Enantioselective gas chromatographic separation of methylsulfonyl PCBs in seal blubber, pelican muscle and human adipose tissues

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Introduction

Methyl sulfone derivatives are known to represent primary metabolic products of PCBs ($MeSO_2$-CB) and DDE ($MeSO_2$-DDE). These metabolites are formed via mercapturic acid pathway[1-3] and belong to persistent, lipophilic compounds which accumulate in the adipose, lung, liver and kidney tissues of mammals exposed to PCBs. In 1976 Jenssen and Jansson[4] reported the identification of PCB methyl sulfones as metabolites of PCBs in Baltic grey seal blubber. Methyl sulfones are moderately polar compounds that are only slightly less hydrophobic than the parent PCBs, and their partition coefficients fulfill the requirements for bioaccumulation. The highest concentrations have been found in kidney and lung tissues of seals, otters, beluga whales, polar bears, fishes and in human tissues[5-7].

Methods and Materials

In the present investigation two samples of seal blubber, two pelican muscles and eleven human adipose tissue samples were analysed with regard to their concentrations of PCB parent compounds as well as to the respective chiral methylsulfonyl metabolites 3-$MeSO_2$-CB 91, 4-$MeSO_2$-CB 91, 3-$MeSO_2$-CB 95, 4-$MeSO_2$-CB 95, 3-$MeSO_2$-CB 149, 4-$MeSO_2$-CB 149, 3-$MeSO_2$-CB 132, 4-$MeSO_2$-CB 132, 3-$MeSO_2$-CB 174, 4-$MeSO_2$-CB 174 and the achiral metabolites 3-$MeSO_2$-CB 49, 4-$MeSO_2$-CB 49, 3-$MeSO_2$-CB 101, 4-$MeSO_2$-CB 101, 3-$MeSO_2$-CB 110, 4-$MeSO_2$-CB 110 and 3-$MeSO_2$-DDE. Human adipose tissue samples were obtained from the Department of Pathology, Faculty Hospital of Charles University Prague. Seal blubber samples and pelican muscles were obtained from ZOO Prague and ZOO Usti nad Labem, both seals have died after escape during flooding in 2002.

Homogenized samples were dehydrated by grinding with sodium sulphate. Isolation and sample preparation was performed by extraction with *n*-hexane : acetone (2 : 1, v/v). Enrichment and cleanup procedure involved gel permeation chromatography using Bio Beads S-X3 gel and chromatography on 33% KOH/silica gel, Florisil, and 2.3% H_2O deactivated basic alumina

columns. The GPC elution curves of PCB methyl sulfones are similar to curves of parent PCB congeners when using Bio Beads S-X3 gel.

$MeSO_2$-CB were determined in biological samples by gas chromatography with electron capture detection using simultaneous injection in splitless mode onto two capillary columns with different polarity of stationary phases, DB – 5 (5 % diphenyl - 95% dimethyl polysiloxane) and DB – 17 (50% diphenyl – 50% dimethyl polysiloxane) both with the same parameters (60m, 0.25mm, 0.25 m film thickness) installed in a HP 5890 series II gas chromatograph with electronic pressure control, dual automatic liquid sampler and two electron capture detectors. The oven temperature was programmed from 80°C (splitless period 2.5 min) to 220 °C at a rate of 30°C/min and then 2°C to 280°C, hold for 30 min.

Due to the possibility of false positive results when using ECD detection, all the results were confirmed by GC/MS operated in negative chemical ionization mode using an Agilent 6890N gas chromatograph with 5973N mass selective detector. Separation was performed on a HP-5MS fused silica capillary column (5 % diphenyl - 95% dimethyl polysiloxane, 30m, 0,25mm, 0,25μm) and the oven temperature was programmed from 90°C (splitless period 2 min) to 230 °C at a rate of 25°C/min and then 1.5°C to 280°C, hold for 10 min. GC/MS-NICI (reagent gas methane, ion source 150°C, quadrupole 150°C) was used to confirm $MeSO_2$-CB and $MeSO_2$-DDE identities by total ion current scanning (TIC) from 50 to 550 amu. For quantitation of trace amounts of the analytes MS-NICI was operated in the selected ion monitoring mode (SIM, dwell time 100 ms per ion) scanning for m/z 370 for $MeSO_2$-tetraCB, 404 for $MeSO_2$-pentaCB, 438 for $MeSO_2$-hexaCB, 472 for $MeSO_2$-heptaCB and 396 for 3-$MeSO_2$-DDE.[8]

In order to verify enantioselective transformation processes and to compare the different enzymatic transformation pathways in birds and different kinds of mammals, the enantioselective excesses of the chiral PCB-metabolites were determined by enantioselective gas chromatography with electron capture and mass spectrometric detection using modified cyclodextrin phases including heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-)-β-cyclodextrin/OV1701 (1:1) for the parent PCBs and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-)-β-cyclodextrin/SE52 (1:4) for the metabolites, respectively. [9] Enantioselective gas chromatography was performed on HP 5890II and Agilent 6890N instruments equipped with chiral columns mentioned above. Oven temperature was programmed from 70°C (splitless period 2.5 min) to 200 °C at a rate of 20°C/min and then hold for 200 min.

Method validation and quantitation of results was performed by using reference solutions prepared from commercially available methyl sulfone standards (Cambridge Isotope Laboratories).

Results and Discussion

The individual congeners of PCB methyl sulfones were identified, separated and quantified successfully in human adipose tissues, pelican muscle and seal blubber by gas chromatographic methods with ECD and MS-NICI detection.

In human adipose tissue samples 3-$MeSO_2$-DDE, 4-$MeSO_2$-CB 49, 4-$MeSO_2$-CB 101 and 3-$MeSO_2$-CB 110 were detected as predominant methyl sulfonyl metabolites. With the exception of 3-DDE all analyte levels were below 5 ng/g of lipids, the concentration of which is too low for further enantioselective gas chromatographic analysis using a chiral stationary phase.

Figure 1: GC/MS-NICI chromatogram of a selected standard mixture (4-4.4ng/mL)of $MeSO_2$-CBs recorded in SIM mode. The numbers refer to the $MeSO_2$-CBs specified in Table 1.

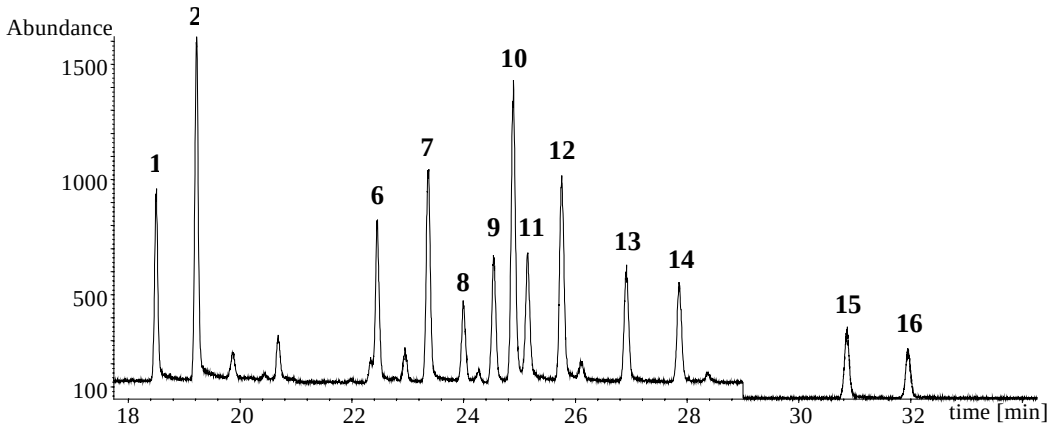

Table 1: The numbers refer to the $MeSO_2$-CBs in chromatograms

number	1	2	3	4	5	6	7	8
$MeSO_2$-CBs	3-49	4-49	3-91	4-95	4-91	3-101	4-101	3-DDE
number	9	10	11	12	13	14	15	16
$MeSO_2$-CBs	3-110	3-149	4-110	4-149	3-132	4-132	3-174	4-174

Figure 2: GC/MS-NICI chromatogram of seal blubber recorded in SIM mode

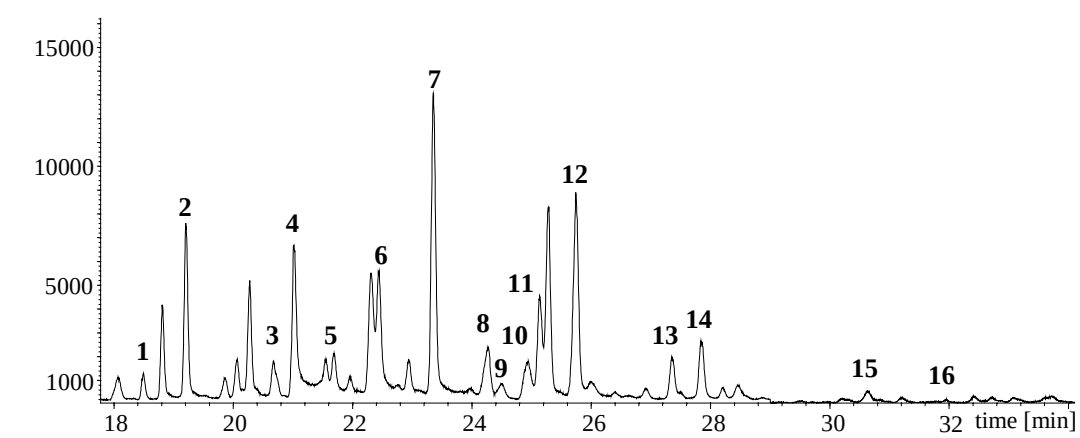

Figure 3: GC/ECD chromatogram of a standard mixture (25 ng/mL) of selected chiral $MeSO_2$-CBs

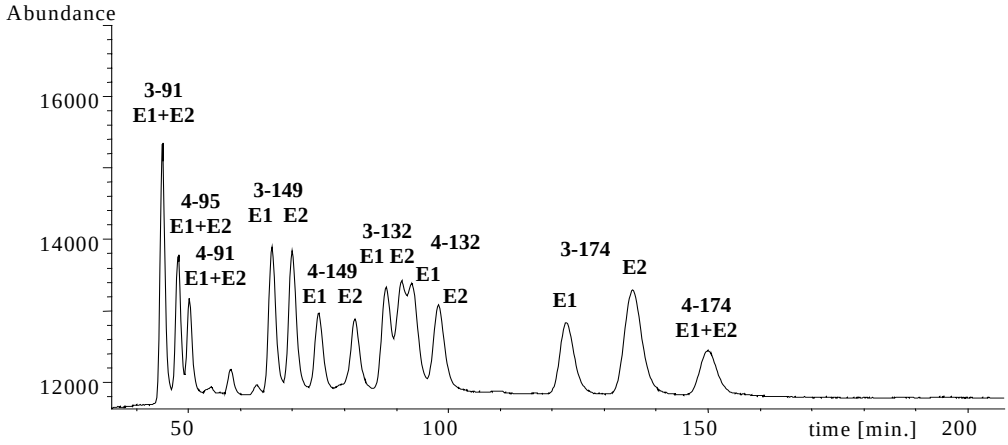

Table 2: Quantitation of enantiomers and enantiomeric ratios for pelican muscles

$MeSO_2$-CB	E1 (ng/g of lipids)	E2 (ng/g of lipids)	ER (E1/E2)
3-132	n.d.	n.d	-
4-132	2.3	1.1	2.10
3-149	2.7	3.6	0.75
4-149	11.8	7.3	1.60

Table 3: Quantitation of enantiomers and enantiomeric ratios for seal blubber

$MeSO_2$-CB	E1 (ng/g of lipids)	E2 (ng/g of lipids)	ER (E1/E2)
4-91	7.2	2.3	3.13
3-132	n.d.	n.d	-
4-132	11.8	3.1	3.81
3-149	2.9	8.2	0.36
4-149	31.4	27.3	1.15

The following conclusions can be drawn:

- Enantiomers of selected atropisomeric PCB methyl sulfones in pelican muscle tissue and seal blubber were separated successfully by enantioselective capillary gas chromatography using a modified cyclodextrin as stationary phase.
- Enantiomers of congeners of methyl sulfones with 3-$MeSO_2$ substitution were better resolved using chiral stationary phases then the corresponding 4-$MeSO_2$ pairs.
- Concentrations of chiral metabolites in human samples were below the limit of detection using a chiral stationary phase. In both pelican and seal samples the enantiomers with *R*-conformation were found in higher concentrations than those exhibiting the complementary *S*-structure. Possible explanations may include enantioselective enzymatic formation, transformation or transport mechanisms.

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