RAPID SEPARATION OF HBCDD ENANTIOMERS USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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INTRODUCTION

The brominated flame retardant hexabromocyclododecane (HBCDD) is commonly monitored for its presence in the human population as well as the environment. HBCDD shows a complex stereochemistry, with six stereogenic centers. The three most commonly detected forms of HBCDD are the α-,β- and γ- diastereomers, each comprised of a +/- enantiomer pair (Figure 1). Here we describe a rapid method using supercritical CO_2 with an organic co-solvent to separate the three most abundant HBCDD enantiomer pairs on a chiral cellulose column. The presented method offers an advantage over existing LC methodolgies by increasing through put time and decreasing solvent usage whilst maintaining the resolution required for quantification.

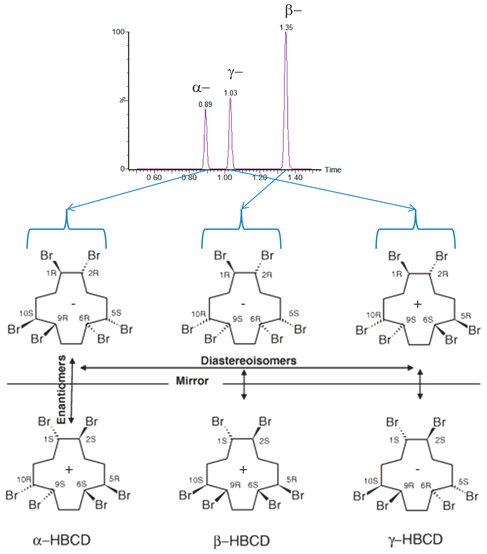

Figure 1. Achiral separation and structures[1] of most common occurring HBCDD diastereomer and their respective enantiomers. Chromatographic separation was achieved using super-critical fluid CO_2[2] with methanol co-solvent on a HSS C18 SB column.

METHOD DEVELOPEMENT

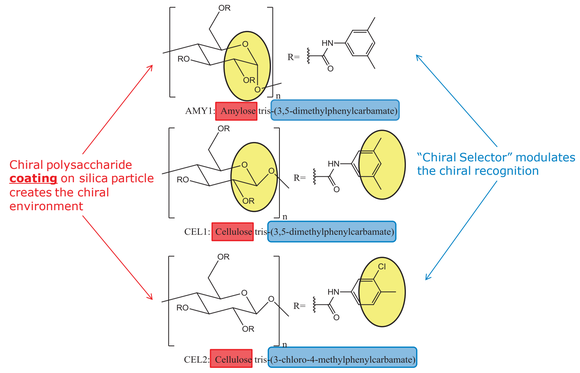

Figure 2. Chiral stationary phases used in this study. Column dimensions were 2.1x150 mm with 2.5µm particle diameters.

Method development was performed on three chiral stationary phases (Figure 2). CEL-2 was found to provide the optimum separation. Table 1 highlights the screening approach and resulting optimum conditions. Table 2a shows the MS/MS MRM method and 2b the final chromatography method.

Table 1: Optimization parameters for supercritical fluid CO_2 method implemented on all three column chemistries. Mobile phase and column temperature had the most significant impact on HBCDD enantiomer separations.

Optimization Parameter	Conditions	Optimum Result
Co-solvent	Methanol, Acetonitrile, Isopropanol and Ethanol	**Isopropanol**
Column Temperature (°C)	5-60 (increments of 5)	**55**
Flow Rate (mL/min.)	1-2.5 (increments of 0.25)	1
ABPR Pressure (psi)	1500-2500 (increments of 500)	2500

Table 2a: MRMs optimized in previously developed achiral method[2].

Compound	Precursor (m/z)	Product (m/z)	Dwell (s)	Cone (V)	Collision (V)
HBCDD	640.6	78.9	0.05	30	20
HBCDD	640.6	80.9	0.05	30	20

Table 2b: Final chromatographic gradient.

Time (min)	%A (CO_2)	%B (Isopropanol)
Initial	90	10
3	70	30
3.5	70	30
3.6	90	10
6	90	10

RESULTS AND DISCUSSION

The final method was developed first using commercially available α-,β- and γ- HBCDD diastereomers (Figure 3a). Individual enantiomer standards, isolated using a permethylated β-cyclodextrin stationary phase by liquid chromatography, were injected in order to establish enantiomer elution order (Figure 3b). RSDs of peak areas were measured across 9 injections of mixed standard (Table 3)

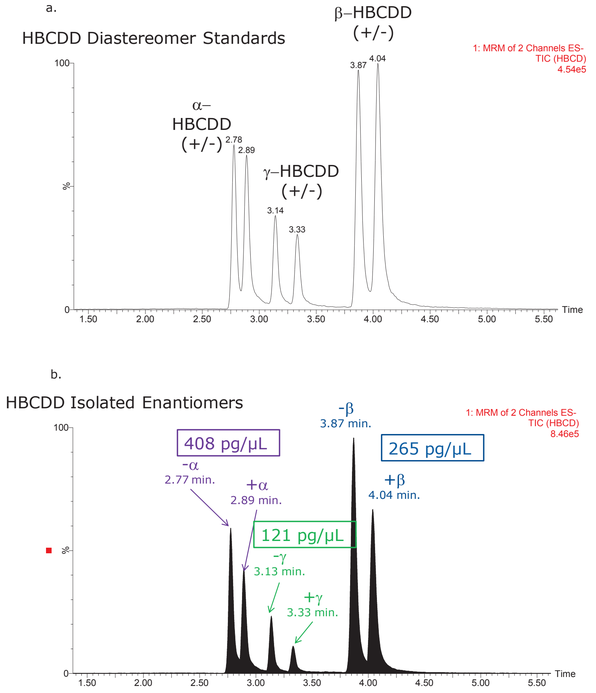

Figure 3. Enantiomer separation using supercritical CO_2 results in the same general elution order as in the achiral separation using the same approach. Separation was first seen using the developed method in mixed diastereomer standards (a), then separated enantiomer standards (b). Enantiomer separation was performed using liquid chromatography fraction collection. An injection volume of 1µL was used for all experiments.

Table 3: RSDs across 9 injections of a 100 pg/µL standard for all enantiomers.

Compound	Number of Samples	Mean	Std.Dev.	%RSD	Bias
- alpha HBCDD	9	2198.1358	98.1031	4.4630	2198.1358
+ alpha HBCDD	9	2230.4431	104.6355	4.6912	2230.4431
- gamma HBCDD	9	944.8965	43.9056	4.6466	944.8965
+ gamma HBCDD	9	970.3125	48.9067	5.0403	970.3125
- beta HBCDD	9	1372.6246	59.6799	4.3479	1372.6246
+ beta HBCDD	9	1373.0282	60.1151	4.3783	1373.0282

Whale blubber samples which had been prepared for a previous brominated flame retardant analysis[3] were analyzed and quantified against a solvent standard calibration curve. Ranges of concentration were found between 5.6 to 38.5 pg/µL for -α and 7.4 to 31.4 pg/µL for +α enantiomers. Enantiomeric fractions (EF) were determined for these detections based on chromatographic peak area (Figure 4). Variable trends reflect findings in previous studies[4]. Further analysis in various biological and abiotic samples could more rapidly be executed utilizing the rapid method presented in this work.

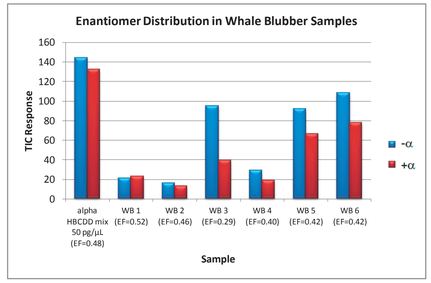

Figure 4: EFs for a-HBCDD in standard and whale blubber (WB) samples indicating slightly higher presence of -a, though further sample points are required.

CONCLUSIONS

- **A rapid and efficient method for separating HBCDD enantiomers is achieved using supercritical fluid CO_2 chromatography**
- **Sample analysis throughput could be greatly increased using this method**
- **Supercritical CO_2 chromatography requires the use of significantly less solvent than lengthy chiral LC methods, reducing costs and laboratory waste**

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