PCB/TOXAPHENE GROUP SEPARATION BY HP-GPC AND HPLC PRIOR TO CONGENER SPECIFIC ANALYSIS OF TOXAPHENE

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Introduction

Toxaphene is a persistent chlorinated pesticide which is distributed ubiquitously especially in the aquatic environment. The analysis of this lipophilic environmental pollutant in complex biological matrices such as fat containing food and biota samples needs extensive analyte purification and concentration. In general the clean-up methods are similar to those for the classical analysis of organochlorine pesticides and PCBs, followed by simultaneous analysis of all polychlorinated pollutants by GC/ECD or GC/MS. In many environmental samples the individual toxaphene congeners are masked by a bulk of PCBs and, in addition, PCBs may create interferences during the GC/NCI/MS analysis of toxaphene (when oxygen is present in the ion source). Therefore a powerful pre-separation of PCBs is necessary for a precise quantification of toxaphene congeners. In particular adsorption chromatography on silica gel columns are widely used for PCB/toxaphene group separation (reviews in [1,2]). These approaches differ mainly in the used water content of the silica gel, mostly the PCBs are separated in the apolar first fraction by hexane or iso-octane. Also separations on florisil and aluminium oxide [1,2] are described. All these methods by adsorption chromatography suffer from several general drawbacks, inter alia:

- lot-to-lot variations of the commercial available adsorbents,
- strong dependence on the water content of the adsorbent,
- change of elution by already moderate amounts of lipids or matrix,
- slow elution, therefore time-consuming and laborious procedures,
- high consumption of solvents,
- some toxaphene congeners elute in the PCB fraction to a varying degree,
- manual and non-automated techniques.

Therefore, the general aim of our work is to optimise and automate the clean-up of fatty food and biota samples by e.g. gel permeation chromatography (GPC), also called size exclusion chromatography (SEC), and HPLC systems or combinations of them. Here we present the automated PCB/toxaphene group separation by high performance (HP)-GPC and HPLC.

Materials and Methods

Standards

The analytical standards were supplied by Promochem, Wesel, Germany; the solvents specified for residue analysis were purchased from E.Merck, Darmstadt, and Promochem, Wesel, both Germany.

HP-GPC Instrumentation and Conditions

For the HP-GPC the following instrumentation was used: pump Gilson 307 with 10 ml pump head, autosampler: Gilson 231 with dilutor 401 (5 ml syringe) and 2 ml sample loop; fraction collector: Gilson 201 with electric 3-port valve. All parts were purchased from Abimed, Langenfeld, Germany. The chromatographic separation was carried out on a preparative HP-GPC column Plgel

10µm 50Å, 600x25 mm and a precolumn 25x24 mm filled with the same material (Polymer Laboratories Ltd., Church Stretton, UK) with dichloromethane as mobile phase at a flow-rate of 5 ml/min, injection volume: 1.0 ml.

HPLC Instrumentation and Conditions

The used HPLC system was described in detail in [3]. The chromatographic separation was carried out under the following conditions: column: Nucleosil 50Å 7µm, 250x8 mm (Macherey-Nagel, Düren, Germany), mobile phase (flow-rate, time): separation with 10% toluene in n-hexane (4 ml/min for 16 min), backflush with 20% acetone in toluene (2 ml/min for 50 min) reconditioning with 10% toluene in n-hexane (4 ml/min for 30 min).

GC/ECD analysis

The instrumentation and conditions used for the GC analysis of the various fractions were described in [3].

Results and discussion

GPC represents an excellent chromatographic technique for quantitative separation of small compounds (e.g. polychlorinated pesticides, PCBs) from macromolecular compounds such as lipids by their molecular size. As a reversible chromatographic technique GPC can be easily automated to run in a continuous and unattended mode. Many laboratories use a combination of GPC and subsequent silica-gel column chromatography for the multiresidue analysis of food, biota and soil [4-6].

Mostly traditional low pressure GPC on soft gels such as Bio-Beads S-X Beads (Bio-Rad, Richmond, CA, USA) with a particle size of 40 - 80 µm is used. GPC on soft gels is characterised by relatively high matrix capacity and low resolution, thus the organochlorine compounds eluate in one fraction without significant group separation. GPC on rigid gels such as Plgel (Polymer Laboratories Ltd., Church Stretton, UK) with a highly crosslinked porous polystyrene/ divinylbenzene matrix offers several advantages: high resolution, compatibility for frequent exchange of solvent system, extended lifetimes, high stability and therefore usage like HPLC columns. On a preparative HP-GPC column not only the lipid matrix was separated quantitatively but also group separation of the organochlorines could be observed (Fig.1). The toxaphene congeners No. 26, 50 and 62, the DDT group (DDE, DDD and DDT) together with dieldrin and endrin are eluating from the column before the PCB congeners, HCB and beta HCH. This fractionation of very similar chemical compounds with comparable molecular size can be explained by additional π - π^* interactions with the gel matrix resulting in a stronger retention of aromatic systems. Therefore polyaromatic hydrocarbons (PAHs) with their typical large aromatic systems eluate with extremely high retention times from GPC columns. In addition, the used preparative HP-GPC column possessed a high matrix capacity of about 400 mg pure fat. The elution of 6 PCB and 3 toxaphene congeners was studied in depth with standard solutions (concentrations per PCB congener: 15 ng/ml, per toxaphene congener: 12.5 ng/ml) and subsequent GC analysis of both fractions (Tab.1). Low recoveries were observed for PCB 28 and 52 which normally do not occur in high and relevant concentrations in biota or food samples. On the other hand both PCB congeners do not interfere the GC analysis of toxaphene. The elution profile of Parlar 62 overlapped partially with the PCB fraction resulting in a lowered recovery

Tab.1: Recoveries of 6 PCB and 3 toxaphene congeners in the toxaphene and PCB fractions of the HP-GPC column (mean of 5 tests)

	Toxaphene Fraction	PCB Fraction

PCB 28		69.0 %
PCB 52		64.9 %
PCB 101		97.6 %
PCB 138		88.4 %
PCB 153		81.7 %
PCB 180		94.4 %
Parlar 26	86.7 %	
Parlar 50	85.4 %	
Parlar 62	80.6 %	

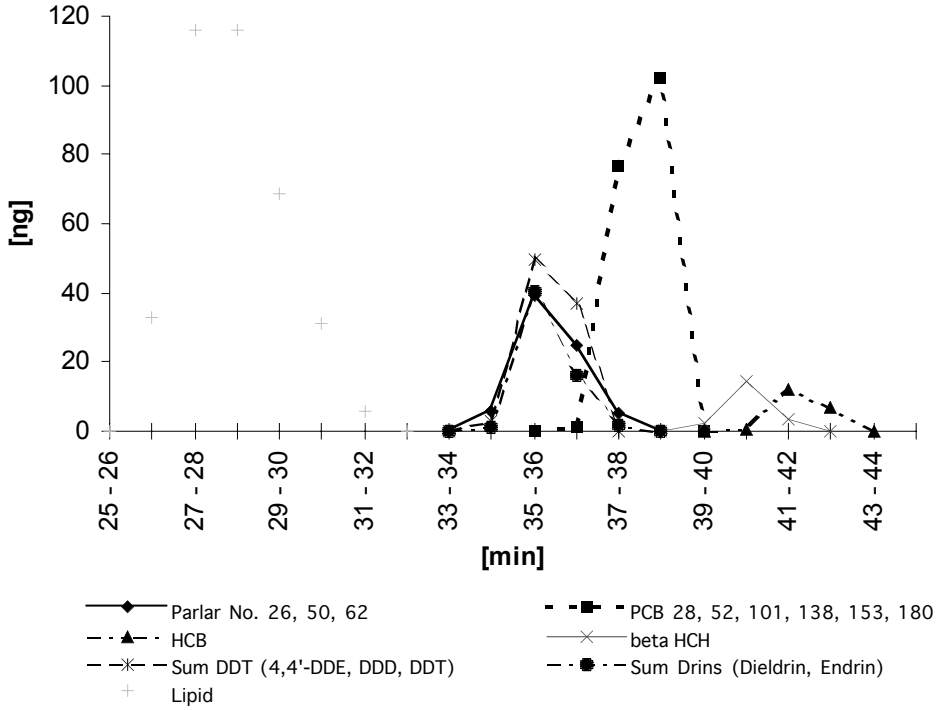

Fig 1 Elution profiles of lipid (butter fat) and groups of organochlorine compounds on a HP-GPC column

(80.6 %). To increase the recoveries of the toxaphene congeners to an optimum the toxaphene fraction has to be broadened with the effect of more PCB inferences in this fraction. Thus the fractionation can be directed variably in dependence on the aim of the analysis. Hitherto comparable HP-GPC separation was only described for the clean-up of soil matrix with a rough separation of technical toxaphene and PCB followed by an additional SPE clean-up [7]. In

summary, HP-GPC offers a simple and efficient one-stage clean-up procedure for toxaphene combining the removal of fat matrix and group separation from the main part of PCBs.
A GPC-HPLC combination as an automated clean-up technique for the multiresidue analysis of fats and fatty biota samples was developed in our laboratory some years ago [3]. In this clean-up method the manual silica gel chromatography was automated by an HPLC system with an analytical normal phase HPLC column. 35 Analytes (mostly organochlorine compounds) were collected in a relatively small HPLC fraction in order to analyse them time-savingly in one GC run. Now we adapted this HPLC system to achieve a PCB/toxaphene group separation by the use of a semipreparative normal phase HPLC column and a binary solvent system. The toxaphene fraction is not only separated from the PCB fraction, but also from 4,4'-DDD, dieldrin, endrin, HCB, lindane and partially from DDE (Fig. 2). In contrast to a described clean-up method with change of the solvent system [8] we used only a binary solvent system in the isocratic mode performing a very robust technique. After each sample the HPLC column is cleaned automatically by backflushing with polar solvents and reconditioned before the next injection. Although this procedure is relatively time-consuming, the HPLC is running as an automatic and unattended system with a high degree of reproducibility. In

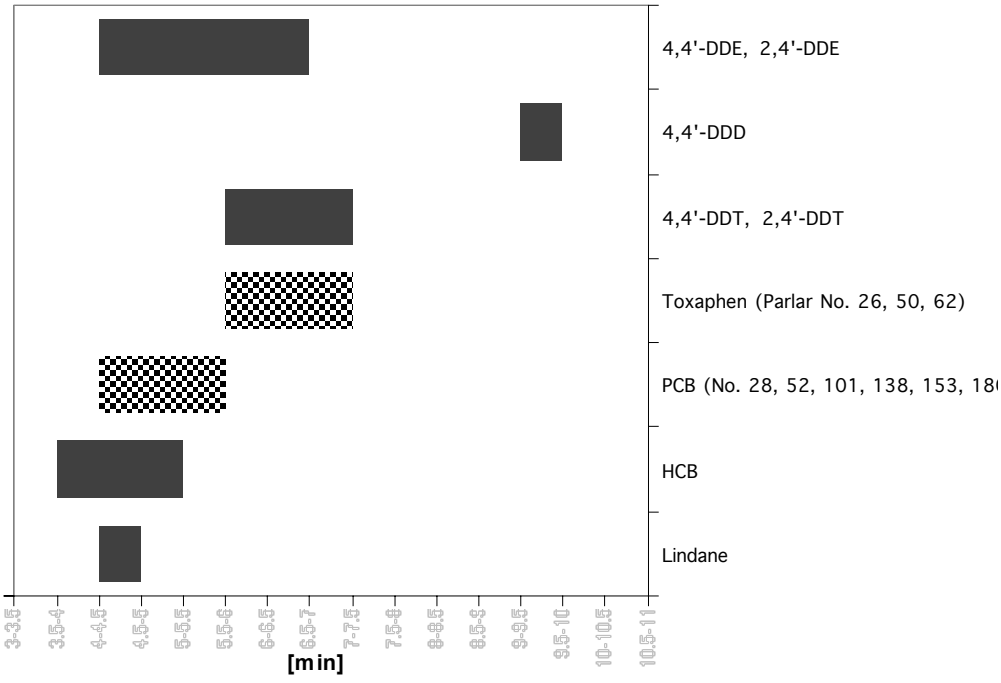

Fig.2 : Elution fractions of some groups of organochlorine compounds on a semipreparative HPLC column

general, adsorption chromatography is relatively sensitive to small amounts of fat matrix and, therefore, the HPLC clean-up needs an almost quantitative pre-separation of lipids by e.g. GPC

or concentrated sulfuric acid. The described HPLC method delivers reproducibly and efficiently very clean extracts for the accurate toxaphene analysis.

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