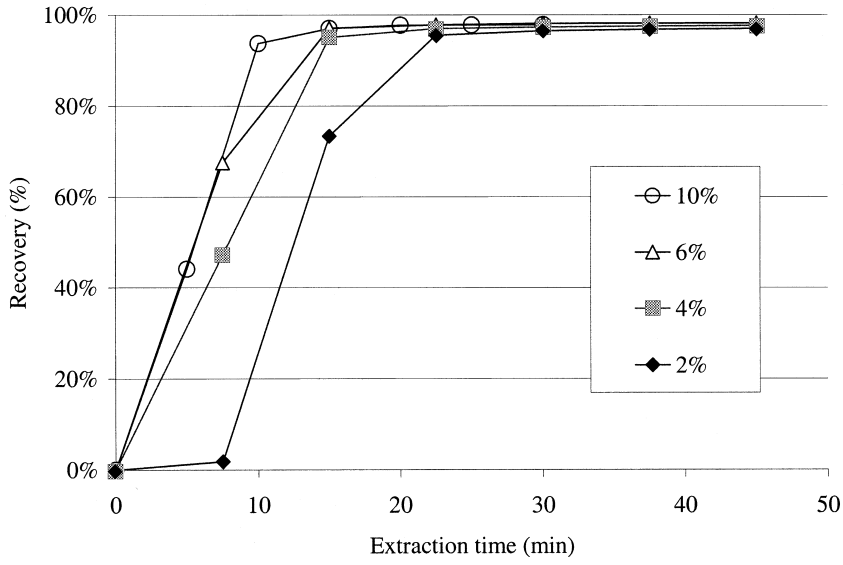

Figure 2. Recovery of triglycerides extracted through 2g of alumina with various amounts of methanol added as modifier (n=3).

which the triglycerides are extracted should be related to the methanol concentration. Determination of a suitable methanol concentration was done by preparing extractions cells containing only stainless steel beads, pure triglycerides (100 mg), and 2 g of alumina. The results from these experiments are presented in Figure 2.

The experiments showed that all methanol concentrations investigated give full recovery within 30 min, with low RSD-values (<3%, n=3). It would be preferable to use a relatively low amount of methanol to minimise co-extraction of unwanted substances, and also to be able to keep the trap at a relatively low temperature. The 2% methanol concentration gave a too steep slope. This is not preferable since it might result in an overload of the trap, as to much fat is extracted in a single step, leading to analyte losses and decreased recovery.[14] The 4% methanol concentration gave a good extraction profile, and therefore, this modifier concentration was chosen for the extraction of triglycerides.

Final Method

The possibility of selectively extracting PCBs from a model fat sample was studied by adding 100 mg of lard fat and 20 mg phospholipids on top of the

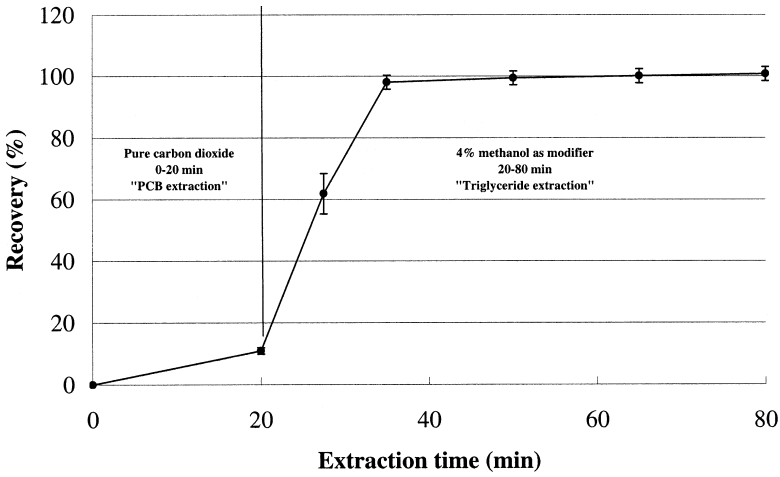

Figure 3. Recovery of triglycerides extracted through 2g of alumina, with 4% methanol added as modifier, (n=3). PCBs were collected during the 20 min PCB extraction window.

PCBs, followed by 2g of alumina. The average recovery of the eight investigated congeners after 20 min of extraction was 94%. Average recovery was based on triplicate measurements with an RSDs below 5%. After the 20 min PCB-extraction-step (using pure carbon dioxide as extraction fluid), methanol was added as a modifier in order to extract the triglycerides in the model fat sample. Results from this subsequent selective triglyceride extraction with the extraction cell containing 2g of fat retainer are seen in Figure 3.

The triglyceride recovery after 15 min of methanol modified extraction is 98%, (RSD 2.3%, n=3), and after another 15 min it only increases to 99% (RSD 2.3%, n=3). This clearly demonstrates that it is possible to quantitatively extract all triglycerides within 20 min from the point when modifier was first added (20 min in Figure 3). The selectivity for triglycerides towards the added phospholipids was investigated by performing separate extractions with 20mg of phospholipids applied on stainless steel beads, with 2g of alumina added on top. The obtained extraction profile, using 10% methanol as modifier, showed that after 80 min of extraction less then 4% (<1 μg) of the phospholipids were recovered (RSD 6%, n=3). Consequently, the selectivity for triglycerides towards phospholipids is also high at large modifier concentrations, meaning that triglycerides can be separated and independently determined from phospholipids.

In Figure 3, it can be observed that some triglycerides are able to pass the alumina layer during the first 20 minutes of PCB extraction. This means that

the length of this layer might be insufficient to completely remove all fat molecules present. However, in this case, using MS for the quantification of PCBs, the amount of co-extracted triglycerides did not cause problems in the final analysis. A more detailed investigation of the relation between the amount alumina needed to retain triglycerides, as well as other fat classes, in both model samples and real world matrices, is the subject of future investigations within our laboratory.

CONCLUSION

Addition of fat retainers, such as basic alumina to the extraction cell in supercritical fluid extraction, gives possibilities to design an extraction method where both minor components (e.g. PCBs) and major sample constituents (e.g. triglycerides) can be separated and quantitatively extracted with a minimum of sample handling. This demonstrates a great potential for environmental applications, where, today, tedious methods are used for measuring organic contaminants and fat in various organisms.

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