ENANTIOSELECTIVE SEPARATION OF CHIRAL METHYLSULFONYL-PCB STANDARDS BY PREPARATIVE HPLC USING METHYLATED CYCLODEXTRIN PHASES

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Introduction

Approximately ten years after the discovery of PCBs in the environment [1], Jensen and Jansson reported on the identification of methylsulfonyl-PCBs ($MeSO_2$-PCBs) in Baltic Grey seal blubber [2]. Over the years thereafter, $MeSO_2$-PCBs have been detected in fish, birds and mammals including humans [3]. Some of the $MeSO_2$-PCBs in biota have also been observed to be selectively and strongly retained in lung and liver tissue of mammals including man [3]. The $MeSO_2$-PCBs formed are persistent and only slightly less hydrophobic than their parent compounds which make them long lasting contaminants in the biosphere. From a toxicological point of view several of the 3-$MeSO_2$-PCBs have been shown by Kato and co-workers to induce P-450 cytochrome enzymes such as P450 2B1, 2B2, 3A2 and 2C6 [4,5]. As a consequence, it is tentatively assumed that a part of the toxic effects induced by PCBs in the environment may be subject to the presence of these PCB metabolites. Furthermore, the main metabolites mentioned above are chiral and, accordingly, enantioselective transformation as well as differential toxic effects of the enantiomers have been postulated [6]. A verification of this conjecture, however, requires enantioselective separation of $MeSO_2$-PCB enantiomers as well as systematic investigations of differential toxic effects of the $MeSO_2$-PCB enantiomers. The present study aimed at filling this gap by the separation of four of the most prevalent chiral $MeSO_2$-PCB standards into their enantiomers using preparative enantioselective HPLC (Fig. 1): 3-$MeSO_2$-2,2´,4´,5,5´,6-hexachlorobiphenyl (abbreviated 3-149; see [7]), 3-$MeSO_2$-2,2´,3´,4´,5,5´,6-heptachlorobiphenyl (3-174), 3-$MeSO_2$-2,2´,3´,4´,5,6-hexachlorobiphenyl (3-132), 4-$MeSO_4$-2,2´,3,3´,4´,6-hexachlorobiphenyl (4-132).

Experimental

The preparation of the methylsulfonyl-PCB standards 3-149, 3-174, 3-132 and 4-132 (Fig. 1) was published elsewhere [8,9]. The enantiomeric pairs of each sulfone were separated by enantioselective HPLC using a chiral 250 x 8 mm Nucleodex ß-PM® column (permethylated b-cyclodextrin bound to Nucleosilâ silica gel, particle size: 5mm) from Macherey & Nagel, Düren, Germany.

Methanol and water, both in quality grade Lichrosolv® from Merck, Darmstadt, Germany, were applied at a ratio of 80: 20 with a flow rate of 1,5 mL/min at a column temperature of 278 K. An UVD-340S Detector from Gynkotech, Germering, Germany was used.

Results and discussion

The HPLC chromatograms obtained by application of methylated cyclodextrins for the methylsulfonyl PCBs of 3-149, 3-174, 3-132 and 4-132 are shown in Fig. 2. It is noteworthy that in the

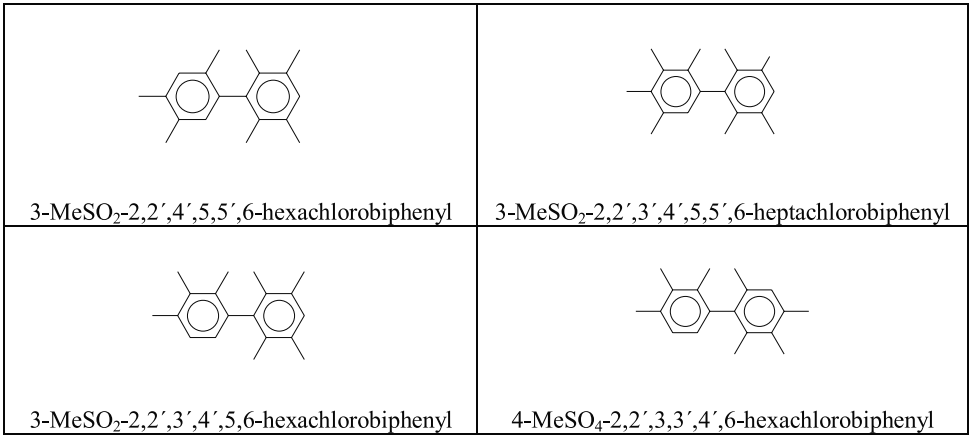

Figure 1. Structures of the $MeSO_2$-PCBs investigated in this work

case of 3-149 and 3-174 optimum enantioselective separation was achieved on an accidently incompletely permethylated Nucleodex ß-PM® column, while the 3-132 and 4-132 enantiomers were most effectively separated on a completely permethylated Nucleodex ß-PM® column. The enantiomeric purity of the separated $MeSO_2$-PCB standards comprised about 99.8 % with separation factors summarised in Table 1. After having noticed the incomplete methylation of the cyclodextrin phase, a comparative study with both the incompletely and the completely methylated cyclodextrin phase was carried out. Comparison of the results (e.g., Fig 3) clearly showed that different methylation degrees are assumed to be an important factor for the optimisation of the enantioselective separation efficiency of modified cyclodextrins for $MeSO_2$-PCBs. To the best of our knowledge this was the first observation of this effect for enantioselective HPLC applications.

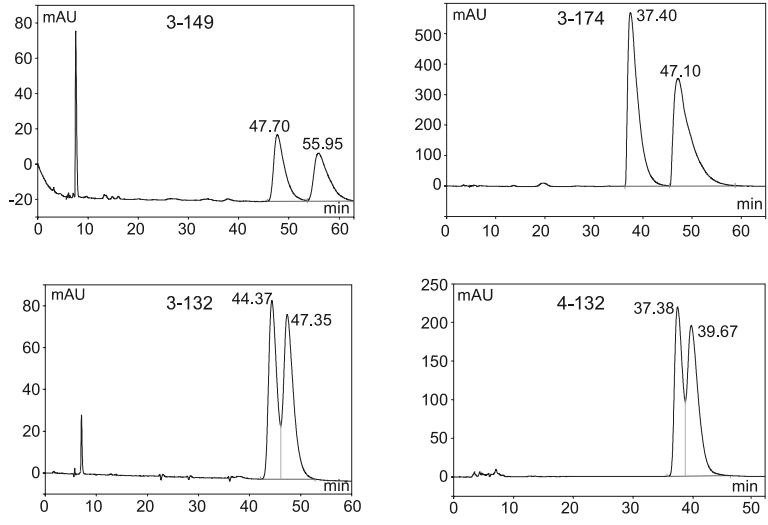

Figure 2. HPLC chromatograms of the four $MeSO_2$-PCBs investigated in this work

Table 1. Retention times of the first (R1) and the second eluting enantiomers (R2) as well as the separation factors a = R2/R1 of the four $MeSO_2$-PCBs investigated in this work.

Substance	Retention time R1 [min]	Retention time R2 [min]	Separation factor a
3-149	47.7	56.0	1.17
3-174	33.7	47.7	1.41
3-132	44.4	47.4	1.06
4-132	40.0	42.5	1.06

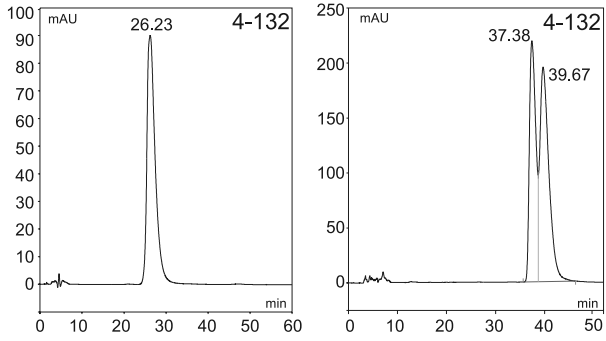

Figure 3. Enantioselective separation of 4-132 using the completely (right) and the incompletely permethylated Nucleodex b-PMâ column (left) under the same experimental conditions.

Thus far, the influence of the purity of chiral stationary phases has been discussed by other authors for gas chromatographic applications only: König pointed out that even if the chiral stationary phase is not a pure enantiomer this would not affect the accuracy of the result; the separation factors a would merely be diminished [10]. On the other hand, variable separation properties between columns containing the same cyclodextrin derivative originating from different batches or manufacturers have caused problems [11-13]. The separation of polychlorinated pesticides may react very sensitively to small changes in the selectivity and polarity of the stationary phase. Differences in the functional group distribution of incompletely derivatised heptakis(2,3,6-*O*-*tert*-butyldimethylsilyl)-b-cyclodextrin molecules may even lead to reversed elution orders of *cis*-chlordane [13] and a-HCH [12] enantiomers. A significant influence of phase purity and composition on the separation properties was also reported for other cyclodextrins such as heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-b-cyclodextrin (TBDMS-CD). Substantial differences in the enantioselectivity for toxaphenes were observed between a semi-raw and a purified product [14]. Other commercially available cyclodextrin derivatives are also often mixtures of differently substituted products [15]. From these observations Jaus and Oehme drew the conclusion [11] that only cyclodextrin derivatives of known and reproducible composition should be used. This requires a control of purity and composition by methods such as high performance TLC, high temperature GC and HPLC combined with refractive index or evaporative light scattering detection (ELSD), supercritical fluid chromatography (SFC) with ELSD, fast bombardment mass spectrometry, electrospray ionisation (ESI) MS (also combined with HPLC) and capillary electrophoresis (CE) coupled to ESI-MS [11]. Unfortunately, none of these methods could be applied to the characterisation of the chiral selector used in the present study because it was already fixed to the silica gel when we got the first indication about its incomplete methylation. Therefore, we only

qualitatively verified the methylation degree of the two phases used herein by application of a set of standard substances, a routine method of Macherey & Nagel.

Different octakis(2,3,6-tri-*O*-ethyl)-g-cyclodextrin (TEG-CD) batches were characterised by HPLC-ELSD or HPLC-MS by Jaus and Oehme [11,16], and the influence of the composition on the enantioselectivity for the polychlorinated pesticides was studied by the same authors. In accordance with the present study, their results seem to indicate that the purity of the chiral selector is not necessarily a crucial parameter for *optimum* selectivity, however, very different performances of columns may be encountered when applying batches of the same material, but different derivatisation grades. Incompletely derivatised batches especially showed a comparable enantioselectivity and thermal stability. In contrast to TBDMS, no degradation over time was observed, and columns could be reproduced from the same batch over a period of months.

After the successful separation of the four $MeSO_2$-PCB enantiomers 3-149, 3-174, 3-132 and 4-132 we intended to carry out subsequent investigations including the determination of the absolute structures and differential toxic effects. It turned out that X-ray studies were problematic, because the single crystals were not sufficiently well-shaped. Therefore, we decided to use vibrational circular dichroism spectroscopy (VCD) in conjunction with quantum chemical *ab initio* calculations. The method, which has for the first time been used for the investigation of such relatively complex chiral molecules, is described in a separate publication [17]. For the enantiomers of the four different methylsulfonyl-PCB congeners investigated in this work a good agreement between the experimental and the simulated VCD spectra has been found.

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