Quantitative Analysis of HBCD Diastereomers in Technical HBCD Mixtures using UPLC-MS/MS

Song-Yee Baek*, Sunyoung Lee, Seonghee Ahn, Byungjoo Kim

Center for Organic Analysis, Korea Research Institute of Standards and Science (KRISS), 267 Gajeong-ro, Yuseong-gu, Daejeon, Republic of Korea, 34113

Introduction

Hexabromocyclododecane (HBCD) is an additive flame retardant, used in expanded and extruded polystyrene for building insulation, in textile backcoatings, and in high-impact polystyrene (HIPS) for electrical appliances.

The technical HBCD is produced by the bromination of cyclododeca-1, 5, 9-trienes theoretically results in 10 diastereomers, *α, β, γ, δ, ε, ζ, η, θ, ι, κ*-HBCD. However, the technical HBCD mixture consists of three predominant isomers: 10-13% of *α* -HBCD, 1-12% of *β* -HBCD, and 75-89% of *γ*-HBCD [1, 2].

Because HBCD diastereomers transform at temperatures over 160 °C, the HBCD diastereomers cannot be separated in the gas chromatography (GC) [3]. Thus, liquid chromatography coupled mass spectrometry (LC-MS) has been frequently used for diastereoselective analysis of HBCD in many studies.

In most previous studies, only *α*, *β*, *γ* isomers are selected as the target analytes, which are separated by commonly C18 LC column. However, earlier work has shown that two isomers, *δ*-HBCD and *ε*-HBCD are present in technical HBCD mixtures [1].

In our recent paper, we suggested the proper liquid chromatographic conditions for the separation of HBCD diastereomers using both C18 and phenyl-hexyl ultra-performance liquid chromatography (UPLC) columns and identified not only *δ*- and *ε*-HBCD but also *η*- and *θ*-HBCD in two types of technical HBCD mixtures [4]. In this study, this LC separation methodology applied for the quantitative analysis of individual HBCD diastereomers in technical HBCD mixtures.

Material and methods

Materials

We purchased standard stock solutions of *α*-HBCD, $^{13}C_{12}$-*α*-HBCD, *β*-HBCD, $^{13}C_{12}$- *β*-HBCD, *γ*-HBCD, and $^{13}C_{12}$-*γ*-HBCD (50 μg/mL in toluene) from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Individual standard stock solutions of *δ-, ε-, ζ-, η-, θ-, ι-,* and *κ*-HBCD (50 μg/mL in toluene) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). HPLC-grade methanol (MeOH) was purchased from Burdick and Jackson (Muskegon, MI, USA). HPLC-grade water was purified in a Milli-Q system (Millipore, Billerica, MA, USA). The powder type of technical HBCD mixture was purchased from Tokyo Chemical

Industry (TCI) Co., Ltd. (Chuo-ku, Tokyo) and the other powder type of technical HBCD mixture was purchased from a local market.

Standard solutions

For the quantification of *α, β, γ, δ, ε, η, θ*-HBCD, HBCD solution mixtures (a mixture of 0.07 mg/kg of *δ*-, 0.07 mg/kg of *θ*-HBCD, 0.37 mg/kg of *β*-HBCD, and 0.45 mg/kg of $^{13}C_{12}$- *β*-HBCD, a mixture of 0.07 mg/kg of *ε*-, 0.07 mg/kg of *η*-, 0.37 mg/kg of *α*-HBCD, 0.37 mg/kg of $^{13}C_{12}$-*α*-HBCD, a mixture of 5 mg/kg of *γ*-HBCD, 5 mg/kg of $^{13}C_{12}$-*γ*-HBCD were prepared by gravimetrically mixing weighed aliquots of individual HBCD stock solutions with MeOH.

LC/MS/MS analysis

The measurements were made on a Waters Acquity UPLC system/Xevo TQ-S triple quadrupole mass spectrometer (Manchester, UK) with electrospray ionization interface.

For chromatographic separation, Waters AQUITY CSH C18 (100 mm length, 2.1 mm i.d., 1.7 μm particle size) and Waters AQUITY CSH phenyl-hexyl (100 mm length, 2.1 mm i.d., 1.7 μm particle size) (Manchester, UK) were used. The injection volume for sample extracts was 1 μL per LC run. Detailed instrumental conditions were described in our previous paper [4].

Results and discussion

When using the C18 column, which is commonly selected in the most HBCD analysis studies, the good separation of the three primary diastereomers (*α, β, γ*) was obtained. However, *δ*- and *ε*-HBCD were not easily separated from *α*- and *γ*-HBCD (Fig. 1(a)). We tested phenyl-hexyl column to separate HBCD diastereomers and found enhanced resolution for HBCD diastereomers with the phenyl-hexyl column. Finally, we confirmed the presence of minor diastereomers in the TCI HBCD mixture and the technical HBCD mixture using the phenyl-hexyl UPLC columns (Fig. 1(b)) [4].

For the quantitative analysis of HBCD diastereomers, two types of technical HBCD mixtures and standard solutions were analyzed on the phenyl-hexyl UPLC column. Both technical HBCD mixtures were observed to have high portion of *γ*-HBCD and trace amounts of minor HBCDs (*δ, ε, η, θ*) as well. Specifically, 10.4 % of *α*-HBCD, 5.3 % of *β*-HBCD, 82.1 % of *γ*-HBCD, 1.87 % of *δ*-HBCD and trace amounts of *ε, η, θ*-HBCD were observed in the technical HBCD mixture. 7.8 % of *α*-HBCD, 7.4 % of *β*-HBCD, 84.3 % of *γ*-HBCD, 0.29 % of *δ*-HBCD and trace amounts of *ε, η, θ*-HBCD were observed in the TCI HBCD mixture (Fig. 2). This result is consistent with a previous report for major diastereomers [2].

This separation method using the phenyl-hexyl column also can be used for qualitative and quantitative analysis of the individual HBCDs in various environmental and biological samples.

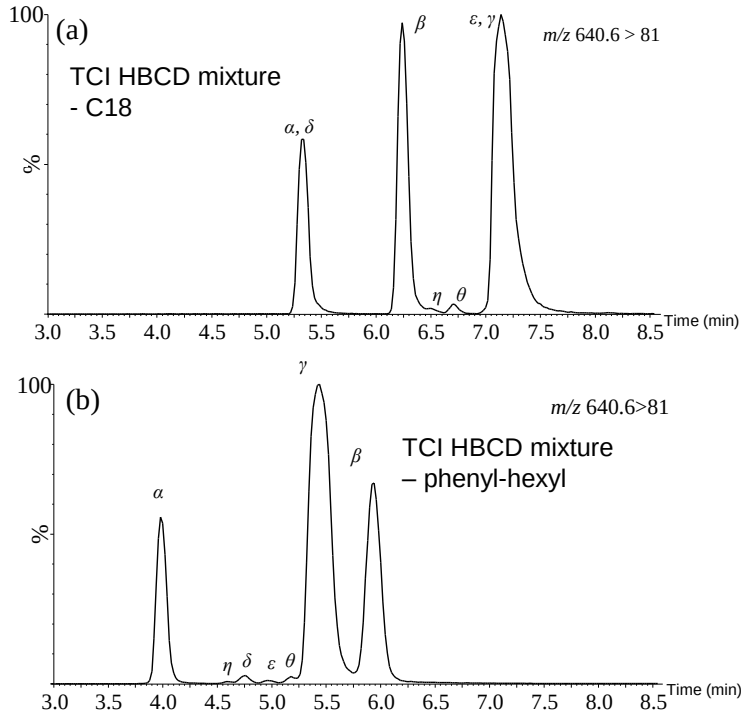

Fig. 1. LC-MS/MS chromatograms for HBCD diastereomers in TCI HBCD mixtures analyzed using (a) a Waters CSH C18 column and (b) a Waters CSH phenyl-hexyl column

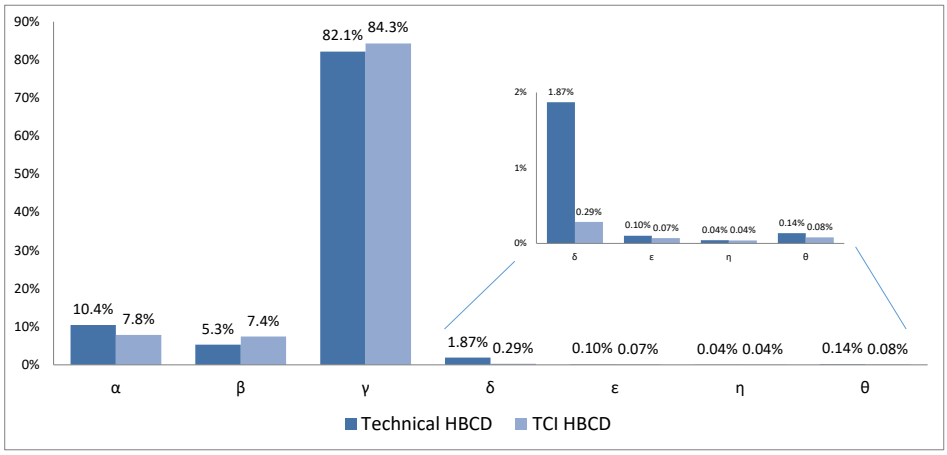

Fig. 2. The compositions of *α, β, γ, δ, ε, η, θ*-HBCD diastereomers in two technical HBCD mixtures analyzed by using the Waters CSH phenyl-hexyl column

Acknowledgements

This research was supported by the Korean Agency for Technology and Standards (KATS) under the project 'Development of commercial reference materials, Grant 14201025' and Korea Research Institute of Standards and Science (KRISS) under the project 'Establishing measurement standards for organic analysis, Grant 17011053'.

References

1. N.V. Heeb, W.B. Schweizer, M. Kohler, A.C. Gerecke. (2005) *Chemosphere*, **61**, 65-73.
2. A. Covaci, A.C. Gerecke, R.J. Law, S. Voorspoels, M. Kohler, N.V. Heeb, H. Leslie, C.R. Allchin, J. de Boer. (2006) *Environmental Science & Technology*, **40**, 3679-3688.
3. F. Barontini, V. Cozzani, L. Petarca. (2001) *Industrial & Engineering Chemistry Research*, **40**, 3270-3280.
4. S.-Y. Baek, S. Lee, B. Kim. (2017) *Journal of Chromatography A*, **1488**, 140-145.