FAST ION TRAP EI-MS/MS DETECTION OF POLYCHLORINATED
n-ALKANES IN BIOTA SAMPLES

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Introduction

Polychlorinated *n*-alkanes (PCAs) are complex technical mixtures with a chlorination degree between 30 and 70% and a linear chain length of C_{10}-C_{13} (short chain PCAs or sPCAs), C_{14}-C_{17} (medium chain PCAs or mPCAs) or $>C_{17}$ (long chain PCAs). Due to their physical properties, PCAs found application as fire retardants, plasticizers in PVC, adhesives, and as extreme pressure additives in lubricants and cutting oils[1]. PCAs can be found in aquatic and terrestrial food webs in rural and remote areas. Most PCA analyses have been performed by high resolution gas chromatography (HRGC) coupled to electron capture negative ionization (ECNI) mass spectrometry (MS) and based on the detection of the [M-Cl]⁻ ions[2]. Due to the many PCA congeners and homologues, this often requires several injections of the sample making PCA analysis expensive and time-consuming. This is one of the reasons for the currently limited information about environmental levels of PCAs.

Electron ionization (EI) of PCAs leads to a strong fragmentation of PCA molecules giving mass spectra without compound specific information such as chain length or degree of chlorination. This work revealed that some of these fragment ions are common to all PCAs, however, at rather low mass-to-charge ratios (*m/z*). Therefore, they were fragmented further by ion trap tandem mass spectrometry (MS/MS) to obtain a satisfactory selectivity and sensitivity as already successfully demonstrated for the analysis of other polychlorinated compounds such as toxaphenes[3]. This technique was then used for the determination of the total PCA concentration in biota.

Methods and Materials

Chemicals and sample clean-up. sPCA (C_{10-13}, Cl contents 51.0%, 55.5% and 63.0%) as well as mPCA (C_{14-17}, Cl contents 51.0%, 55.5% and 63.0%) technical mixtures were purchased from Ehrenstorfer (Augsburg, Germany) as solutions. [$^{13}C_{10}$]-*trans*-chlordane (Cambridge Isotope Laboratories, Mandover MA) was used as internal standard for quantification. Technical chlordane and technical toxaphene were purchased by LGC-Promochem (Wesel, Germany).

Fish samples were collected in the North Sea and Baltic Sea (see Table 2) by the Federal Research Center for Fisheries (Hamburg, Germany) in August 2002. Pool samples were homogenized with a 10-fold excess of Na_2SO_4 after the addition of 10 ng of [$^{13}C_{10}$]-*trans*-chlordane (internal standard) and extracted with 250 ml n-hexane/cyclohexane (1+1, v/v). A sequential clean-up of the extracts was carried out consisting of removal of fat by column chromatography on 40 g silica gel impregnated with 44% H_2SO_4 and elution with 100 ml *n*-hexane/CH_2Cl_2 1+1 (v/v) followed by adsorption chromatography on 16 g Florisil (1.5% H_2O, Fraction1: 60 ml *n*-hexane, Fraction2: 5 ml CH_2Cl_2, Fraction3: 60 ml CH_2Cl_2). Fraction3 contained

the PCAs. Quantification was carried out using the technical sPCA mixture with 55.5% chlorine content as reference. Further details about the clean-up method are given elsewhere[4].

Instrumentation. Gas chromatographic separations were performed on a Star 3400 CX (Varian, Wallnut Creek, USA) gas chromatograph equipped with a fused silica capillary (9 cm length, 0.05 mm id) without coating connected the injector and to a fused silica capillary column (5 m length, 0.25 mm id) coated with 0.25 μm of DB35-MS (35% crosslinked phenyl-methylpolysiloxane, J&W Scientific, Folsom, USA). The injector was equipped with a glass liner filled with glass wool and set to 250 °C. Helium was used as carrier gas at an inlet pressure of 68.9 kPa (10 psi). The temperature program was: 100 °C isothermal for 1 min, then 50 °C/min to 300 °C and isothermal for 4 min. Splitless injections of 2.5 μl volume were carried out with a Varian 8200 autosampler.

A Saturn 2000 (Varian, Wallnut Creek, USA) ion trap MS detector was employed. For all experiments the transfer line temperature was set to 280 °C, the ion trap temperature to 250 °C and the manifold temperature to 40 °C. EI-MS Spectra were recorded at 70 eV and a scan range of *m/z* 50-500 (emission current 10 μA, scan time 0.8 s, trap target 25000 counts). EI-MS/MS spectra were registered at 70 eV, a scan range of *m/z* 50-300 (emission current 70 μA, scan time 0.3 s, trap target 20000 counts and maximal ionization time 25 ms) and collision induced dissociation (CID, isolation time 5 ms, excitation time 20 ms) working in the resonant waveform. Further ion trap MS/MS parameters are listed in Table 1. For the detection of $[^{13}C_{10}]$-*trans*-chlordane the precursor ion *m/z* 383 and the product ion *m/z* 169 were selected (excitation amplitude 0.85 V and excitation storage level *m/z* 169.0).

Table 1. Ion trap MS/MS parameters for the selected PCA fragment ions.

Precursor ion (*m/z*)	Product ion (*m/z*)	Excitation amplitude (V)	Excitation storage level (*m/z*)
77 $[C_6H_5]^+$	51 $[C_4H_3]^+$	0.2	35.0
79 $[C_6H_7]^+$	51 $[C_4H_3]^+$	0.2	35.0
81 $[C_6H_9]^+$	79 $[C_6H_7]^+$	0.2	35.4
91 $[C_7H_7]^+$	65 $[C_5H_5]^+$	0.2	39.9
91 $[C_7H_7]^+$	53 $[C_4H_5]^+$	2.0	39.9
102 $[C_5H_7Cl]^+$	65 $[C_5H_5]^+$	0.6	44.7
104 $[C_8H_8]^+$	77 $[C_6H_5]^+$	0.7	45.6

Results and Discussion

Choice of typical PCA fragments as precursor ion. The EI mass spectra of PCAs show a strong fragmentation and do not contain any compound specific information like chain length or degree of chlorination. However, the patterns of the chromatographic signals of technical PCA mixtures were very similar to the total ion current for the mass chromatograms of some selected ions (see Figure 1). This means that these fragment ions are common to all components of the technical PCA mixtures. Typical ions were *m/z* 77, 79, 81, 91, 102 and 104 (Table 1). A good linearity could be obtained for all CIDs ($R^2 > 0.998$, 6 measuring points between 2 to 100 ng technical PCA

mixture). The limits of detection were in the range of 1-5 ng/µl and the limit of quantification between 2.5-15 ng/µl depending on the selected fragmentation reaction.
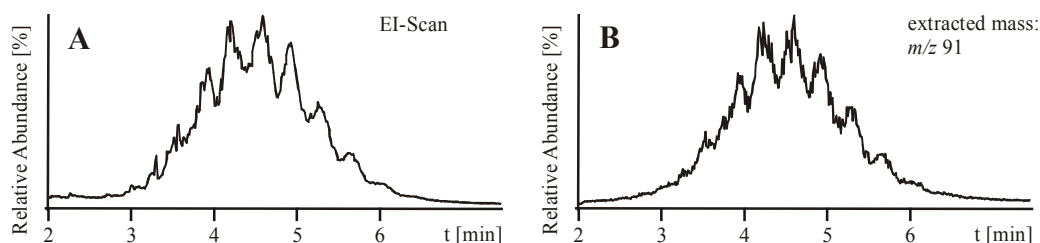

Figure 1. A) EI-scan and B) extracted mass chromatogram (*m/z* 91) of a sPCA technical mixture with 55.5% Cl content. The fragment ion at *m/z* 91 corresponding to $[C_6H_7]^+$ is common to all PCAs.

Use of ion trap MS/MS to increase selectivity. Due to the huge amount of different congeners, isomers and diastereoisomers formed during the synthesis of PCAs, a chromatographic separation of the components of technical PCA mixtures is impossible. Consequently, PCAs give broad ranges of overlapping GC signals, which are likely to interfere with other polychlorinated compounds. The most abundant EI fragment ions of PCAs have low *m/z* values. Therefore, the risk of interferences from fragment ions of other compounds present in the sample extracts is considerable. This lack of selectivity could be compensated by tandem mass spectrometry. In order to evaluate matrix interferences, PCAs were spiked to fish oil. After sample clean-up PCAs were quantified with the different MS/MS fragmentations. The results obtained by CID of *m/z* 91 to 53 and of *m/z* 102 to 65 deviated less then 20% from the spiked amount. All the other fragmentations listed in Table 1 were affected by interferences from the matrix or other polychlorinated compounds present in the sample extract leading to recoveries of >150%. Therefore, these two CID fragmentation reactions were selected for the analysis of real samples.

Simultaneous detection of sPCAs and mPCAs. The relative response factors of three sPCA technical mixtures (51.0, 55.5 and 63.0% Cl content) and three mPCA technical mixtures (42.0, 52.0 and 57.0% Cl content) were determined. Figure 2 shows that they varied less than 20% allowing to determine the sum of short and medium chain PCAs in a semiquantitative way.

Analysis of fish samples. The developed EI-MS/MS detection method allowed the quantification of PCAs in fish liver. The total PCA concentrations (sum of sPCAs and mPCAs) are summarized in Table 2. The values ranged between 210 and 1450 ng/g (wet weight) which is comparable to the values reported in the literature for fish (100-1700 ng/g wet weight) as determined mainly by HRMS[5]. Duplicate analysis deviated less than 10%. sPCA determination by NICI-MS in these samples resulted in comparable levels (Table 2) for all samples except sample 1.
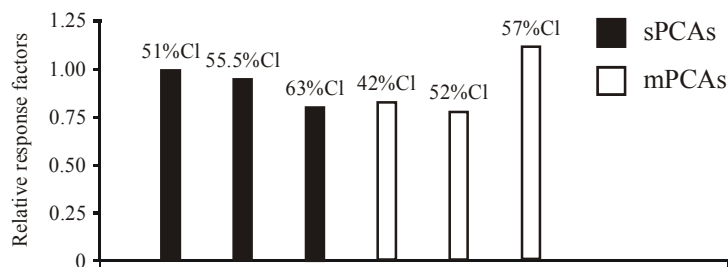
Figure 2. Response factors of six technical mixtures relative to the sPCA mixture with 55.5 % Cl.

Table 2. PCA levels (sum of sPCAs and mPCAs) determined in liver of North Sea dab and cod (ng/g of wet weight) from the North Sea and Baltic Sea.

No.	Fish species	Capture location	Sex	CID (*m/z*)	PCA conc (ng/g)
1	North Sea dab (*Limanda limanda*)	54°15´N/7°29´E (North Sea)	f	$m/z\ 91 \rightarrow 63$ $m/z\ 102 \rightarrow 65$	1210 1450
2	North Sea dab (*Limanda limanda*)	54°39´N/2°12´E (North Sea)	f	$m/z\ 91 \rightarrow 63$ $m/z\ 102 \rightarrow 65$	226 213
3	Cod (*Gadus morhua*)	54°39´N/2°12´E (North Sea)	nd	$m/z\ 91 \rightarrow 63$ $m/z\ 102 \rightarrow 65$	243 283
4	Cod (*Gadus morhua*)	54°45´N/13°20´E (Baltic Sea)	nd	$m/z\ 91 \rightarrow 63$ $m/z\ 102 \rightarrow 65$	351 535

nd: not determined

The lack of information about homologues and congeners of the EI-MS/MS method is compensated by its fast screening potential. In contrast to the SIM-NICI-MS method applied so far[2,4,5], it required only one injection, and the overall analysis time was maximum 10 min. Moreover, sensitivity was situated between high resolution and low resolution NICI-MS.

References

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