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Offline coupling of high-speed counter-current chromatography and gas chromatography/mass spectrometry generates a two-dimensional plot of toxaphene components

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ABSTRACT

High-speed counter-current chromatography (HSCCC), a separation technique based solely on the partitioning of solutes between two immiscible liquid phases, was applied for the fractionation of technical toxaphene, an organochlorine pesticide which consists of a complex mixture of structurally closely related compounds. A solvent system (*n*-hexane/methanol/water 34:24:1, v/v/v) was developed which allowed to separate compounds of technical toxaphene (CTTs) with excellent retention of the stationary phase ($S_f = 88\%$). Subsequent analysis of all HSCCC fractions by gas chromatography coupled to electron-capture negative ion mass spectrometry (GC/ECNI-MS) provided a wealth of information regarding separation characteristics of HSCCC and the composition of technical toxaphene. The visualization of the large amount of data obtained from the offline two-dimensional HSCCC–GC/ECNI-MS experiment was facilitated by the creation of a two-dimensional (2D) contour plot. The contour plot not only provided an excellent overview of the HSCCC separation progress, it also illustrated the differences in selectivity between HSCCC and GC. The results of this proof-of-concept study showed that the 2D chromatographic approach involving HSCCC facilitated the separation of CTTs that coelute in unidimensional GC. Furthermore, the creation of 2D contour plots may provide a useful means of enhancing data visualization for other offline two-dimensional separations.

1. Introduction

The term counter-current chromatography (CCC) refers to a separation technique developed in the late 1960s which relies solely on partitioning processes between two immiscible liquid phases [1]. Since the introduction of the J-type planetary centrifuge by Ito in the early 1980s [2], the technique – from then on described as high-speed counter-current chromatography (HSCCC) – has gained both efficiency and popularity [3]. In these hydrodynamic instruments, the stationary phase is kept inside a rotating coiled column by centrifugal force, whereas the mobile phase is pumped through the system. In contrast to conventional column chromatography, CCC techniques do not involve the use of solid media. Therefore, adsorption processes on the stationary phase surface, which may lead to peak tailing or even sample loss due to irreversible adsorption, are avoided in CCC [4]. As the interaction of solutes with the stationary phase is furthermore not restricted to the surface area of a solid but occurs within the whole volume of liquid stationary phase retained in the column, HSCCC features a high sample loading capacity which is beneficial for preparative purposes. Since the scale-up of established separations can be easily accomplished and since HSCCC does not require extensive sample pretreatment, the technique is very popular in the isolation of natural products from crude plant extracts [3,5,6].

Toxaphene, also known as camphechlor, is a complex mixture of organohalogen compounds that has been heavily used worldwide because of its insecticidal properties [7,8]. The production volume of toxaphene has been estimated to exceed one million metric tonnes [9]. Its usage has been discontinued in most parts of the world since the 1990s because of environmental and toxicological concerns. Today, both the production and use of toxaphene is banned by the Stockholm convention on persistent organic pollutants (POPs). However, considerable levels of toxaphene residues are still reported in marine biota [10–13]. Toxaphene has been produced by exhaustive photochlorination of camphene which leads to a multicomponent mixture comprising more than 1000 structurally closely related compounds [14]. Of this vast number of constituents, only approximately fifty are structurally known and commercially available as standards for congener-specific analysis. Typical compounds of technical toxaphene (CTTs) are polychlorinated bornanes, bornenes, camphenes or dihydrocamphenes (Fig. 1) bearing between six and ten chlorine substituents per molecule [7,8]. Bioac-

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Table 1
Compositions (volume ratios) of the solvent systems tested for suitability to separate technical toxaphene by HSCCC in preliminary shake-flask experiments. The partition coefficients $K_{U/L}$ for toxaphene were estimated from the total peak areas of upper and lower phase, as determined by GC/ECD analysis.

No.	*n*-Hexane (H)	Ethyl acetate (E)	Methanol (M)	Water (Wat)	Settling time (s)	$K_{U/L}$
#1	1	–	1	–	8	1.98
#2	9	1	9	1	10	2.48
#3	19	1	19	1	12	1.36
#4	34	–	24	1	8	0.81
	n-Hexane (H)	*tert*-Butyl methyl ether (*ter*)	Acetonitrile (Ac)	Water (Wat)		
#5	1	–	1	–	7	4.90
#6	4	1	4	1	26	2.76
#7	9	1	9	1	7	0.44
#8	19	1	19	1	7	0.95

cumulation or environmental transformation, e.g. by reductive dechlorination, leads to residue patterns which differ from the original technical mixture. In biota of high trophic levels, usually a few octa- to nonachlorinated CTTs dominate [8], whereas the congener profile in sediment samples is shifted towards lower-chlorinated CTTs, often with pronounced enrichment of the hexa- and heptachlorobornanes B6-923 (HxSed) and B7-1001 (HpSed), respectively [15]. In contrast to the recalcitrant congeners which are bioaccumulated e.g. in marine mammals, comparably little is known about the lower-chlorinated CTTs resulting from anaerobic degradation. The quantitation of toxaphene levels is often performed relative to the technical mixture or to selected persistent congeners. However, due to the lack of suitable standards, it is difficult to determine reliable residue levels for sediment, soil or sewage sludge where the residue pattern is shifted towards CTTs with less chlorine substituents [8,15,16].

The idea behind this study was to evaluate whether the unique separation characteristics of CCC can be utilized to elicit more information from the technical toxaphene mixture. To investigate this, the first step consisted of the search for a suitable HSCCC solvent system that would facilitate the separation of toxaphene. Secondly, it had to be clarified whether HSCCC can be used to simplify as highly complex mixtures as toxaphene in general. HSCCC has successfully been applied to isolate constituents of complex mixtures before, but its separation capabilities have rarely if ever been tested with complex mixtures that showed such limited structural variety as does toxaphene. Since the number of unknown CTTs seems to be particularly high in weathered toxaphene profiles where the congener pattern is shifted towards lower-chlorinated homologs, we chose to focus our investigations on the early eluting fractions under reversed-phase HSCCC conditions. A comprehensive evaluation of the fractionation was attempted by the construction of a two-dimensional contour plot showing HSCCC retention data plotted against GC/MS chromatogram data of the second-dimension analyses.

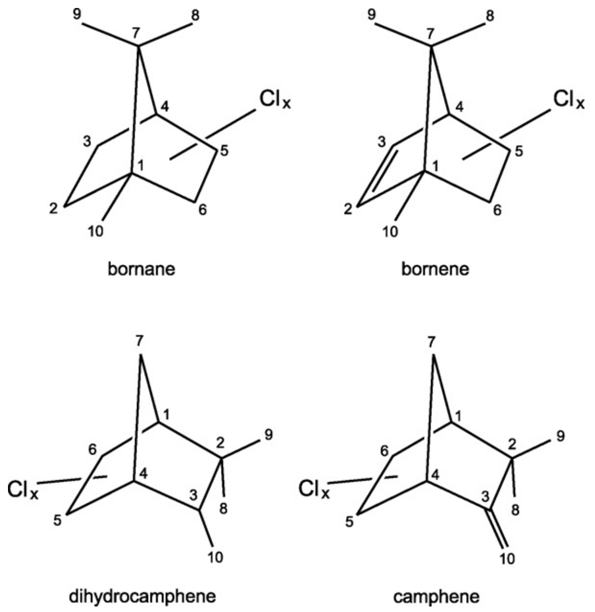

Fig. 1. Carbon skeleton structures of the most prominent compound classes of technical toxaphene with carbon numbering according to IUPAC. The degree of chlorination typically varies between $6 \leq x \leq 10$.

2. Materials and methods

2.1. Reagents

All organic solvents used for this study were of analytical grade or better. Purified water (18 MΩ cm) was obtained from a Milli-Q system (Millipore, Schwalbach, Germany). The toxaphene used for the fractionation originated from a 50-kg sample of the technical product Melipax (Fahlberg-List, Magdeburg, former GDR) which was discovered in Slovakia in 2006. According to the label, the Melipax powder contained 10% of technical toxaphene with a chlorine content of 67–69% (charge number 1263-11/79). The active ingredient was isolated from 25.17 g of the formulation by ultrasonic extraction with eight 100 mL portions of *n*-hexane. After evaporation of the solvent, 2.11 g of an amber, waxy solid were obtained.

2.2. High-speed counter-current chromatography (HSCCC)

HSCCC was performed on a CCC-1000 high-speed counter-current chromatograph (Pharma-Tech Research, Baltimore, MD, USA), equipped with a semi-preparative column consisting of three serially connected coils of 1.6 mm i.d. PTFE tubing. The total column volume was 325 mL. The distance *r* from the coil holder shaft to the coil varied from 3.8 to 5.7 cm. The distance between the coil axes and the central axis of the centrifuge was R = 7.6 cm. The resulting β value ($\beta = r/R$) ranged from 0.50 (internal terminal) to 0.75 (external terminal). The HSCCC system was equipped with a quaternary P580 HPLC pump (Dionex, Idstein, Germany) for solvent delivery. Fraction collection was accomplished by an Isco Retriever 500 (Teledyne Isco, Lincoln, NE, USA).

2.3. Selection of an appropriate two-phase solvent system

Several solvent systems (Table 1) were tested in preliminary partitioning experiments for the applicability in the HSCCC fractionation of toxaphene. These shake-flask tests were performed by adding 1.5 mL of upper and lower phase of a pre-equilibrated solvent system to a 4 mL vial containing 300 μg of toxaphene. The vials were thoroughly shaken and the settling time was measured. After equilibration, 1 mL aliquots were taken from both