Isomer-specific analysis of hexabromocyclododecane by LC/MS/MS

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INTRODUCTION

Hexabromocyclododecane (HBCD, $C_{12}H_{18}Br_6$) is the most widely used of the cycloaliphatic BFRs. In 1999, the global demand for HBCD (~16 kilotons) was more than double that of the penta-bromodiphenyl ether (~8.5 kilotons) mixture [1]. HBCD is used as thermal insulation in the building industry, and is the principal flame retardant in extruded (XEPS) and expanded (EPS) polystyrene foams [2]. Secondary uses of HBCD are in upholstery textiles including residential and commercial furniture, upholstery seating in transportation, draperies, and wall coverings [1,2].

Like the brominated diphenyl ethers (BDEs), HBCD is an additive type BFR. Thus HBCD is not covalently bonded to the material leading to the risk of migration out of the product during use or disposal.

Technical HBCD is industrially synthesized by the addition of bromine to *cis-trans-trans*-1,5,9–cyclododecatriene [2,3]. The resulting technical mixture contains three diastereoisomers (α, β and γ) and tetrabromocyclododecene as an impurity (<2%). There are four grades of the technical mixture produced by industry, each containing different percentages of the three isomers: low melt, medium range, high melt and thermally stabilized [4]. Typically, the γ–isomer is the most dominant (>70%) followed by the α– and then β. The selection of HBCD grade used depends on the usage of the end-product.

Recently, an isomer specific method for the analysis of HBCD has been described by Budakowski and Tomy [5]. Here we build on that earlier work and report on refinements made to the method because of the recent availability of isotopically labelled standards.

MATERIALS AND METHODS

Chemicals. All three native (α, β and γ), deuterium (d_{18}–α, β and γ–HBCD) and carbon-13 mass ($^{13}C_{12}$–α, β and γ–HBCD) labelled HBCD isomers (each >98% purity) were provided by Wellington Laboratories Inc. (Guelph, ON, Canada). The synthesis of the labelled isomers has been described by Arsenault *et al*. [6]. HPLC Optima grade methanol, water and acetonitrile were obtained from Fisher Scientific (Nepean, ON, Canada).

Liquid Chromatography. An Agilent 1100 Series (Agilent Technologies, Wilmington, DE, USA) equipped with a vacuum degasser, binary pump and autosampler was used. A Genesis (Jones Chromatography, Chromatographic Specialties Inc.) C_{18} (10.0 cm × 2.1 mm i.d., 4 μm particle size) analytical column was used for all samples. A mobile phase of (A) water and (B) methanol at a flow-rate of 300 μL/min was used. The elution program started at an initial composition of 30:70 (v/v) and was ramped to 100% methanol in 3 minutes. Thereafter it was held for 3.5 minutes and returned to the starting conditions in 3 minutes. The column was allowed to equilibrate for 6 minutes between runs.

Mass Spectrometry. A Sciex API 2000 triple quadrapole mass spectrometer (ABS, Foster City, CA, USA) was used to run negative mode electrospray ionization. The collision cell was recently upgrade by mSpec (ON, Canada) to improve the instrument sensitivity in the multiple reaction mode (MRM). Further details of the MS/MS operating parameters can be found in Budakowski and Tomy [5]. Quantitation was based on the ion signal from the *m/z* 640.6 → *m/z* 79 MRM transition. MS/MS detection of d_{18}–HBCD and $^{13}C_{12}$–HBCD isomers was based on the analogous ($[M - H]^-$) → Br^- reaction monitored for the native HBCD (*m/z* d_{18}: 657.6 and $^{13}C_{12}$: 652.4 ($[M - H]^-$)).

Extraction and clean-up of environmental samples. Extraction of HBCD from samples is done by accelerated solvent extraction (ASE). 33 mL ASE extraction cells are packed with ~10 g of dry-ice homogenized biota or ~10 g of freeze dried sediment. Cells are then packed with pre-cleaned Ottawa Sand (600°C, 6hrs) to remove any dead volume. Recovery internal standards consisting of the three isomers of $^{13}C_{12}$-HBCD (10 µL of a 1 ng/µL solution) are spiked into the ASE cell. Lipids are removed from biotic extracts using gel permeation chromatography (GPC). A GPC column packed with BioBeads (SX-3, 60 g) is eluted with 50:50 dichloromethane (DCM):hexane. Two fractions (F1, 2) are collected off the GPC: 150 mL fraction contained the lipids (F1), and F2, 200 mL contained HBCD. Lipid free extracts and the sediment extract are then cleaned-up on a Florisil column (1.2% deactivated, 8 g, 300 mm × 10.5 i.d). Two fractions are collected: F1, 32 mL of hexane contains PBDEs, and F2, 70 mL of DCM:hexane (15:85) contains HBCD. Extracts are then exchanged into methanol and spiked with 5 ng (1 µL of a 5 ng/µL solution) of each d_{18}–HBCD to act as an instrument performance internal standard and then concentrated to a final volume of 200 µL

RESULTS AND DISCUSSION

A.) Recommended Analytical Protocol**.**

(1) <u>*Recovery Internal Standards (RIS).*</u> To monitor the extraction efficiency and losses during the work-up procedure, it is recommended that samples be spiked with all three $^{13}C_{12}$–HBCD standards. We have evidence that suggests there are differences in the extraction efficiencies of the $^{13}C_{12}$–isomers. In sediments from Lake Winnipeg, the recoveries of the $^{13}C_{12}$–α–HBCD isomer (108.7 ± 2.6%, arithmetic mean ± standard error)

was consistently higher than the $^{13}C_{12}$– γ–HBCD isomer (46.6 ± 8.0%) (*n=3*)]. This would imply that native HBCD isomers are also recovered differently from samples. Using all three $^{13}C_{12}$–isomers as RIS and correcting the integrated ion signals of native HBCD isomers in samples with its corresponding $^{13}C_{12}$ surrogate, should reduce some of the uncertainty in reporting concentrations.

(2) <u>*Instrument performance internal standards (IPIS*).</u> An often overlooked phenomenon in LC/MS is suppression (or less common, enhancement) of the analyte ion signal as a results of matrix effects [7,8]. Isotopically labelled internal standards, which coelute with the analyte of interest, can account for the ion suppression (and enhancement) of an analyte; diluting the final volume of extracts is also another means of reducing or eliminating matrix effects. The three IPIS used in our laboratory for the analysis of HBCD (*i.e.*, d_{18}–α, β and γ–HBCD) performs a dual role: (*i*) it accounts for instrumental fluctuations of the MS between injections and, (*ii*) accounts for effects arising from the matrix. The ion signal from the IPIS is used to correct both the signals of the corresponding recovery internal standard ($^{13}C_{12}$–isomers) and that of the native HBCD isomer. A systematic study on the potential matrix effects on ion intensity of individual HBCD isomers is currently underway in our laboratory.

B.) Standard Reference Material (SRM) for fish.
SRM are invaluable for laboratory quality control purposes. A natural SRM for the analysis of biota samples for HBCD is that of lake trout homogenate material currently in use for PBDEs analysis (*Cerilliant* – lake trout). When analyzed in our laboratory, lipid normalized concentrations of α– and γ–HBCD isomers in this material were 39.6 and 39.8 ng/g, respectively. The β–isomer was below detection limits. Interlaboratory measurements are urgently needed to validate levels of HBCD in this material. In any event, laboratories should endeavour to include this material in their analytical protocol as this will provide a measure of their laboratories precision.

CONCLUSIONS

The advent of newly synthesized isotopically labelled HBCD standards will circumvent some of the inherent problems often encountered in LC/MS and account for differences in the extraction efficiencies amongst HBCD isomers. Further interlaboratory calibration exercises are warranted to quantify/validate HBCD levels in the lake trout – SRM, as this, along with the isotopically labelled HBCD standards, will ultimately improve our confidence in measuring environmental concentrations of HBCD in biota.

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