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# CONGENER-SPECIFIC ANALYSIS OF HEXABROMOCYCLODODECANE (HBCDD) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ELECTROSPRAY TANDEM MASS SPECTROMETRY

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## Introduction

Hexabromocyclododecane (HBCDD) belongs to the class of cycloaliphatic brominated flame retardants (BFRs) and is used primarily in the building industry as a thermal insulator additive in polystyrene foam[1,2,3]. Secondary uses are in upholstery textiles[2]. HBCDD is synthesized industrially by the addition of bromine to *cis-trans-trans*-1,5,9-cyclododecatriene[2]. The resulting technical mixture contains three diastereoisomers (α, β, γ) existing in proportions of approximately 6, 8 and 80 %, respectively, together with other lower brominated impurities[2].

Recent reports suggest that usage of HBCDD is increasing[1]. In Europe, HBCDD is already being used to replace other BFR formulations, namely the polybrominated diphenyl ethers (PBDEs)[4]. The physical chemical properties of HBCDD are similar to those of BDEs and other persistent organic pollutants, including polychlorinated biphenyls (PCBs), which are known to be persistent, bioaccumulative and toxic[1,3,5,6].

At an international workshop on brominated flame retardants, the need for more reliable analytical measurements on HBCDD was highlighted[7]. Current methods are all based on gas chromatography (GC)[3,7]. Because the congeners of HBCDD are thermally labile, elution from a capillary GC column usually results in a broad, diffuse peak[8]. In addition, the high GC-oven and injector temperature are also thought to cause interconversion among isomers thus precluding congener-specific analysis[7,8].

To investigate whether high performance liquid chromatography (HPLC) might be a useful tool for the congener specific analysis of HBCDD, we developed in this study a method based on HPLC-coupled with electrospray (ES) introduction-tandem mass spectrometry.

## Methods and Materials

***Chemicals.*** The γ-HBCDD congener (1R,2R,5R,6S,9S,10R) was purchased from CIL (Andover, MA, USA). The α-HBCDD (1R,2R,5S,6R,9R,10S) and β-HBCDD congeners (1S,2S,5R,6S,9R,10S) were a kind gift from Dr. Tom Harner (Meteorological Services of Canada, Downsview, ON, Canada).

***Liquid Chromatography***. Two analytical columns were used: (a) Genesis $C_{18}$ analytical column (5.0 cm × 2.1 mm i.d., 4 μm particle size; Chromatographic Specialties Inc., Brockville, ON, Canada) and (b) a Vydac 201TP54 reversed-phase column (15 cm × 4.1 mm i.d , 5 μm particle size; Mandel Scientific Ltd., Guelph, ON, Canada) was used. With the Genesis column a mobile phase methanol/water was used, while an acetronitrile/water mobile phase was used on the Vydac column.

***Mass Spectrometry.*** A Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) was used in the ES negative ion mode. Infusion experiments utilized the built-in Harvard syringe pump with a flow rate of 10 µL/min. The Q1 scan range was m/z 630-650. Source parameters were as follows: ionspray voltage, –4000 V; curtain gas flow, 25 a.u. (arbitrary units); sheath gas flow, 35 a.u.; turbo gas flow and temperature, 30 a.u. and 500 oC, respectively; declustering potential, –5.0 V. For MS acquisition, Q1 was operated with unit resolution with a scan time of 0.5 seconds. All gas supplies were provided from a nitrogen generator (Peak Scientific, Renfrewshire, Scotland, UK)

MS/MS detection used MRM conditions for the m/z 640.6 ($[M - H]^-$) $\rightarrow$ $Br^-$ reaction (both isotopes), utilizing unit resolution on the first and third quadrupoles and a 200 ms dwell time. Collision activated dissociation gas pressure was 8 a.u. and the collision energy was –50 eV.

**Results and Discussion**

***Elution from HPLC.*** Efforts were made to separate the three isomers of HBCDD from both HPLC columns (Figure 1). The best chromatography off the Genesis $C_{18}$ column was achieved using a 70:30 methanol:water and increasing to 100% methanol in 3 minutes (flow-rate 300 µl/min). Under these conditions, the α-isomer was adequately separated from the other two, while the β- and γ-isomers were sufficiently resolved at half their peak heights. Baseline separations of all three isomers was achieved using an isocratic mobile phase of acetronitrile:water (80:20) off the Vydac column (flow-rate 1 mL/min).

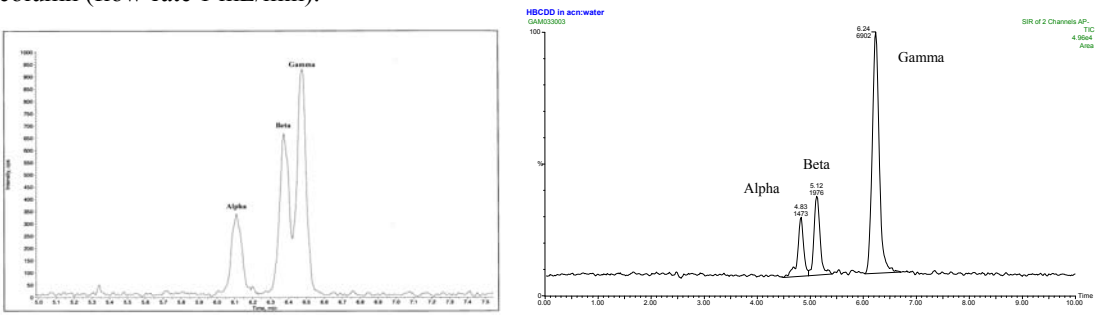


**Figure 1**. Elution of α, β, γ-HBCDD isomers off a Genesis $C_{18}$ (left) and a Vydac column.

***Optimization of MS conditions***. As a starting point for optimization, a Q1 scan with a range of m/z 630-650, for injection of a 200 pg/µL γ-HBCDD methanol solution, was recorded using multiple count addition (MCA) mode. Figure 2 shows the Q1 spectrum of γ-HBCDD after an accumulation of 20 MCA scans.

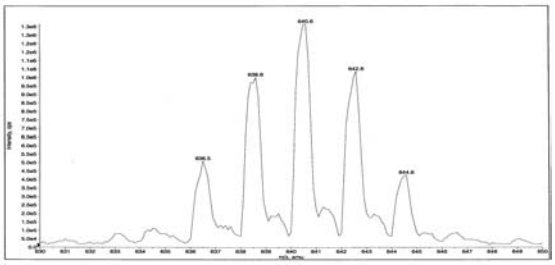

**Figure 2**. Q1 mass spectrum (accumulation of 20 MCA scans) of γ-HBCDD (200 pg/µL in methanol, 10 µL/min infusion flow)

Many peaks are present, with the dominant m/z 640.6 peak corresponding to the $[M - H]^-$ ion ($^{12}C_{12}H_{17}{}^{79}Br_3{}^{81}Br_3$, calculated mass 640.6 Da). The other peaks result from other bromine isotopic contents and $^{13}C$ contributions. When the ES results were compared to a similar experiment utilizing an atmospheric pressure chemical ionization (APCI) source (which displayed a weak intensity m/z 642 peak) it was found that the ES intensities were greater. Thus, the ES source was used for all further experiments.

Following the detection of the $[M - H]^-$ ion, the selected ion monitoring (SIM) mode was employed to optimize the Q1 parameters. The signal is greatest at low values (0 – 15 V) of the declustering potential. The ionspray voltage caused one of the largest variations in signal intensity. A drastic decrease in the ion signal was observed upon reducing the potential from –4500 V to –1500 V.

Once the source parameters were optimized, the product ion scan for the *m/z* 640.6 precursor (which yielded $Br^-$ as the only fragment ion) was recorded. Its yield was strongly influenced by the collision voltage setting, decreasing above 50 V. By utilizing the MS/MS capabilities of the instrument, multiple reaction monitoring (MRM) mode was optimized for γ-HBCDD for the *m/z* 640.6 → 79 and → 81 reactions. For quantitation purposes, the MRM signal from the *m/z* 640.6 → 79 transition was used.

The optimum temperature of the turbo gas was about 500 ˚C even though HBCDD is thermally labile, the primary reason it is difficult to detect via GC (Figure 3). However, the nature of the ES source allows the solvent to successfully 'cushion' the HBCDD allowing it to be ionized and enter the mass spectrometer, despite the high temperature. Perhaps too, the transit time through the 'hot-zone' is sufficiently short that enough heat is not transferred to HBCDD to cause thermal decomposition. In fact, the highest signal was observed with a source temperature between 500-550 ˚C. It should be noted that this increase in signal for the MRM transition is not due to temperature induced in-source fragmentation and subsequent detection of $Br^-$. The MRM method operates by monitoring of a specific parent to fragment reaction, thus the parent must enter the first quadrapole in order for the transition to be recorded. Any in-source fragmentation would lower the yield of the parent ion, thus reducing the overall signal associated with the transition.

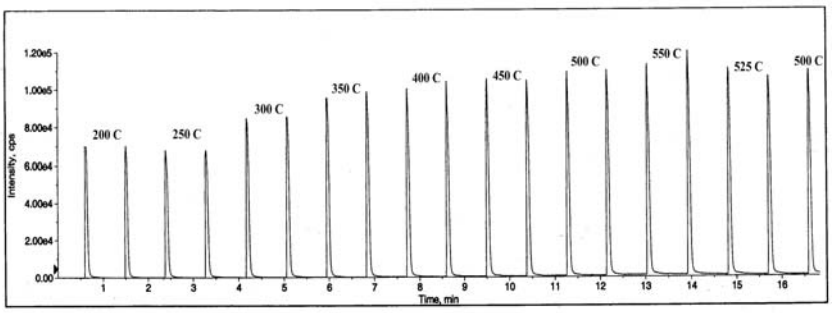


**Figure 3.** Flow injection analysis experiments on γ-HBCDD with different turbo gas temperatures (all other parameters constant). Each experiment was run twice to ensure temperature stability

***Quality Parameters***. Injection of γ-HBCDD standards ranging from 10 to 500 pg on-column produced a linear relationship up to 250-300 pg ($r^2$>0.998). Above 300 pg the MRM peak areas

began to plateau. The limit of detection (LOD) on column (S/N=3) was determined to be 4–6 pg. Inter-run variability was tested with injections of both 113 pg and 170 pg samples on column. Run-to-run and day-to-day (n=3) variability was minimal, with relative standard deviations of 2.6–4.1% and 2.4–4.4%, respectively. Sample blanks run using the above parameters displayed no signal, and sample carryover was not observed.

***Application of the method***. The analytical method is currently being applied to biotic and abiotic samples from the Great Lakes. These results are being presented at the meeting.

**Acknowledgements**
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