Determination of N-containing halogenated natural products using gas chromatography in combination of a nitrogen-phosphorus-detector

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Introduction

In the last few years several nonpolar halogenated natural products (HNPs) such as Q1, MHC-1, BC-2, BC-3, BC-10 were detected at elevated concentrations in marine biota samples [1,2]. In addition, there are still some abundant peaks of halogenated compounds frequently found in the gas chromatograms of many marine samples which have not yet been identified. Some of the known halogenated natural products (Q1, HDBPs including BC-10, bromoindoles) contain N-heterocyclic backbones [1,2,3]. Since nitrogen is scarcely found in anthropogenic halogenated compounds, the detection of N-containing halogenated substances may be used as a first indicator for the presence of HNPs in a sample extract. In the presented method we studied the suitability of a nitrogen phosphorous detector (NPD) for quantification of Q1 and the detection of N-containing compounds in marine biota. Analyses were accompanied with GC/ECD analyses.

Materials and Methods

Samples and Chemicals: Australian samples analysed in this study were from Queensland, North-eastern Australia. Commercial shark liver oil from New Zealand was from Lovely Health (Auckland, New Zealand).
The following solvents were used: cyclohexane (purest; Merck, Germany), ethyl acetate (Acros, Germany), n-hexane (Unisolv®; Merck, Germany), isooctane (ECD tested for pesticide analysis; Acros, Germany). Silica gel (60, purest, for column chromatography) was from Merck, Germany.

Pure Q1 was synthesized and purified as described elsewhere [4]. A stock solution of 50 µg/mL was stepwise diluted to concentrations ranging from 20 to 830 pg/µL (calibration range).

Sample clean-up: Samples except blubber and oils were lyophilized prior to extraction. After spiking with the internal standard perdeuterated α-HCH, samples (0.5 – 30 g) were extracted by accelerated solvent extraction (ASE, Dionex) using the following conditions: temperature 125 °C, pressure 10 MPa, preheat 0 min, heat 6 min, static 10 min, flush 60 %, purge 120 sec, cycles 2. Aliquots were taken for gravimetric determination of the lipid content. Collected extracts were concentrated to 5.0 mL and 4.5 mL were subjected to gel permeation chromatography on Bio-Beads SX-3 (Bio Rad, USA. Cyclohexane/ethyl acetate (1:1, v/v) was used as the mobile phase at a flow of 5 mL/min. The collected organohalogen fraction (20 to 35 min) was concentrated in a rotary evaporator to ~2 mL, and the solvent was changed to isooctane in a gentle flow of nitrogen. The sample in isooctane (~1 mL) was subjected to further purification by adsorption column chromatography on deactivated silica. 3 g of deactivated silica containing 30 % water (w/w) were slurried with hexane into a 1 cm i.d. glass column and covered with dry sodium sulfate. The sample was then rinsed carefully on the column and eluted with 60 mL of hexane. The samples were concentrated to suitable volumes and analyzed by GC/NPD and GC/ECD.

Gas chromatography: Analyses were performed with a Hewlett-Packard 5890 series II system equipped with both, an electron capture detector (ECD) and a nitrogen-phosphorous detector (NPD). Samples were injected with a 7673A autosampler into a split-splitless injector operated in the splitless mode. Helium (purity 5.0) was used as carrier gas and nitrogen (purity 5.0) as make-up gas for the ECD and NPD. The NPD was operated with a hydrogen flow of 3.5 mL/min and a synthetic air flow of 100 mL/min. The active element heater was adjusted to 900.
The GC capillary column used, consisted of a CP-Sil 8/10% C18 column (50 m, 0.25 mm i.d., 0.25 µm d_f; Chrompack, Middelburg/NL). The following conditions were used: injector temperature (250 °C), detector temperature (250 °C), column head pressure 25 psi (const.), oven temperature program: 80 °C (1 min), 40 °C/min to 180 °C (1 min), 2 °C/min to 220 °C (1 min), 4 °C/min to 270 °C (26 min). The injected volume was always 1 µL of sample solutions.

Results and Discussion

Figure 1 demonstrates the selectivity of the GC/NPD for nitrogen containing compounds. As in the GC/ECD analyses (Figure 1a), Q1 was the dominating peak in the GC/NPD chromatogram (Figure 1b). However, the signal-to-noise ratio was worse in the GC/NPD run.

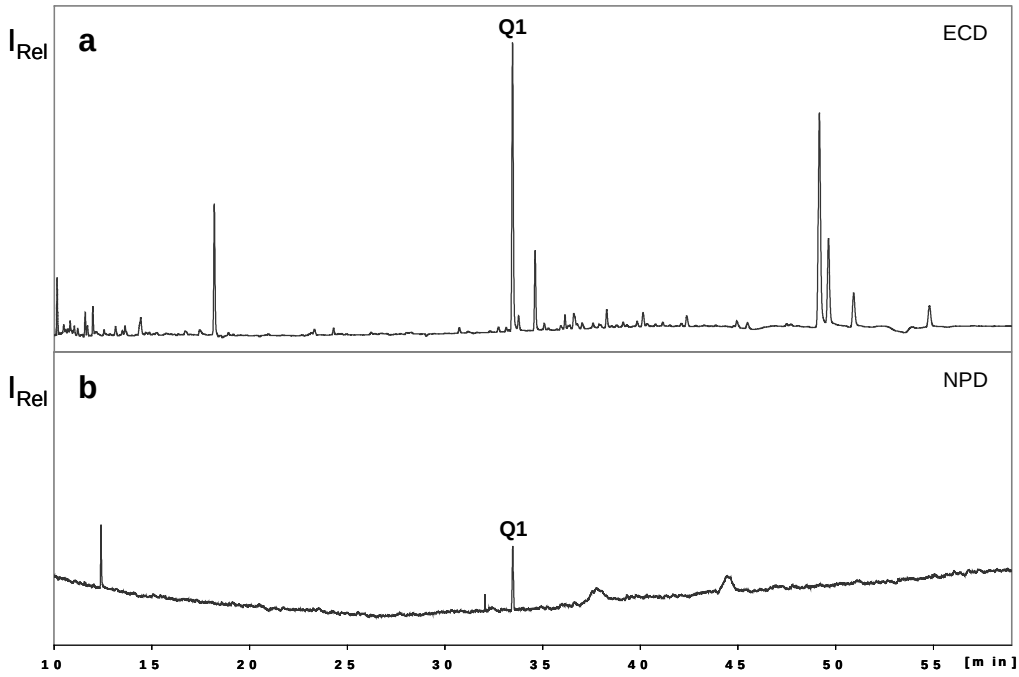

Fig. 1: GC/ECD (a) and GC/NPD (b) chromatograms of an extract of a bottlenose dolphin *(T. truncatus)* from Australia

The GC/NPD calibration curve was linear in the range 20-830 pg (Figure 2) and the system was used for quantification of Q1 in a range of samples from Oceania (Table 1).

Table 1: Concentrations [µg/kg lipids] of Q1 in marine biota determined by GC/NPD

Species	Origin	Q1 concentrations*
Common dolphin *(D. delphis)*	Australia	7700 (+25%)
Bottlenose Dolphin *(T. truncatus)*	Australia	19200 (+38%)
Melonhead whale *(P. electra)*	Australia	7500 (+31%)
Pygmy sperm whale *(K. breviceps)*	Australia	2500 (+27%)
Humpback dolphin *(S. chinensis)*	Australia	500 (+7%)
Shark liver oil (commercial)	New Zealand	300 (+/-0%)

* percentages in parentheses reflect differences to concentrations determined by GC/ECD

The concentrations determined with GC/NPD were up to 38% higher than those determined by GC/ECD on the same system, although both detectors were used in the calibrated range. The data suggests that the differences correlated with increasing concentration of Q1 in the sample extract (Table 1). No explanation was found for the differences.

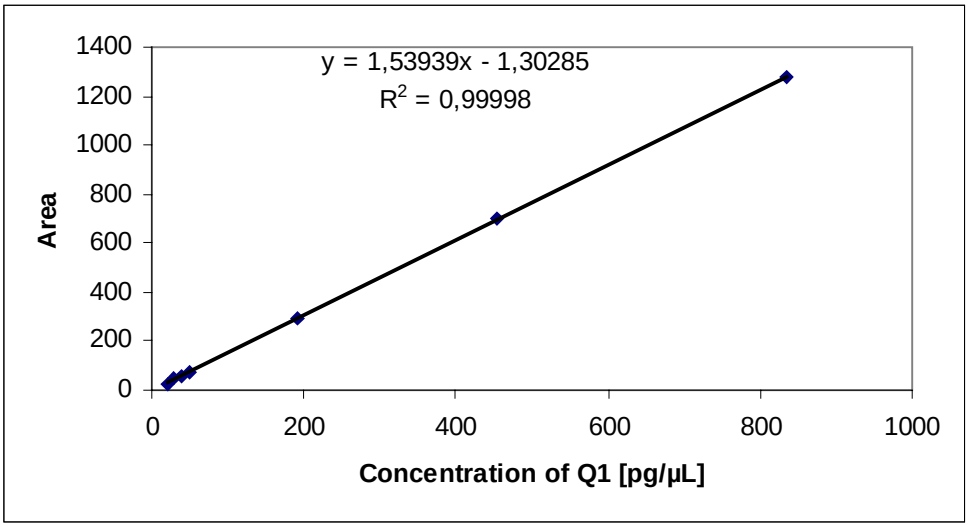

Fig. 2: Calibration curve (10-800 pg) for the determination of Q1 by means of GC/NPD

In addition, we studied different samples for the presence of further N-containing compounds which may be potential HNPs. Figure 1 only showed response for one additional N- (or P-) containing compound at short retention time which was found in most of the investigated samples, but does not seem to be halogenated. However, GC/NPD helped to identify further N-containing compounds in other samples (Figure 3b). Studies are ongoing to determine whether or not these compounds are halogenated. The analyses performed by GC/MS will provide insights in the chemical structure of the compounds. Although the sensitivity of the NPD was significantly lower than that of the ECD, GC/NPD analyses are a valuable tool for both the quantification of known HNPs such as Q1 and detection of N-containing compounds in food and environmental samples.

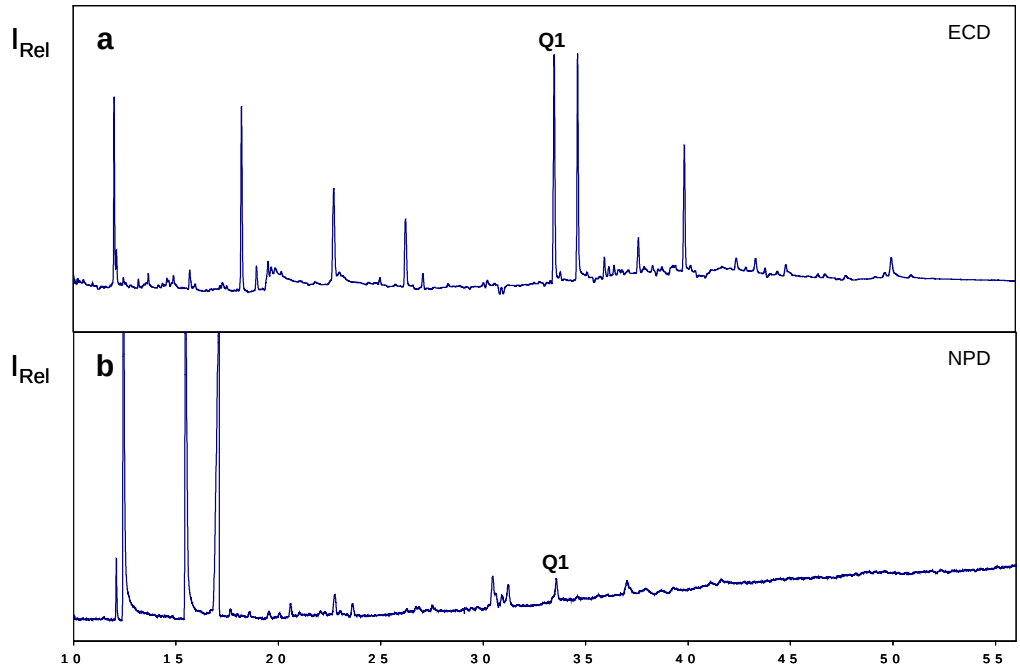

Fig. 3: GC/ECD (a) and GC/NPD (b) chromatograms of an extract of commercial avaiable shark liver oil capsules from New Zealand

References

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