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Enantiomer-specific determination of hexabromocyclododecane in fish by supramolecular solvent-based single-step sample treatment and liquid chromatography–tandem mass spectrometry

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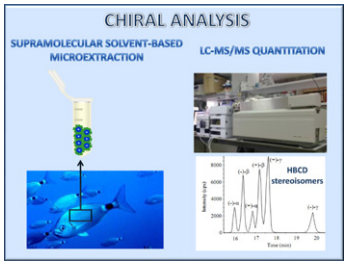
HIGHLIGHTS

- A solvent consisting of decanoic acid aggregates is proposed for microextraction.
- Hexabromocyclododecane stereoisomers are quantitatively extracted from fish samples.
- The sample treatment approach is simple, rapid, inexpensive and eco-friendly.
- Sample extracts are directly analyzed by chiral LC–MS/MS.
- The method provides quantitation limits at the low ng g^{-1} level.

GRAPHICAL ABSTRACT



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ABSTRACT

A single-step, environmentally friendly sample treatment was developed and used in combination with liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the quantitation of hexabromocyclododecane (HBCD) stereoisomers in fish. It was based on the microextraction of the stereoisomers with a supramolecular solvent (SUPRAS) made up of reverse aggregates of decanoic acid (DeA). The procedure involved the stirring of the fish sample (750 mg) with 600 μL of SUPRAS for five minutes, subsequent centrifugation for extract separation from matrix components and direct analysis of the extract after dilution 1:1 with methanol. Individual enantiomers of α-, β- and γ-HBCD were separated on a chiral stationary phase of β-cyclodextrin and quantified by monitoring of the $[M-H]^- \rightarrow Br^-$ transition at m/z 640.9→80.9. Driving forces for the microextraction of HBCD in the SUPRAS involved both dispersion and dipole–dipole interactions. Quantitation limits for the determination of individual HBCD enantiomers in hake, cod, sole, panga, whiting and sea bass were within the intervals 0.5–3.4 ng g^{-1}, 0.9–2.5 ng g^{-1}, 0.6–1.4 ng g^{-1}, 1.0–5.6 ng g^{-1}, 0.8–1.3 ng g^{-1} and 0.5–3.5 ng g^{-1}, respectively. Recoveries for fish samples fortified at the ng g^{-1} level ranged between 87 and 114% with relative standard deviations from 1 to 10%. The sample treatment proposed greatly simplifies current procedures for extraction of HBCD stereoisomers and is a useful tool for the development of a large scale database for their presence in fish.

1. Introduction

Hexabromocyclododecane (HBCD) is an emerging contaminant of very high concern because of its environmental persistence, bioaccumulative properties [1] and adverse effects, mainly derived from its neurotoxic [2], carcinogenic [3], and endocrine-disrupting [4] character. The commercial HBCD primarily consists of racemic

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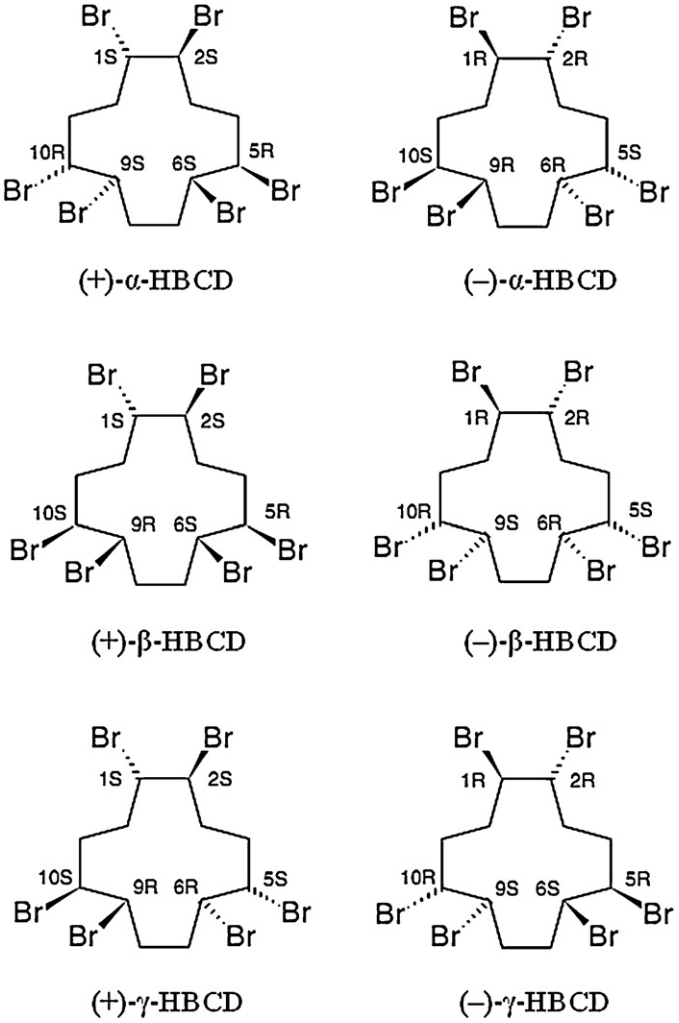

Fig. 1. Stereochemical structures of the three enantiomeric pairs of hexabromocyclododecane (HBCD) diastereoisomers. Bold wedges and wedges of parallel lines correspond to bromine groups above and below the drawing plane, respectively.

mixtures of three diastereoisomeric pairs of enantiomers (Fig. 1), the γ-isomer being the most abundant (75–89%), followed by the α- (10–13%) and β- (0.5–12%) isomers [5]. It is used as an additive in polystyrene foams for thermal insulation of buildings, in upholstery textiles and in electrical equipment housings [6]. Similarly to other brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) or polybrominated biphenyls (PBBs), HBCD reduces the risk of fire by releasing bromine free radicals under heating that scavenge other free radicals taking part in the flame propagation process [7]. HBCD can enter the environment by emission during its production or the manufacture of flame-retarded products, by leaching from consumer products or following disposal [8]. Since the Europe Union prohibited the use of PBDEs and PBBs in 2006 [9], the production of HBCD has continuously raised (it currently exceeds 20,000 tons per year [10]), and as a result, the abundance of this pollutant in the environment has steadily increased in the last years.

Fishes are often contaminated with HBCD owing to their low metabolizing capability for this pollutant and their high position in the food chain. HBCD has been found in fishes at concentrations ranging from the low ng g^{-1} level to about 20 μg g^{-1} [8,11], α-HBCD being the most abundant isomer in the majority of fish species [8]. Differences in the absorption and metabolization rate between the different diastereoisomers [12] and their bioisomerization, with a preferential formation of the α-HBCD isomer [13], have been pointed out as the most probable reasons for alteration of the HBCD composition in fish from that of commercial products. In addition, deviation of the enantiomeric ratios [ERs, defined as the molar ratio of (+)- to (−)-enantiomers] from their original value (i.e. ER = 1 for racemic mixtures) has been observed for the three diastereoisomeric pairs, the ER values found greatly varying among species and tissues and within populations [14,15]. Enantiomer specific accumulation of HBCD in fish indicates that, similarly to other chiral contaminants [16,17], the enantiomers may be selectively degraded in biota. To further our understanding of the behavior and distribution of HBCD stereoisomers in life organisms, which is essential for assessment of their environmental and health risks, analytical methods able to reliably determine HBCD diastereoisomers and enantiomers in biota are required.

Total concentration of HBCD has been traditionally determined by gas chromatography (GC) coupled to electron-capture detection (ECD) or mass spectrometry (MS) [8,16]. This technique is not suitable for resolution of individual stereoisomers because of their interconversion at temperatures above 160 °C and the fact that they do not elute from GC columns at lower temperatures [10]. Liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) [1,15,18–20] has been the choice for isomer- and/or enantiomer-specific analyses by using C_{18} [1,15,18,19] and β-cyclodextrin [1,11,18] stationary phases, respectively. With regard to the MS detection, triple quadrupole has been the mass analyser more frequently employed [1,11,15,18–20], and electrospray sources, operating in the negative ion mode, have provided the highest sensitivity [19]. Extraction of HBCD in fish is usually performed by Soxhlet utilizing 100–150 mL of pure organic solvents (e.g. ethyl acetate [11], dichlorometane [20]) or their mixtures (e.g. n-hexane/acetone [15,21]). Accelerated solvent extraction (ASE) [1,18,19] has been also proposed. ASE spends shorter times than Soxhlet extraction (times are reduced from several hours to a few minutes) but both techniques require clean-up involving multiple steps (typically gel permeation chromatography followed by SPE and/or sulphuric acid treatment [1,11,15,18–21]) before analysing the extracts by LC/MS. Isotopically labeled standards and the standard addition method are usually used for HBCD quantitation.

Supramolecular solvents (SUPRASs) are nanostructured liquids made up of three-dimensional aggregates of amphiphilic compounds that have outstanding properties for analytical extractions, such as high capability to effectively extract compounds in a wide polarity range from both liquid and solid samples, and low volatility and inflammability, which permits the development of extraction processes safer and more environmentally friendly than those based on the use of organic solvents. SUPRASs generated from aqueous solutions of non-ionic surfactants at temperatures above a threshold value (the cloud point) [22,23] have been extensively used to extract both inorganic and organic compounds, primarily from environmental and biological liquid samples [24,25], while ionic surfactant-based SUPRASs [26,27] have not been extensively applied [28,29]. Recently, alkyl carboxylic acid-based SUPRASs made up of vesicular and inverse aggregates have been described [30,31] and successfully applied to the extraction of a variety of contaminants (viz. PAHs [32,33], phenols [34], bisphenols [32], pesticides [35], bioactive compounds [36], dyes [37] and mycotoxins [32]) in aqueous environmental [33–35] and food [32,36,37] samples. SUPRASs have not been used in enantiomer-selective analysis so far.

In this research, a SUPRAS synthesized by a self-assembly process from the mixing of decanoic acid, THF and water, was assessed for the microextraction of HBCD in fish prior to its