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Determination of ten hexabromocyclododecane diastereoisomers using two coupled reversed-phase columns and liquid chromatography/tandem mass spectrometry

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RATIONALE: Hexabromocyclododecanes (HBCDs) are raising concern due to their potential persistence, bioaccumulation and toxicity. Apart from the widely reported isomers α-, β-, γ-HBCD, other HBCD diastereoisomers such as δ-HBCD have been also recently found in environmental media and biota. These newly reported diastereoisomers might give more insight into the degradation and biotransformation of HBCDs.
METHODS: A reversed-phase C_{18} column coupled to a C_8 column was used to improve the chromatographic resolution. A gradient mobile phase consisting of methanol, acetonitrile, and water, as well as tandem mass spectrometry parameters, were optimized. Ten HBCD diastereoisomers were finally determined by liquid chromatography/tandem mass spectrometry in multiple reaction monitoring mode with negative electrospray ionization.
RESULTS: Eight of the ten HBCDs could be chromatographically separated by using the coupled reversed-phase columns. Results of the method validation indicate high reproducibility and good sensitivity. The limit of detection ranged from 0.4 to 0.8 pg, and the relative standard deviations of intra- and inter-day injections ranged from 1.8 to 5.1% and from 2.7 to 9.5%, respectively. The developed method was further applied to the analysis of HBCDs in HBCD commercial products and soil samples. δ-, η-, θ-, and ε-HBCD were detected in commercial products and soil samples.
CONCLUSIONS: The present study revealed that δ-, η-, θ-, and ε-HBCD might occur ubiquitously in environmental media and biota. These newly reported diastereoisomers may help us to better understand the fate and transformation of HBCDs in the environment. Copyright © 2014 John Wiley & Sons, Ltd.

Hexabromocyclododecanes (HBCDs), the third largest group of brominated flame retardants in production volume, are mainly used as additives in extruded and expanded polystyrene foam insulation, upholstery textiles, and electronic equipment.[1,2] Their applications have increased as an alternative since the production and use of penta- and octa-bromodiphenyl ethers were banned in Europe and North America.[3] At present, HBCDs have become ubiquitous contaminants. They have been detected in various environmental media, biota, and humans.[3–5] Because of their toxicity, persistence, and tendency for bioaccumulation, HBCDs are classified by the European Union Chemicals Management Regulations (REACH) as substances of very high concern, and are currently assessed for inclusion in the Stockholm Convention.[6,7]

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Theoretically, HBCDs have 16 stereoisomers, which comprise 6 pairs of enantiomers and 4 meso forms with different axial–equatorial substitution patterns around the ring.[8] Technical-grade HBCD mixtures mainly consist of α-HBCD, β-HBCD, and γ-HBCD. However, two minor diastereoisomers, δ- and ε-HBCD, which occur at very low relative ratio of total HBCDs, have been reported recently.[8] δ-HBCD was also found in fishes in the UK and air particles in China,[9,10] and ε-HBCD was found in sediment samples in China.[11] Harrad *et al.*[9] firstly detected δ-HBCD in 13 fish samples from 9 English lakes, the relative abundance of δ-HBCD to total HBCDs ranged from 1.0 to 11%. Li *et al.*[10] detected δ-HBCD in all air particle samples in Shanghai City, and discovered that the relative contribution of δ-HBCD to total HBCDs was 2.6–15%. Evidently, the relative abundances of δ-HBCD in fish and air samples exceed the reported values for technical-grade HBCD mixtures (0.5%).[8] However, the mechanism of transformation among HBCDs is still unclear. Harrad *et al.*[9] attributed the occurrence of δ-HBCD to biotransformation in fish samples on the basis of its absence in ambient water and sediment. Furthermore, some studies showed that isomerization of HBCDs could

occur during thermal exposure.[12–15] Heeb *et al.*[15] found that β-HBCD could probably form small amounts of δ-HBCD and other minor diastereoisomers during thermal treatment. Therefore, minor HBCD diastereoisomers might be useful in tracing their transformation process.

Currently, liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) is the preferred technique for HBCD analysis because of thermal rearrangement of HBCDs at temperatures above 160 °C and thermal decomposition of HBCDs at temperatures above 240 °C.[16] Many studies have focused on the optimization of analytical methods for HBCDs.[10,17–21] However, most of such studies detected only α-, β-, and γ-HBCD, and only a few developed method for δ- and ε-HBCD. Li *et al.*[10] used two different mobile phases to quantify the five HBCDs α-, β-, γ-, δ-, and ε-HBCD. All reported methods could not achieve baseline chromatographic separation of the above five HBCDs. Thus, it is necessary to develop a new method for simultaneous determination all ten HBCD diastereoisomers to understand better the source, distribution, and fate of HBCDs.

In the present study, we developed a new LC/MS/MS method for analysis of ten HBCD diastereoisomers using two coupled reserved-phase columns. The mobile phase and MS/MS parameters were optimized. The method was validated and applied to the determination of HBCDs in commercial HBCD products and in soil samples. To the best of our knowledge, this is the first work to report the occurrence of δ-, η-, θ-, and ε-HBCD in soils.

EXPERIMENTAL

Chemicals and solvents

The HBCD diastereoisomers α-, β-, γ-, δ-, ε-, ζ-, η-, θ-, ι-, and κ-HBCD (50 mg/L in toluene), as well as deuterated HBCD diastereoisomers (50 mg/L in toluene) and $^{13}C_{12}$-labeled HBCD diastereoisomers (50 mg/L in toluene), were purchased from Wellington Laboratories (Guelph, ON, Canada). All solvents used in extraction and analytical procedures were HPLC grade. Hexane, methanol, acetonitrile were obtained from Merck (Darmstadt, Germany). Dichloromethane and acetone were purchased from J.T. Baker (Phillipsburg, NJ, USA). Three HBCD commercial products were purchased from manufacturers in China.

Sample collection

In August 2009, three soil samples were collected in a public park in Weifang City, Shandong Province. Each of the surface soil samples was collected by a pre-cleaned stainless steel scoop and was constituted by five subsamples. Before extraction, all soil samples were freeze-dried and homogenized after removal of pebbles, weeds and sticks. After being screened through a 1-mm sieve, the samples were stored in brown glass bottles at –20 °C until analysis.

Sample extraction and preparation

The procedures used for sample extraction and cleanup have been described in detail elsewhere.[22,23] Briefly, soil samples (5 g) were spiked with $^{13}C_{12}$-α-, β-, γ-HBCD (12 ng) and then Soxhlet extracted in a mixture of acetone/n-hexane (1:1, v/v) for 48 h. Activated copper powder was added in the solvent to remove elemental sulfur. The concentrated extracts were cleaned on multilayer silica/alumina columns which composed of 6 cm of aluminum, 2 cm of silica gel, 5 cm of basic silica gel (3:1 silica gel: 1M NaOH, w/w), 2 cm of silica gel, 8 cm of acid silica gel (1:1 silica gel: sulfuric acid, w/w), and 2 cm of anhydrous Na_2SO_4 from the bottom to the top. The HBCDs were eluted with 70 mL hexane/dichloromethane (1:1, v/v). The eluent was evaporated and dried by a nitrogen steam and then reconstituted in 300 μL of methanol. HBCD commercial products were dissolved in methanol and the final concentrations were 250 μg/L. The final concentrations at 40 μg/L deuterated α-, β-, γ-HBCD were added in the soils and HBCD commercial products as internal standards.

Instrumental analysis

Liquid chromatography

An 1100 series HPLC system (Agilent, Palo Alto, CA, USA) consisting of a vacuum degasser, a quaternary pump and an autosampler was used. HBCD diastereoisomers were separated using an Eclipse Plus-C18 reversed-phase column (250 mm × 4.6 mm i.d., 5 μm; Agilent) coupled to a Zorbax Eclipse XDB-C8 reversed-phase column (150 mm × 4.6 mm i.d., 5 μm; Agilent). The gradient mobile phase consisted of methanol (A)/acetonitrile (B)/Water (C). The flow rate was set at 0.5 mL/min. The gradient program was as follows: an initial composition of 3:87:10 A/B/C (v/v) was maintained for 25 min and changed linearly to 100% B in 1 min and was held for 9 min, followed by a return to the initial composition in 3 min. The column was equilibrated for a further 10 min.

Mass spectrometry

The quantification of the ten HBCD diastereoisomers was performed with a API 4000 (Applied Biosystems, Foster City, CA, USA) triple quadrupole mass spectrometer equipped with a TurboIonSpray ionization interface running in electrospray ionization negative ion mode with multiple reaction monitoring (MRM) for $[M–H]^- \rightarrow Br^-$ (*m/z* 640.7 → 78.8 for HBCDs). The unit resolution and a 200 ms dwell time per transition were used. Details of the optimized MS/MS parameters are listed in Table 1.

RESULTS AND DISCUSSION

Optimization of chromatographic resolution

Previous studies using LC/MS/MS for HBCD analysis have reported that organic solvents such as methanol and acetonitrile might affect the retention time.[22] For example, β-HBCD was retained in the C_{18} reversed-phase column longer when methanol was used as the mobile phase than when acetonitrile was used.[22] Therefore, the elution order of the ten HBCD diastereoisomers was investigated by using three different mobile phases.

Retention times and LC/MS/MS chromatograms of the ten HBCD diastereoisomers obtained by using the two mobile phases are presented in Table 2 and Fig. 1, respectively. We

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Table 1. Optimized MS/MS parameters for the determination of HBCD diastereoisomers

Parameter	Optimized value
Source temperature, TEM (°C)	400
Ionization voltage (V)	–4500
Ion source (GS1) settings	50
Ion source (GS2) settings	55
Curtain gas settings	10
CAD gas settings	25
Declustering potential (V)	–70
Entrance potential (V)	–10
Collision energy (V)	–50
Collision cell exit potential (V)	–2

Table 2. Retention times (min) of ten HBCD diastereoisomers in different mobile phases

	Methanol/water (9:1, v/v)	Acetonitrile/water (9:1, v/v)	Gradient mobile phases in this study
δ-HBCD	23.6	16.4	16.8
ι-HBCD	22.7	17.0	17.2
α-HBCD	22.7	17.1	17.3
ζ-HBCD	23.8	17.1	17.3
β-HBCD	27.0	18.3	18.7
η-HBCD	29.0	19.2	19.5
κ-HBCD	28.4	20.3	20.8
θ-HBCD	29.1	22.0	22.7
γ-HBCD	29.7	23.7	24.3
ε-HBCD	29.1	24.5	25.1

can see that the effects of different mobile phases on the separation of the ten HBCDs are remarkable. With the methanol/water mobile phase, only six HBCD diastereoisomers could be separated, whereas α-HBCD could not be separated from ι-HBCD and δ-HBCD could not be separated from ζ-HBCD. Meanwhile, η-, θ-, and ε-HBCD had very similar retention times. However, eight HBCD diastereoisomers could be completely separated and only α-HBCD could not be separated from ζ- and ι-HBCD when methanol was replaced by acetonitrile. Also, the elution orders of the ten HBCDs varied with the mobile phases. With the methanol/water mobile phase, the initial chromatographic peak coeluted with the peaks for α- and ι-HBCD, and the final peak was for γ-HBCD. And with the acetonitrile/water mobile phase, the first peak and the last peak were δ-HBCD and ε-HBCD, respectively. In summary, the retention times of HBCDs were significantly altered by modifying the mobile phases. This result is also confirmed by the findings of Riddell *et al.*[24] On the basis of these observations, a gradient composition (listed in the Experimental section) based on methanol, acetonitrile, and water was established.

Another important parameter relevant to chromatographic resolution is the column efficiency and peak capacity. Theoretically, the column efficiency and peak capacity have a highly linear relationship with column length.[25] However, because of the high backpressure generated due to the packing procedure, preparing a longer column is difficult. Thus, several studies attempted to use serial coupled columns to increase the separation efficiency.[26,27] In the present study, we used one C_8 reversed-phase column coupled to one C_{18} reversed-phase column to improve the resolution of the ten HBCD diastereoisomers. To verify the advantage of the new method, we compared its separation efficiency with that of the method in our previous study.[22] As indicated in Fig. 2, the developed method exhibited significantly higher

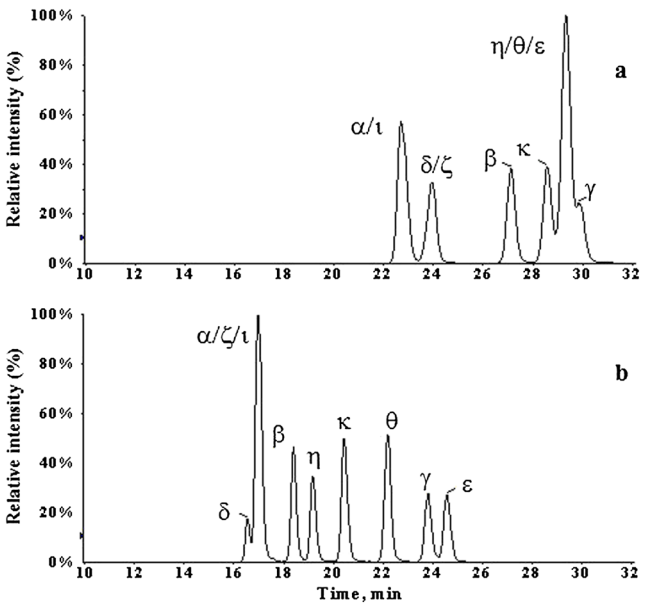

Figure 1. LC/MS/MS chromatograms of ten HBCD diastereoisomers using different mobile phases: (a) methanol/water (9:1) and (b) acetonitrile/water (9:1).

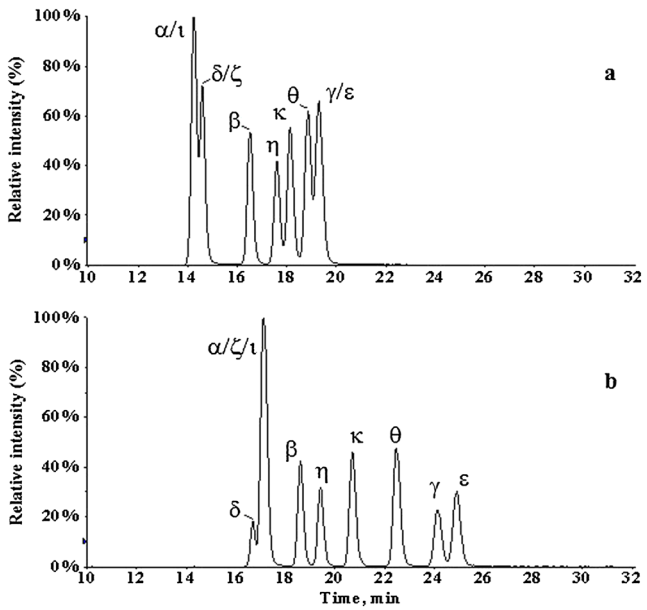

Figure 2. LC/MS/MS chromatograms of ten HBCD diastereoisomers using (a) the method of Yu *et al.*[22] and (b) the method used in this study.

resolution. δ-, γ-, and ε-HBCD were separated, whereas they were found to coelute in our previous study. This improvement might be explained by the effect of the different mobile phases. It was also much more improved than that reported by Riddell *et al.*[24]

Optimization of MS

Optimal parameters for mass spectrometry vary with the mobile phase.[22] Although optimization of the MS/MS parameters for α-, β-, and γ-HBCD was performed in our previous study,[22] MS/MS parameters for the other seven HBCDs were not optimized yet. Thus, MS parameters were optimized in the present study. Flow injection analysis was used to optimize MS/MS parameters for HBCDs. Five runs were done for each parameter set to ensure response stability. Table 1 lists the final optimized MS/MS parameters. Compared with the parameters in our previous study, optimized MS/MS parameters here are unique for each HBCD. As shown in Fig. 3, the source temperature affected the sensitivity of HBCD detection. δ-HBCD produced the highest signal response when the source temperature was 450 °C whereas for ε-, θ-, ι-, and κ-HBCD, the optimum source temperature was 400 °C. Meanwhile, the sensitivity for detecting ζ- and η-HBCD was highest at a source temperature of 350 °C. In our previous study, optimal source temperature for detection of β-HBCD was 400 °C, and that for α-HBCD and γ-HBCD was 300 °C.[22] Except for α-, β- and γ-HBCD, the various diastereoisomers might be present at low environmental concentrations; therefore, the source temperature was set at 400 °C to improve the sensitivity toward these diastereoisomers. Moreover, optimal values for the declustering potential for the ten HBCDs were also different. The optimal value for the declustering potential of δ-, θ-, and ι-HBCD was –70 V while the other seven HBCDs showed the highest signal response when the declustering potential was set at –80 V. No significant difference in the other MS/MS parameters was observed.

Method performance

The performance of the method developed in the present study was evaluated by examining its reproducibility (intra- and inter-day precision) and limit of detection (LOD) (Table 3). The intra-day reproducibility was established by performing six injections of the ten HBCD standard solutions at concentrations of 3 and 10 μg/L. The inter-day reproducibility was established by three repeated injections on six consecutive days. As Table 3 indicates, the method exhibited good reproducibility. The relative standard deviations for intra-day analyses of the ten HBCD diastereoisomers at 3 and 10 μg/L ranged from 1.9 to 3.6% and from 1.8 to 5.1%, respectively. The relative standard deviations for inter-day analyses of the ten HBCD diastereoisomers at 3 and 10 μg/L ranged from 2.8 to 5.7% and from 2.7 to 9.5%, respectively.

The LOD for analysis of the ten HBCDs was defined as a signal-to-noise ratio of 5:1. As indicated in Table 3, the LOD for analysis of the ten HBCD diastereoisomers on column ranged from 0.4 to 0.8 pg. As the concentrations of α-, β-, and γ-HBCD in environmental samples were much higher than those of other HBCDs, we used two calibration curves based on different concentration ranges of HBCD standards. The calibration curves for α-, β-, and γ-HBCD use concentrations of 2.5 to 250 μg/L whereas the curves for the other seven HBCDs use concentrations of 1.0 to 25.0 μg/L. The instrumental response indicates that the linearity were generally higher than 0.993 (Table 3).

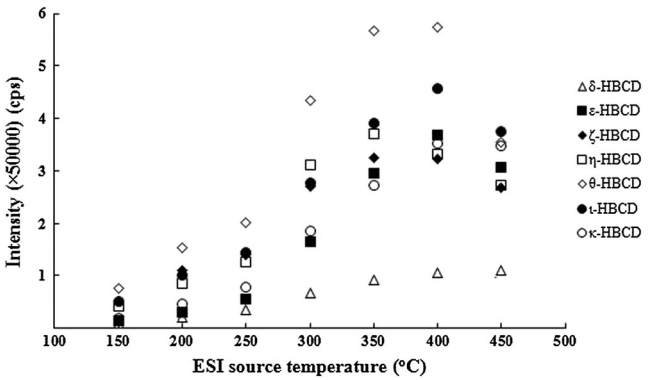

Figure 3. Effects of different source temperatures on the sensitivity of HBCDs using MRM for $[M–H]^-$ (*m/z* 640.7) → Br^- (*m/z* 78.8).

Application to analysis of real samples

Three HBCD commercial products from China and three soil samples were analyzed to assess the performance of the LC/MS/MS method developed in this study. As shown in Table 4 and Fig. 4, the concentrations of total HBCDs in soils from urban areas in Weifang City ranged from 9.39 to 11.12 ng/g dry weight (dw). Minor amounts of δ-, η-, θ-, and ε-HBCD were also detected in commercial products. Proportions of α-, β-, γ-, δ-, η-, θ-, and ε-HBCD in the three products were in the ranges of 6.2–9.2%, 7.6–9.6%, 80.2–85.8%, 0.3–0.5%, 0.0–0.1%, 0.1–0.2%, and 0.0–0.3%, respectively. However, the profiles of α-, β-, and γ-HBCD in the three soil samples were different from commercial product. γ-HBCD was the dominant isomer in one of the three soil samples (69.4%), whereas α-HBCD was the most abundant diastereoisomer in the other two soils (41.8 and 45.7%). The

Table 3. Limits of detection (LODs) and the relative standard deviations (RSDs) of HBCDs in intra-day and inter-day injections

Analytes	LOD (pg)	R^2	Intra-day precision (RSD, %)		Inter-day precision (RSD, %)	
			3 μg/L	10 μg/L	3 μg/L	10 μg/L
δ-HBCD	0.8	0.997	3.1	5.1	3.0	3.7
ι-HBCD	0.4	0.993	1.9	2.8	3.8	5.1
α-HBCD	0.4	0.999	1.9	2.8	3.8	5.1
ζ-HBCD	0.6	0.993	1.9	2.8	3.8	5.1
β-HBCD	0.4	0.998	2.3	3.9	5.7	9.5
η-HBCD	0.4	0.995	2.3	4.0	4.7	6.8
κ-HBCD	0.4	0.999	3.5	5.0	3.9	6.6
θ-HBCD	0.4	0.999	3.6	4.5	2.8	3.6
γ-HBCD	0.6	0.999	3.5	1.8	5.3	2.7
ε-HBCD	0.6	0.999	2.6	3.7	3.3	7.7

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Table 4. The concentrations of HBCD diastereoisomers in three soil samples (ng/g dw) and three commercial products (μg/L) in this study

	Concentration							
Sample name	α	β	γ	δ	η	θ	ε	∑HBCDs
Soil-1	1.71	1.47	7.72	0.10	0.04	0.02	0.07	11.12
Soil-2	4.29	1.38	3.39	0.17	0.10	0.02	0.06	9.39
Soil-3	3.48	1.96	4.04	0.08	0.07	0.02	0.02	9.67
Product-1	18.8	19.2	210.2	1.04	0.35	0.41	0.00	250.0
Product-2	15.6	19.0	214.4	0.71	0.00	0.29	0.00	250.0
Product-3	23.0	24.0	200.6	1.23	0.00	0.43	0.78	250.0

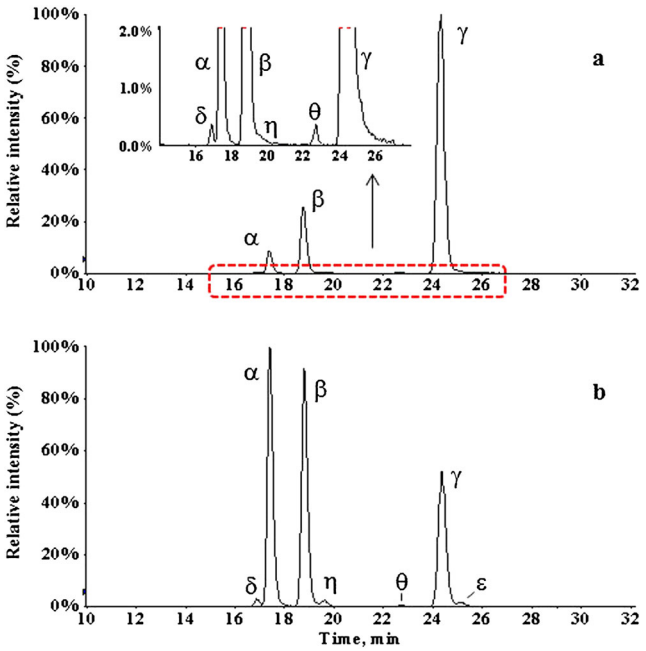

Figure 4. LC/MS/MS chromatograms of HBCD diastereoisomers in (a) the commercial product and (b) the soil sample from Weifang City.

relative contributions of δ-, η-, θ-, and ε-HBCD to the ten HBCDs in the soils were in the ranges of 0.9–1.8%, 0.3–1.0%, 0.1–0.2%, and 0.2–0.6%, respectively. To our knowledge, this is the first report of δ-, η-, θ-, and ε-HBCD in soil samples.

In fact, other HBCD diastereoisomers have been reported by only a few studies. Harrad *et al.*[9] found δ-HBCD in 13 fish samples from English lakes, the relative abundances of which ranged from 1.0 to 11%. Li *et al.*[10] reported δ-HBCD in all air particle samples from Shanghai, the proportions of which ranged from 2.6 to 15%. Zhang *et al.*[11] found δ-HBCD and ε-HBCD in sediment samples from Dagu Drainage Canal at Bohai Bay. In our study, the relative abundances of δ-, η-, θ-, and ε-HBCD in soils were obviously different from those in the commercial products, implying that isomeric interconversion might be occurred in soils. Some studies reported the occurrence of isomeric interconversion of HBCDs subjected to thermal processing[12–15] or photolysis.[28] However, most of the aforementioned studies were conducted using laboratory simulations. The mechanism of isomeric interconversion of HBCDs in environmental media and biota needs to be further investigated.

CONCLUSIONS

A new method for LC/MS/MS analysis of ten HBCD diastereoisomers using coupled reversed-phase columns was developed. The compositions of mobile phases consisting of methanol and acetonitrile significantly affected the retention times and the response of the ten diastereoisomers. After optimization of chromatographic conditions and MS/MS parameters, the method exhibited good reproducibility and sensitivity, as well as good resolution, compared with those of previously reported methods.

The method was further applied to the analysis of commercial HBCD products and soil samples. The isomers δ-, η-, θ-, and ε-HBCD in the soil samples were first reported in the present study. Our results suggest that δ-, η-, θ-, and ε-HBCD might be ubiquitous in environmental media and biota. These newly reported diastereoisomers may help us to better understand the fate and transformation of HBCDs in the environment.

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REFERENCES

[1] M. Alaee, P. Arias, A. Sjödin, Å. Bergman. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **2003**, *29*, 683.
[2] R. J. Law, M. Kohler, N. V. Heeb, A. C. Gerecke, P. Schmid, S. Voorspoels, A. Covaci, G. Becher, K. Janak, C. Thomsen. Hexabromocyclododecane challenges scientists and regulators. *Environ. Sci. Technol.* **2005**, *39*, 281A.
[3] A. Covaci, A. C. Gerecke, R. J. Law, S. Voorspoels, M. Kohler, N. V. Heeb, H. Leslie, C. R. Allchin, J. de Boer. Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. *Environ. Sci. Technol.* **2006**, *40*, 3679.
[4] R. J. Law, D. Herzke, S. Harrad, S. Morris, P. Bersuder, C. R. Allchin. Levels and trends of HBCD and BDEs in the European and Asian environments, with some information for other BFRs. *Chemosphere* **2008**, *73*, 223.

[5] S. Tanabe. Temporal trends of brominated flame retardants in coastal waters of Japan and South China: Retrospective monitoring study using archived samples from es-Bank, Ehime University, Japan. *Mar. Pollut. Bull.* **2008**, *57*, 267.
[6] POPs Review Committee (POPRC). Recommendation of the POPRC on hexabromocyclododecane, **2012**. Available: http://chm.pops.int/Convention/POPsReviewCommittee/LatestMeeting/POPRC8/POPRC8Followup/HBCD-Recommendation/tabid/2912/Default.aspx (last accessed April 7, 2014).
[7] European Commission. Risk assessment report on hexabromocyclododecane; CAS-No.: 25637-99-4. EINECS No.: 247-148-4, Final Report. The Scientific Committee on Health and Environmental Risks (SCHER), European Commission, Brussels, **2008**.
[8] N. V. Heeb, W. B. Schweizer, M. Kohler, A. C. Gerecke. Structure elucidation of hexabromocyclododecanes – a class of compounds with a complex stereochemistry. *Chemosphere* **2005**, *61*, 65.
[9] S. Harrad, M. A. E. Abdallah, N. L. Rose, S. D. Turner, T. A. Davidson. Current-use brominated flame retardants in water, sediment, and fish from English lakes. *Environ. Sci. Technol.* **2009**, *43*, 9077.
[10] H. Li, L. Mo, Z. Yu, G. Sheng, J. Fu. Levels, isomer profiles and chiral signatures of particle-bound hexabromocyclododecanes in ambient air around Shanghai, *China. Environ. Pollut.* **2012**, *165*, 140.
[11] Y. Zhang, Y. Ruan, H. Sun, L. Zhao, Z. Gan. Hexabromocyclododecanes in surface sediments and a sediment core from rivers and harbor in the northern Chinese city of Tianjin. *Chemosphere* **2013**, *90*, 1610.
[12] N. V. Heeb, W. B. Schweizer, P. Mattrel, R. Haag, A. C. Gerecke, P. Schmid, M. Zennegg, H. Vonmont. Regio- and stereoselective isomerization of hexabromocyclododecanes (HBCDs): Kinetics and mechanism of gamma- to alpha-HBCD isomerization. *Chemosphere* **2008**, *73*, 1201.
[13] R. Köppen, R. Becker, C. Jung, I. Nehls. On the thermally induced isomerisation of hexabromocyclododecane stereoisomers. *Chemosphere* **2008**, *71*, 656.
[14] N. V. Heeb, H. Graf, W. B. Schweizer, P. Lienemann. Thermally-induced transformation of hexabromocyclododecanes and isobutoxypenta bromocyclododecanes in flame-proofed polystyrene materials. *Chemosphere* **2010**, *80*, 701.
[15] N. V. Heeb, W. B. Schweizer, P. Mattrel, R. Haag, M. Kohler, P. Schmid, M. Zennegg, M. Wolfensberger. Regio- and stereoselective isomerization of hexabromocyclododecanes (HBCDs): Kinetics and mechanism of β-HBCD racemization. *Chemosphere* **2008**, *71*, 1547.
[16] F. Barontini, V. Cozzani, L. Petarca. Thermal stability and decomposition products of hexabromocyclododecane. *Ind. Eng. Chem. Res.* **2001**, *40*, 3270.
[17] P. Galindo-Iranzo, J. E. Quintanilla-López, R. Lebrón-Aguilar, B. Gómara. Improving the sensitivity of liquid chromatography–tandem mass spectrometry analysis of hexabromocyclododecanes by chlorine adduct generation. *J. Chromatogr. A* **2009**, *1216*, 3919.
[18] J. Jung, S. Bae, L. Lee, J. K. Shin, J. Choi, S. Lee. Rapid identification of brominated flame retardants by using direct exposure probe mass spectrometry. *Microchem. J.* **2009**, *91*, 140.
[19] H. Wu, H. Chen, W. Ding. Combining microwave-assisted extraction and liquid chromatography–ion-trap mass spectrometry for the analysis of hexabromocyclododecane diastereoisomers in marine sediments. *J. Chromatogr. A* **2009**, *1216*, 7755.
[20] M. J. La Guardia, R. C. Hale, E. Harvey, D. Chen. Flame-retardants and other organohalogens detected in sewage sudge by electron capture negative ion mass spectrometry. *Environ. Sci. Technol.* **2010**, *44*, 4658.
[21] P. Guerra, E. Eljarrat, D. Barceló. Determination of halogenated flame retardants by liquid chromatography coupled to mass spectrometry. *Trends Anal. Chem.* **2011**, *30*, 842.
[22] Z. Yu, P. Peng, G. Sheng, J. Fu. Determination of hexabromocyclododecane diastereoisomers in air and soil by liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. A* **2008**, *1190*, 74.
[23] S. Gao, J. Wang, Z. Yu, Q. Guo, G. Sheng, J. Fu. Hexabromocyclododecanes in surface soils from e-waste recycling areas and industrial areas in South China: Concentrations, diastereoisomer- and enantiomer-specific profiles, and inventory. *Environ. Sci. Technol.* **2011**, *45*, 2093.
[24] N. Riddell, R. Becker, B. Chittim, F. Emmerling, R. Koppen, A. Lough, A. McAlees, R. McCrindle. Preparation and X-ray structural characterization of further stereoisomers of 1,2,5,6,9,10-hexabromocyclododecane. *Chemosphere* **2011**, *84*, 900.
[25] F. Lestremau, A. Cooper, R. Szucs, F. David, P. Sandra. High-efficiency liquid chromatography on conventional columns and instrumentation by using temperature as a variable: I. Experiments with 25 cm × 4.6 mm I.D., 5µm ODS columns. *J. Chromatogr. A* **2006**, *1109*, 191.
[26] M. Herrero, F. Cacciola, P. Donato, D. Giuffrida, G. Dugo, P. Dugo, L. Mondello. Serial coupled columns reversed-phase separations in high-performance liquid chromatography: Tool for analysis of complex real samples. *J. Chromatogr. A* **2008**, *1188*, 208.
[27] D. Tao, G. Zhu, L. Sun, J. Ma, Z. Liang, W. Zhang, L. Zhang, Y. Zhang. Serially coupled microcolumn reversed phase liquid chromatography for shotgun proteomic analysis. *Proteomics* **2009**, *9*, 2029.
[28] S. Harrad, M. A. E. Abdallah, A. Covaci. Causes of variability in concentrations and diastereomer patterns of hexabromocyclododecanes in indoor dust. *Environ. Int.* **2009**, *35*, 573.

