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Short Communication

Enantiomer-specific analysis of hexabromocyclododecane in fish from Etnefjorden (Norway)

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ABSTRACT

High-performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) was applied to sterospecifically quantify the content of α-, β-, and γ-hexabromocyclododecane (HBCD) in six fish species from the Norwegian Etnefjorden. A combination of a β-PM cyclodextrin and an achiral column enabled the paired chromatographic separation of the stereoisomers in the order (−)-α-, (+)-α-, (−)-β-, (+)-β-, (+)-γ- and, (−)-γ-HBCD. The limits of detection were in the range of 6–21 pg g^{-1} depending on the stereoisomer and the concentrations of α-, β-, and γ-HBCD in fillets ranged from <5.4 ng g^{-1} to 11.1 μg g^{-1} lipid weight. α-HBCD enantiomers were throughout dominating, and in most cases the accumulation of the respective first eluted enantiomers ((−)-α-, (−)-β- and (+)-γ-HBCD) was observed. Deviations from the racemic EF-value were considered to be significant if it was outside of the expanded uncertainty range for each of the racemic HBCD-ratios. The composition of HBCD isomers varied between the investigated fish species and the relative high values for the γ-HBCD concentrations for the bottom-dwellers flounder and thorny skate seems to echo the HBCD pattern of ocean sediments.

1. Introduction

In recent years, high-performance liquid chromatography (HPLC) coupled to electrospray ionisation (ESI) mass spectrometry has become an increasingly used tool for the enantiomer-specific quantification of the individual HBCD stereoisomers in biota (Tomy et al., 2004; Janák et al., 2005; Vorkamp et al., 2005; Zegers et al., 2005; Covaci et al., 2006; Law et al., 2006; Stapleton et al., 2006; Ueno et al., 2006; Janák et al., 2008; Peck et al., 2008; Tomy et al., 2008). Herein, the enantiomer-specific quantification of α-, β-, and γ-HBCD in fish in the fillets of a group of fish from a Norwegian fjord by HPLC–ESI-MS/MS using a gradient-free eluent and a combination of a C_{18} and a chiral analytical column. HBCD levels and isomeric patterns are discussed against the background of the absolute configurations of enantiomers (Koeppen et al., 2007).

2. Materials and methods

Native and [$^{13}C_{12}$]-labelled α-, β-, and γ-HBCD standards as racemic solutions in toluene (chemical purity >98%) were provided by Wellington Laboratories, Inc. (Ontario, Canada). Ammonium acetate, sea sand (washed and ignited), HPLC grade acetonitrile, methanol, dichloromethane, and *n*-hexane were obtained from J.T. Baker (Deventer, Netherlands), Picograde® cyclohexane and ethyl acetate from Promochem (Wesel, Germany). Hydromatrix was from (Varian Canada Inc., Ontario, Canada) and SPE cartridges (80 × 15 mm, J.T. Baker, Deventer, Netherlands) were filled with 1 g of activated Florisil (60–100 mesh, Aldrich, Steinheim, Germany, baked at 400 °C for 24 h).

Thorny skate (*Amblyraja radiate*, *n* = 1, length: 70 cm), codfish (*Gadus morhua*, *n* = 1, length: 76 cm), pollack (*Pollachius pollachius*, *n* = 1, length: 43 cm), flounder (*Platichthys flesus*, *n* = 2, length: 30 – 32 cm) and mackerel (*Scomber scombrus*, *n* = 20, length: 30 – 36 cm) were caught in autumn 2006 in Etnefjorden, located in the southwestern part of Norway (Fig. 1). All specimens were eviscerated and heads, scales and skins were detached before cutting into fillets. The fillets were pooled in case of more than one specimen and stored immediately at −20 °C.

Fish fillets were cryo-ground (liquid nitrogen) through a 500 μm sieve on centrifugal mill (ZM 1000; Retsch GmbH, Haan, Germany) and then lyophilised on a Lyovac GT2 (Finn-Aqua Santasalo – Sohlberg GmbH, Hürth, Germany), homogenised and stored at −20 °C. The extraction was performed on an ASE™ 200 Accelerated Solvent Extractor System (Dionex Corporation, Sunnyvale, USA). Samples (0.3–1.5 g) were spiked with 50 μL of a 450 ng g^{-1} solution of $^{13}C_{12}$-labelled α-, β- and γ-HBCD in toluene. The extraction cell was loaded with a glass fibre filter at the cell outlets, followed by 1 g of hydromatrix, the dried fish powder, and sea sand to fill up the dead volume. Extraction solvent was ethyl acetate (100 °C for 5 min; 140 bar; flush volume: 60%; three static cycles).

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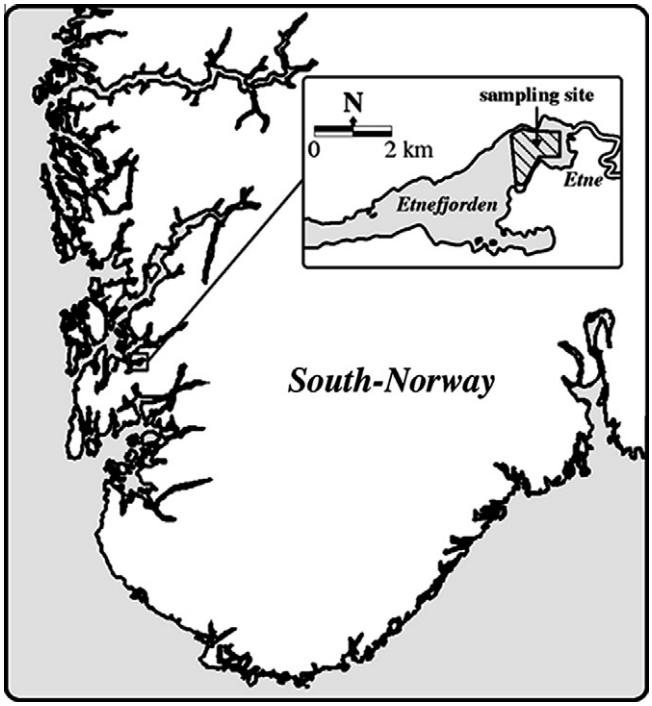

Fig. 1. Map of Etnefjorden on the soutwest coast of Norway, with the sampling site (longitude: 5°54′00″O – 5°55′30″O, latitude: 59°29′29″N – 59°40′17″N).

Extracts were concentrated to 10 mL under a stream of nitrogen (N_2). Lipids were removed using an automated GPC-system (GPC VARIO, LCTech, Dorfen, Germany). Six milliliter of the fish extract was injected into an S-X3 Bio-Beads gel permeation column (500 mm × 40 mm, $L \times$ OD, 50 g of 200–400 mesh). Cyclohexane:ethyl acetate (1:1, v:v) was used as mobile phase with a flow rate of 4 mL min^{-1}. The fraction containing HBCD was collected in a 100 mL GPC bottle, evaporated to dryness, re-dissolved in *n*-hexane and cleaned on 1 g pre-treated Florisil (heated at 160 °C for 24 h) with *n*-hexane (5 mL) followed by *n*-hexane:dichloromethane (1:1, v:v, 13 mL). Extracts were concentrated to dryness using a gentle stream of N_2 and re-dissolved in 300 μL of methanol for HPLC–MS/MS analysis on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an API 4000™ triple-stage-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, California/Concord, Ontario, Canada) run in the electrospray negative ionisation mode. Stereoisomers were separated on a Zorbax XDB-C_{18} (double end-capped, pore size: 80 Å, Agilent Technologies, Waldbronn, Germany) followed by a chiral NUCLEODEX β-PM (pore size: 100 Å, Macherey–Nagel GmbH & Co., Düren, Germany) analytical column (both columns: 5 μm particle size, 200 × 4.6 mm) and using a mixture of 10 mM ammonium acetate buffer and acetonitrile:methanol (90:10, v:v) in the ratio of 90:10 (isocratic, 15 °C; 300 μL min^{-1}). Monitored transitions were 640.6 → 79.0 (native) and 652.6 → 79.0 (labelled). The first and third quadrupoles were set to unit resolution. MS/MS parameters for each monitored transition were optimised using flow injection analysis (ion spray voltage: −4500 V; declustering potential: −30 V; desolvation temperature: 450 °C; ion source gas 1:40 arbitrary units (a.u.); ion source gas 2:30 a.u.; curtain gas: 20 a.u.; collision energy: −40 eV and collision gas: 4 a.u.). Reported concentrations are not surrogate recovery corrected. The lipid contents were determined gravimetrically using the not injected portions of the concentrated extracts after GPC clean-up.

$\delta^{15}N$ and $\delta^{13}C$ isotope ratios were measured in the powdered fish samples using a Vario EL III CHN analyser (Elementar Analysensysteme GmbH, Hanau, Germany) coupled with an isotope-ratio mass spectrometer (IRMS; Isoprime, GV Instruments, UK). The resulting CO_2 and N_2 gases were directly analysed and results expressed in δ notation as the deviation from the standards in parts per thousand [‰]:

$$\delta^{13}C = \frac{\left(\frac{^{13}C_{sample}}{^{12}C_{sample}}\right)}{\left(\frac{^{13}C_{VPDB}}{^{12}C_{VPDB}} - 1\right)} \times 1000 \quad (1)$$

and

$$\delta^{15}N = \frac{\left(\frac{^{15}N_{sample}}{^{14}N_{sample}}\right)}{\left(\frac{^{15}N_{AIR}}{^{14}N_{AIR}} - 1\right)} \times 1000 \quad (2)$$

where the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ for the standard materials are based on the Vienna PeeDee Belemnite for carbon and atmospheric N_2 (ambient inhalable reservoir) for nitrogen.

3. Results and discussion

In recent years, HBCD contents in marine fish were seen to range from the lower ng g^{-1}-level (Morris et al., 2004; Janák et al., 2005; Zegers et al., 2005; Ueno et al., 2006; Johnson-Restrepo et al., 2008; Kakimoto et al., 2008; van Leeuwen and de Boer, 2008) up to 19 208 ng g^{-1} (Law et al., 2006). In deep water fish no HBCD was detected (Webster et al., 2009). Furthermore, significant differences of the HBCD content in biota even from adjacent sampling areas were observed (Morris et al., 2004; Remberger et al., 2004; Janák et al., 2005). The mentioned cohort of fish was regarded as opportunity to add orientating information from a region so far scantly covered in the literature. No comprehensive monitoring was intended.

Etnefjorden is a small branch of Hardangerfjorden, which is the second largest fjord in Norway and one of four major fish farming regions in the world. Water bodies in this area receive discharges from shipping, fish farms, sewage treatment and industry plants. The sampling site in Etnefjorden is of 80–120 m deep and located in front of the promenade, industrial area and harbour of Etne.

The chosen combination of columns led to the ordered elution of enantiomeric pairs as shown in Fig. 2, which is different from that observed in case of the sole use of the chiral phase. All six HBCD stereoisomers were detected in all samples from Etnefjorden well above the detection limits.

The concentration levels summarised in Table 1 range from 5.41 to 11 140 ng g^{-1} lipid weight (lw) and reveal a clear shift of the diastereomeric pattern towards α-HBCD as it was observed by other workers in the field (Tomy et al., 2004; Janák et al., 2005; Covaci et al., 2006; Law et al., 2006; Janák et al., 2008; Peck et al., 2008; Yu et al., 2008). The total HBCD tends to increase with higher trophic level of the fish species as indicated by the comparison with the respective $\delta^{15}N$ values (see Table 1) only the thorny skate deviates from this rule.

The total HBCD content of the cod specimen is extremely high ($\sum_{HBCD}$ = 30.32 μg g^{-1}) and outstandingly, the β-HBCD contents are abnormally high. To the author's knowledge, no similar observation seems to have been reported. The total HBCD concentration in cod was up to three decades higher than values reported in other studies from the Norwegian region (Bytingsvik et al., 2004; Covaci et al., 2006; Sørmo et al., 2006; Jenssen et al., 2007) and was at the high end of the worldwide range. In general, the fish from Etnefjorden show higher HBCD levels compared to available data from other Norwegian fjords (Haukås et al., 2009; Sørmo et al., 2009), suggesting that they were substantially exposed to HBCD. However, no obvious point source discharges were identified in the surrounding area of the sampling site.