GPC clean-up for the analysis of PCBs, PCDDs and their metabolites: a comparison of different mobile phases.

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ABSTRACT

*Clean-up properties of a gel permeation chromatography system (GPC) were studied. A 25 cm long column filled with Bio-Beads SX-3 was used with either acetone or cyclohexane : dichloromethane (CH:DCM, 1:1) as the mobile phase.
*The elution profiles of mesenteric adipose tissue of a cow, 2,2',6,6'-tetrachloro-4,4'-dimethoxy-biphenyl (TCB-$(OCH_3)_2$ and 1,2,3,4-tetrachloro dibenzo-p-dioxin (1,2,3,4-TCDD) were determined in the case of acetone.
*The elution profiles of adipose tissue, TCB-$(OCH_3)_2$, TCDD, 2,2',4,5'-tetrachlorobiphenyl (PCB) and 3-SO_2CH_3-2,2'4,5,5',6'-hexachlorobiphenyl (MSF-HxCB) were determined in the case of CH:DCM.
*The mixture CH:DCM yielded the best separation between fat and the studied compounds, also when compared to hexane:dichloromethane (H:DCM, 1:1).

INTRODUCTION

For the clean-up of hydroxy-metabolites of halogenated benzenes, polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs) and -furans (PCDFs) generated in a microsomal assay (*in vitro*), various methods have been applied. In a previous study, a combination of a florisil column followed by an alumina column yielded satisfactory results for single ion monitoring (SIM) with a GC with mass-spectrometric (GC-MSD) or GC with electron capture detection (GC-ECD)[1]. For full scans, however, the extracts of the microsomal assays are still contaminated with some high molecular compound. Then, problems are encountered like drifting retention-times and high undergrounds in the fragmentation patterns, resulting in data which are difficult to interpret. Since the contaminations behave similar as the PCDDs and the metabolites on both solid phases, no

improvement is to be expected from another clean-up based on chemical affinity. A better solution might be GPC. The seperation by GPC between compounds is based on molecular size. Klasson-Wehler[2] demonstrated satisfactory clean-up of PCBs and their metabolites after extraction from dosed mouse tissues. Kuroki *et al.*[3,4] applied GPC for the clean-up of PCDF metabolites.

The purpose of this study is to determine the elution point and fraction volume for PCDDs and metabolites using a column of of 25*250 mm filled with Bio Beads SX-3. A comparison will be made between CH:DCM as a mobile phase, as suggested by Erickson[5], and acetone. Unpublished data[6] were available on the more frequently advised combination of H:DCM (1:1)[2,7]. Acetone was tested to as possebility since one of the steps in metabolites analysis is methylation. This is done in an acetone solution. It would be convenient, and solvent economising to do this step after GPC clean-up, in the sampled elution volume without any evaporation losses.

MATERIAL AND METHODS

Chemicals

TCB-$(OCH_3)_2$ and 1,2,3,4-TCDD were synthesised according to Rozemeijer *et al.*[1]. TCB was obtained from Promochem, MSF-HxCB was a kind gift from Å. Bergman. Solvents were purchased from Rathburn. The mesenteric adipose tissue was taken from around the kideney of a cow. Bio Beads SX-3 was obtained from Bio-Rad.

GPC-system

A Gilson automatic clean-up system was used. The 231-401 injection system was applied, consisting of the 401 dilutor module (volume 5 ml) and 231 sample injector module. The 302 piston pump module was used as a pump. The column was a Merck-column, with a length of 250 mm, and an internal diameter of 25 mm. Bio Beads SX-3 was used as the stationary phase, swollen in the used solvent. The sample volume was 2.5 ml, and the flow 2.5 ml/min.

Experimental set-up

Adipose tissue was dissolved in the used mobile phase in a final volume of 2.5 ml. The mesenteric adipose tissue was selected as a model for lipids with a relatively small molecular volume. Subsequently, fractions of 10 ml were collected in preweighted vials. The weight was determined again after evaporation of the solvent. The difference yielded the amount of adipose tissue eluted. For the compounds, a standard solution was injected in the GPC system. Different fractions were collected of 5 ml each. The fractions were evaporated under a gentle stream of N_2 gas and transferred to iso-octane. This concentrate was analysed on GC-ECD.

RESULTS & DISCUSSION

The elution profiles of the adipose tissue, 1,2,3,4-TCDD and TCB-$(OCH_3)_2$ are given in fig. 1A, B. The adipose tissue is given in mg/ml. The compounds are given in categories of recovery: category 0=0%, 1=0.1-5%, 2=5-10%, 3=10-20%, 4=20-40%. The adipose

tissue as well as the compounds eluted faster when using CH:DCM as mobile phase. This could be due to either solvent-solute-solid phase interactions or due to the fact that SX-3 swells less in acetone than in CH:DCM. This results in more solid phase to be placed in the column and, consequently, a longer distance to be covered.

Whereas the elution of both adipose tissue and organochlorines is faster with CH:DCM as mobile phase, still, the separation between the adipose tissue and the compounds is better. The bulk of the adipose tissue is in the elution volume till 90 ml (36 min) with a small amount (5%) in the fraction 90-100 ml. The TCB-$(OCH_3)_2$ starts to appear in the fraction 85 ml (only 1% of injected amount). The other compounds (1,2,3,4-TCDD, TCB and MSF-HxCB) start at 36 min. (90 ml, only 1,2,3,4-TCDD shown). The optimal choice between separation and recovery would be to start collecting after 90 ml (=36 min) leading to a loss of only 1% of TCB-$(OCH_3)_2$.

In the case of acetone as mobile phase, the majority of the adipose tissue had passed after 50 min (125 ml). As shown in fig. 1B, both compounds already eluted in substantial ammounts as well. The fractionation point, taking recovery in consideration, is at 110 ml.

The modile phase H:DCM lead to a faster elution of both adipose tissue and PCDDs and PCDFs[6]. The bulk of the adipose tissue was eluted after 32 min with a small amount till 40 min. The PCDDs and PCDFs started to elute in important amounts after 28 min. It can be expected that the non-planar TCB-$(OCH_3)_2$ will elute even earlier leading to an even worse separation.

Concluding, it can be stated that of the three studied mobile phases, two by measurements, the other by literature, CH:DCM (1:1) offers the best separation between adipose tissue and the organochlorines that are studied.

Literature

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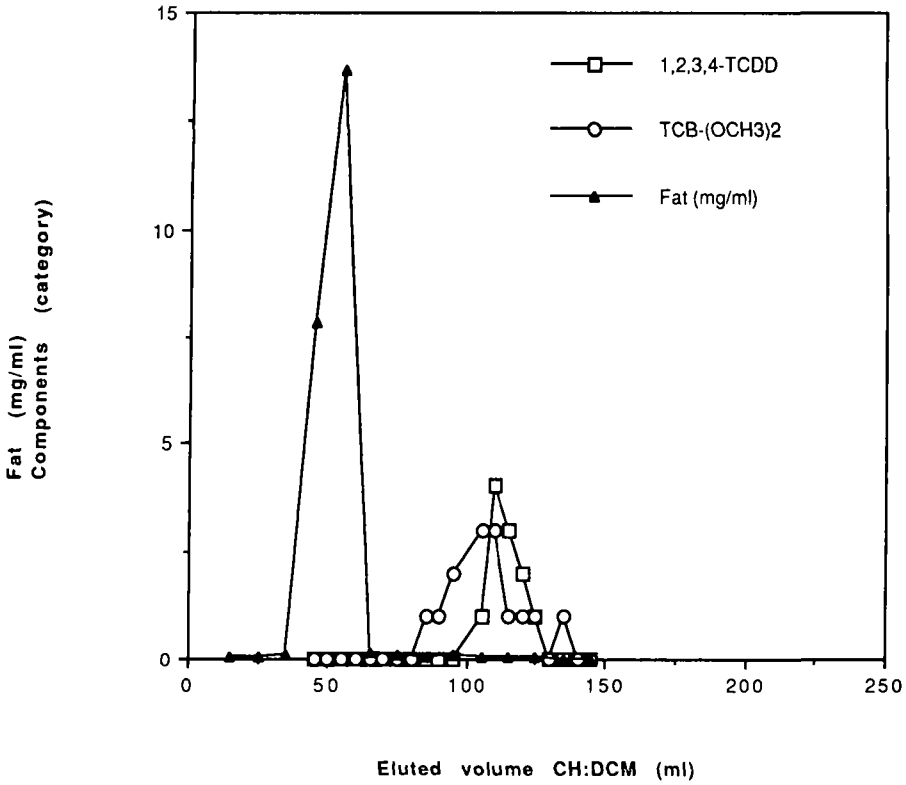
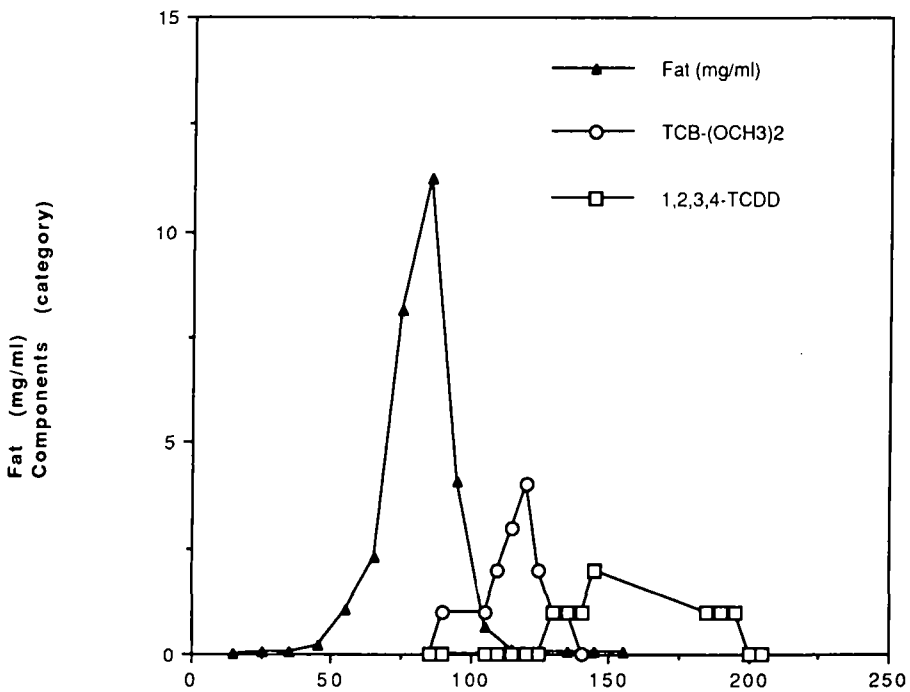

A
15
10
5
0
0
50
100
150
200
250
1,2,3,4-TCDD
TCB-(OCH3)2
Fat (mg/ml)
Fat (mg/ml) Components (category)
Eluted volume CH:DCM (ml)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15
10
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Fat (mg/ml)
TCB-(OCH3)2
1,2,3,4-TCDD
Fat (mg/ml) Components (category)