ANALYSIS OF TOXAPHENE CONGENERS USING ION TRAP MS/MS: SELECTION OF PARENT AND DAUGHTER IONS.

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Introduction

Quantification of toxaphene or chlorobornanes congeners (CHBs) in environmental samples requires highly selective techniques in order to discriminate them from interferences caused by other CHBs or organochlorinated compounds or the sample matrix (1). Such selectivity can be achieved by tandem mass spectrometry (MS/MS) using an ion trap. The major steps occurring in the ion trap, as a sequence of timed events, are 1- formation of parent ion (I_p), 2- isolation of I_p, 3- formation of product ions (I_d) and 4- I_d mass scanning. Ion trap MS/MS has been employed successfully for the analysis of PCBs and pesticides in marine mammals (2). For toxaphene analysis, however, only two studies have shown some applications of ion trap MS/MS. These studies showed that ion trap MS/MS permits reliable analysis of CHBs in biological samples (3) and offers similar sensitivity and better selectivity than the negative ion chemical ionization mass spectrometry (NICI-MS) generally used for the quantification of CHBs (4). These two studies differ by the selection of I_p and I_d. In one case, I_p and I_d of low mass to charge (m/z) ratios were monitored whereas I_p and I_d of high m/z ratios were selected in the other case.
The aim of this study was to assess the analytical performance of two ion trap MS/MS approaches by selecting low or high m/z ratios for I_p and I_d ions for the detection of specific CHBs in biological samples.

Materials and Methods

Origin of standard and samples: A standard solution containing CHBs 26, 40, 41, 44, 50 and 62 was obtained from Dr. Ehrenstorfer, Germany. Blubber samples from one male and one female stranded beluga whales (*Delphinapterus leucas*), one grey seal (*Halichoerus grypus*), and liver samples from one Atlantic tomcod (*Microgadus tomcod*) and one Greenland halibut (*Reinhardtius hippoglossoides*) sampled in the Estuary and Gulf of St. Lawrence, Canada, between 1997 and 1999, were analyzed.

Sample clean-up: Each sample was chemically dried with anhydrous sodium sulfate and spiked with $^{13}C_{12}$ PCB (170). Contaminants and lipids were extracted with 50% dichloromethane in hexane and a fraction of the extract was spiked with a mixture of labeled surrogate compounds including D_8-4,4'-DDT. Lipids were then removed from the spiked fraction of the extract by gel permeation chromatography (GPC). The GPC fraction containing organochlorinated compounds was subjected to alumina/neutral silica and neutral silica column chromatographies. The final extract was concentrated and spiked with $^{13}C_{12}$ PCB (101) for instrument performance control.

Gas chromatography: Analyses were performed using a Varian 3400CX gas chromatograph (GC) equipped with a programmable split/splitless injector. Chromatographic separations of 5 µL sample injections were made on a DB-5MS column (30 m x 0.25 mm id x 0.25µm film thickness). The temperature program for the injector was as follow: 85°C during 0.3 min then raised to 300°C at 200°C/min and held for 57.8 min. The GC oven temperature was kept at 85°C during 1.4min, then raised to 200°C at 40°C/min and held for 40 min. It was raised again to 270°C at 5°C/min and held for 14 min. Finally, the GC oven temperature was raised to 300°C at 40°C/min and held for 8 min.

Ion trap MS/MS detection: The GC was coupled with a Varian Saturn 4D MS/MS ion trap mass spectrometer. The ionization was done in electronic impact mode. The ion trap was operated in MS/MS mode. The multiplier voltage and trap target were set at 1800 volts and 5000 ions, respectively. Depending on the low or high m/z approach, different I_p and I_d selections for CHBs were performed as described in Table 1. In both approaches, I_p for CHBs were isolated (isolation window = 1 m/z) and stabilized in the resonant wave-form mode for 20 msec. Collision induced dissociation of I_p in I_d was made at the different amplitudes (CID_{rf}) shown in Table 1. The m/z ranges, scanned to monitor I_d, were selected in order to have a range of 20 m/z for all CHBs (Table 1). A scan time of 0.34 s was applied to all monitored CHBs I_d. Parameters for PCB 101 detection were identical in low and high m/z approaches in order to normalize the instrument's performance.

Table 1: MS/MS parameters selected for low and high m/z approaches.

	Low m/z				High m/z			
CHBs	I_p (m/z)	CID_{rf} (V)	I_d (m/z)	Scan range (m/z)	I_p (m/z)	CID_{rf} (V)	I_d (m/z)	Scan range (m/z)
26	125	0.30	89	80-100	305	0.35	269	260-280
40+41	125	0.30	89	80-100	305	0.30	269	260-280
44	125	0.30	89	80-100	305	0.30	269	260-280
50	125	0.30	89	80-100	279	0.20	243	233-253
62	125	0.30	89	80-100	303	0.25	267	257-277

Results and Discussion

Ions selection: The I_p selected for the low m/z approach corresponds to a monochlorotropylium structure, which is considered to be characteristic of CHBs (3). For the high m/z approach, the selection of I_p was based on the EI mass spectrum of the CHBs studied (results not shown). Higher m/z ion clusters that present high abundance were considered and the most abundant ion from a given isotopic cluster was chosen. In both approaches, I_d corresponds to the loss of HCl from I_p.

Response factors (RF): The RFs (RF = specific CHBs area / PCB 101 area) obtained in low and high m/z approaches are reported in Figure 1. In order to compare relative differences between both approaches for each of the CHBs, RFs were normalized to the highest RF. Results for the standard solution show high RF with the low m/z approach for octachlorinated CHBs 26, 40+41 and 44 congeners and with the high m/z approach for nonachlorinated CHBs 50 and 62 congeners. The same trends are observed in the biological samples examined. The most significant difference between the two approaches is obtained with the CHB 62 congener, an abundant CHB generally observed in marine mammals and fish (1). The high m/z ratio approach assessed in this study

allows better detection limits for CHB 62 congener. For instance, this congener is detected in all samples only when the high m/z approach is used. The difficulty of measuring CHB 62 has been reported with NICI-MS of toxaphene (1).

It has been suggested that quantification of total toxaphene with low m/z ion trap MS/MS approach is problematic due to the variability of RFs among CHBs (3). The average RFs calculated from the different CHBs in the standard solution are characterized by coefficients of variation of 91% and 58% for the low and high m/z approaches, respectively (RF for CHB 40+41 was not included in the calculations because of their non chromatographic resolution). Therefore, using an average RF to quantify toxaphene congeners for which no analytical standards are available will result in poor estimations of their concentrations and, then, of the total toxaphene concentration.

S/N ratios: Figure 2 shows the S/N ratios (S/N = specific CHBs signal / baseline noise) calculated from the chromatograms used to determine RFs. Results for the standard solution indicate that both approaches give similar S/N ratios for CHBs 26, 40+41 and 44 congeners. However, S/N ratios obtained with the high m/z approach for CHB 50 and 62 congeners are 7 to 8 times higher than those obtained with the low m/z approach. For biological matrices, results indicate that the high m/z approach is better for all specific CHBs measured. These differences can be explained by the presence of interfering ions in the trap; the lower the m/z of the ions monitored, the higher the probability of detecting signals from fragments associated with the bulk matrix of the sample. As a result, a more intense baseline noise and consequently a low S/N ratio is observed. The different trend observed for CHB 26, 40+41 and 44 congeners, for which both approaches give similar results for the "clean" standard solution, supports this explanation. Moreover, this expresses the need for an efficient sample clean-up procedure when the low m/z ratio approach is adopted.

Quantification: In the samples analyzed, concentrations of specific CHBs determined with the high m/z approach represent, on average, 106 ± 28 % of those obtained with the low m/z approach. Both approaches give very similar results for all CHBs except for the CHB 62 congener, whose quantification was not possible with the low m/z approach in most samples analyzed in this study.

References

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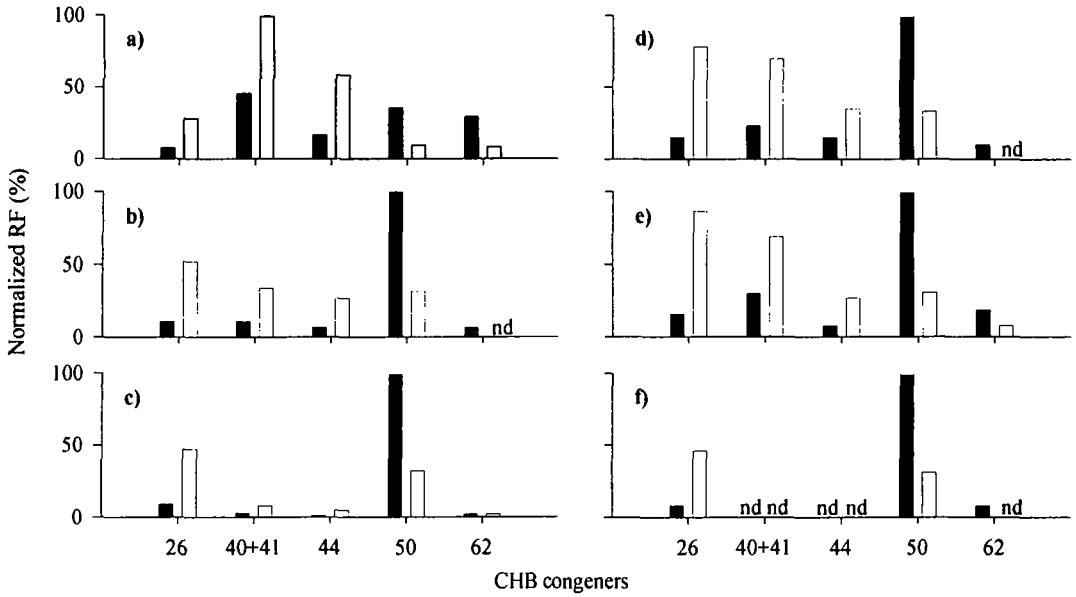

Figure 1: Normalized RFs of CHBs determined with low ( ) and high ( ) m/z approaches by ion trap MS/MS for a) standard solution at 100 pg/uL per congener, b) Atlantic tomcod liver, c) male beluga blubber, d) grey seal blubber, e) Greenland halibut liver and f) female beluga blubber. (nd: not detected)

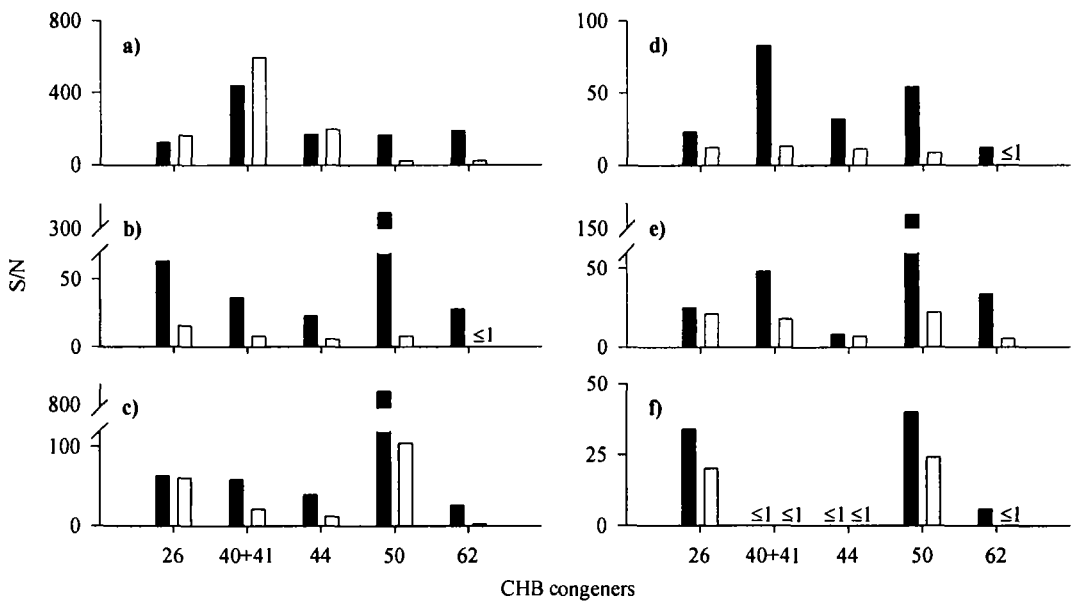

Figure 2: S/N ratios determined for CHBs with low ( ) and high ( ) m/z approaches by ion trap MS/MS for a) standard solution at 100 pg/uL per congener, b) Atlantic tomcod liver, c) male beluga blubber, d) grey seal blubber, e) Greenland halibut liver and f) female beluga blubber.