RAPID SEPARATION OF HEXABROMOCYCLODODECANE DIASTEREOMERS AND TETRABROMOBISPHENOL-A USING A NOVEL METHOD COMBINING CONVERGENCE CHROMATOGRAPHY AND MS/MS DETECTION

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Introduction

The brominated flame retardants hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBPA) are compounds which are monitored for their presence in the human population as well as the environment (1). The ability to resolve the various HBCD isomers from one another is an important facet in the analysis of this compound, due to the differences in isomeric distribution in biota, abiotic systems and technical formulations (2). Separation of the three α–, β–, and γ– diastereomers of HBCD and TBBPA can be achieved using reversed phase liquid chromatography (RP-LC) and this is currently the method of choice (3,4). Convergence chromatography (CC) is a chromatography technique based on the use of a supercritical fluid and has shown to have enhanced efficiency due to higher diffusivity and lower viscosity of supercritical fluid compared to liquids (5), making it well suited to isomeric separation. The development of a CC method that analyzes these two BFR's also offers the advantage of lower solvent usage, as well as the ability to inject a variety of solvents that are not typically compatable with RP-LC analysis. The latter feature has the potential to remove the time consuming solvent exchange step that accompanies many sample preparation procedures.

Here we describe a method using supercritical CO_2 and methanol to baseline separate the three most abundant HBCD diastereomers and TBBPA within a three minute run time using an HSS C18 1.8 µm particle size column. A tandem quadrupole mass spectrometer using negative mode electrospray ionization was used for detection, operating in multiple reaction monitoring (MRM) mode. Ionization was enhanced by the addition of a make-up flow, which is introduced to the post-column effluent. Method limit of detection (LOD) and limit of quantification (LOQ) for α–, β–, and γ– HBCD were based on peak-to-peak signal to noise ratios of greater than 3 or 10, respectively. The LOD for all HBCD diastereomers was 100 fg/µl, and LOQs 500 fg/µl for α– and γ– HBCD and 250 fg/µl for β–HBCD. In order to test the efficacy of this method, a small subset of complex human serum extracts were analyzed, and the detection of a sub-pg/µl concentration of α–HBCD was made.

Materials and methods

Convergence Chromatography Conditions

We performed the method optimization and analysis of samples on a Waters Ultra Performance Convergence Chromatography System (UPC^2). The total run time was 3 minutes. Upon finalization of method development, we used a High Strength Silica (HSS) C18 SB 1.8 µm particle size 3.0 x 100 mm column and operated at 40 °C. The sample temperature was maintained at 10 °C and a 1 µl injection volume was taken for all standards and samples. We used the following gradient conditions, with CO_2 as phase A and methanol as phase B.

Gradient:

Time (min.)	% A	% B	Flow Rate (mL/min.)
Initial	98	2	2
2	90	10	2
2.01	90	10	2.5
2.3	98	2	2.5
2.31	98	2	2

A Waters 515 HPLC pump was used to introduce a 0.2 mL/min. make-up flow of 0.1% ammonium hydroxide solution in isopropanol (IPA). This flow was introduced to the post column effluent prior to introduction into the MS.

MS Conditions

MS detection was performed on a Waters Xevo TQ-S operating in MRM mode. Electrospray ionization (ESI) in the negative mode was used. The optimized capillary voltage was found to be 2.0 kV. The source temperature was 150 °C, desolvation temperature 500°C, cone gas 150 L/hr and desolvation gas 1000 L/hr. MRM mode was used, with the following transitions for each compound studied.

Compound	Parent (m/z)	Daughter (m/z)	Dwell Time (s)	Cone (V)	Collision (V)
HBCD	640.6	78.9	0.05	30	20
HBCD	640.6	80.9	0.05	30	20
TBBPA	542.5	78.9	0.05	30	50
TBBPA	542.5	80.9	0.05	30	50
TBBPA	542.5	419.5	0.05	30	40

Results and discussion

Rapid chromatographic separation of α–, β–, and γ– HBCD and TBBPA in a single injection was achieved using CC. The final run time achieving full separation was three minutes, compared to typical RP-LC run times of at least 10 minutes. Figure 1 compares the chromatograms of the HBCD isomers at 100 pg/µl using UPLC versus CC. As can be seen in the figure, the CC separation surprisingly results in a different elution order than that seen using a C18 column in LC conditions. This selectivity could be used as an additional confirmation of isomeric identification when used in conjunction with LC analysis.

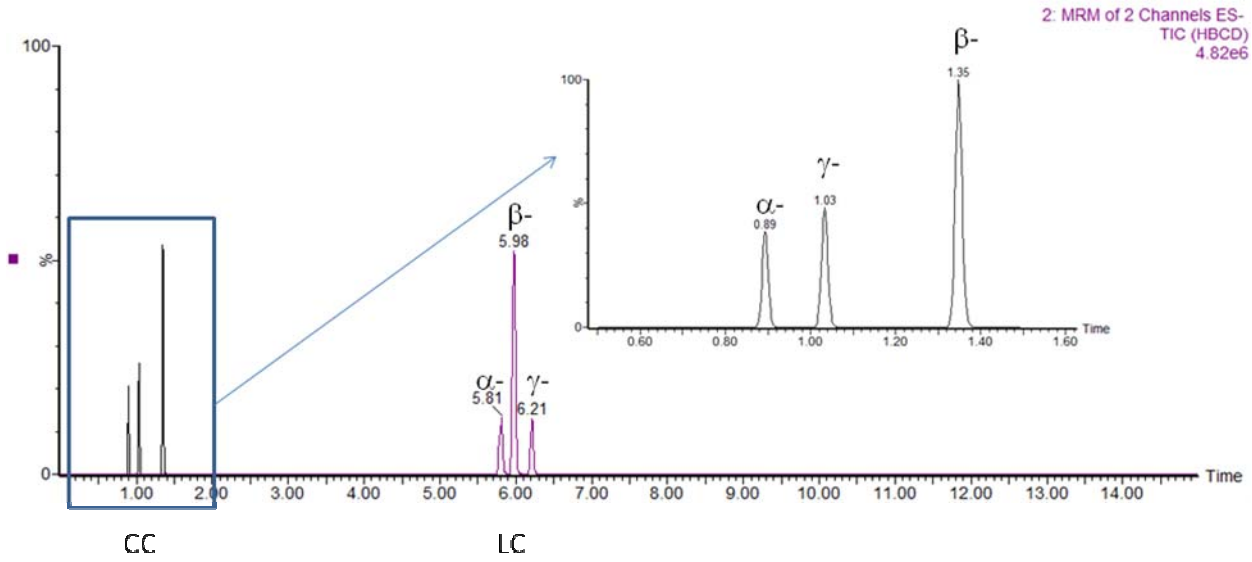

Figure 1: Overlaid chromatograms of CC and LC separations of α–, β–, and γ– HBCD illustrating the enhanced speed of chromatographic resolution afforded in this method. In the case of the CC separation, 1 µl of a 100 pg/µl toluene solvent standard was injected, while the LC injection used a 10 µL injection of a100 pg/µl methanol solvent standard.

Three columns with different chemistries were screened during method optimization under both isocratic versus gradient conditions. A HSS C18 column, Ethylene Bridged Hybrid (BEH) and BEH 2-ethylpyridine (BEH 2-EP) columns were investigated for their ability to effectively resolve the three HBCD isomers studied, as well as TBBPA. Chromatographic peak tailing of standards was also assessed as an additional parameter for column suitability in which the peak symmetry at 5% of peak height was measured. The HSS C18 column was found to be the optimum column for this method based on quality of resolution and minimum peak tailing of HBCD diastereomers. Additional modifications to the gradient method were made to enhance the signal of TBBPA.

MS detection of the HBCD isomers was enhanced by the use of a make-up flow, which adds additional solvent and basic additive in the form of 0.1% ammonium hydroxide solution in isopropanol. Other ionization techniques were investigated including atmospheric pressure chemical ionization (APCI) and electrospray chemical ionization (ESCi™), both operating in the negative mode. For the comparison of ESI, APCI and ESCi™ modes, the $[M-H]^-$ parent ions were monitored, with the most intense signal for HBCD diastereomers achieved using ESI.

Linearity of the HBCD calibration curves using solvent standards were good, with r^2 values > 0.995 for standards ranging from 0.5 pg/µl to 100 pg/µl for α–HBCD and γ–HBCD, and 0.25 pg/µl to 100 pg/µl for β–HBCD. Reproducibility across five injections at each point was also assessed. To demonstrate the utility of the method for the analysis of biological samples, a small subset of human serum extracts were screened and a sub-pg/µl identification of α–HBCD was made. The presence of this analyte was confirmed by the conservation of the expected ratio between the two MRM transitions (Figure 2); β– and γ–HBCD isomers were present but below the LOD. This distribution confirms expected isomer occurrence in biotic samples, and also provides a basis for the continued study of CC to analyze complex samples for HBCD isomers. Future analyses are intended to include a range of environmental, human and other biotic extracts.

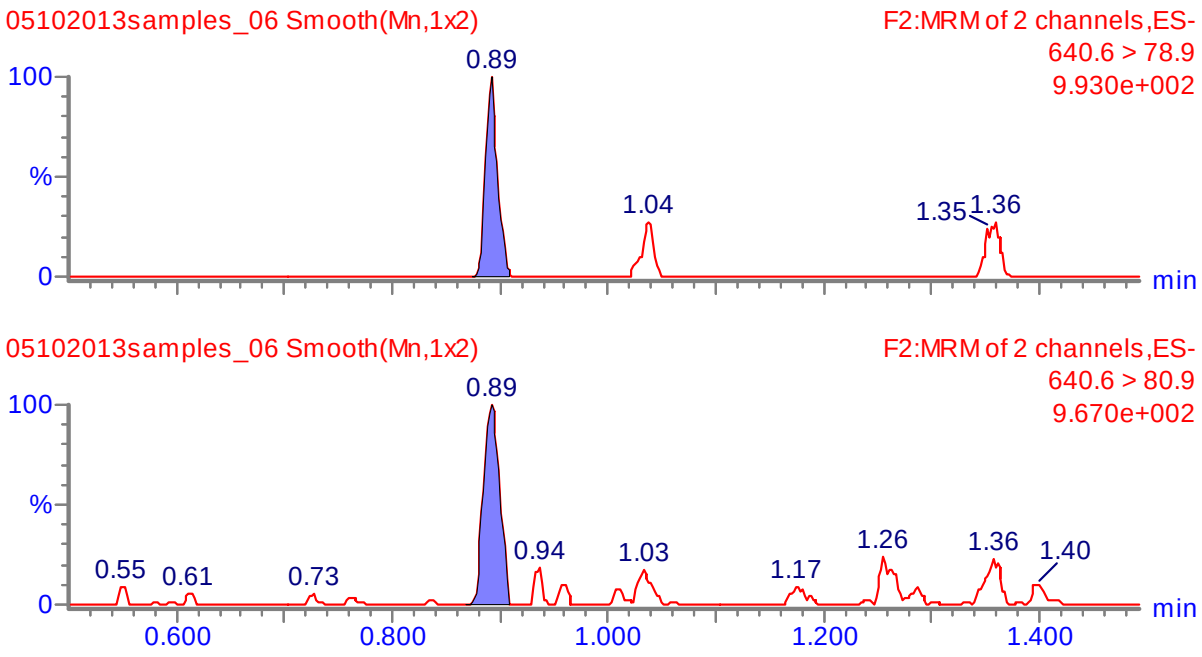

Figure 2: Processed MRMs of α–HBCD identified in human extracts. The quantification transition (640.6 > 78.9) is the top chromatogram, and confirmatory transition (640.6 > 80.9) on the bottom.

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