Enantiomer specific determination of HBCD diastereomers by LC-MS-MS

Karel Janák, Cathrine Thomsen and Georg Becher

Division of Environmental Medicine, Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, NO-0403 Oslo, Norway

Introduction

1,2,5,6,9,10-Hexabromocyclododecane (HBCD) is an additive flame retardant used in various polystyrene resins and textiles[1]. HBCD is produced by bromination of 1*Z*,5*E*,9*E*-cyclododeca-1,5,9-triene (*cis*,*trans*,*trans*-CDT), a butadiene trimer[2]. The resulting technical product consists to more than 90% of a mixture of three enantiomeric pairs of diastereomers of 1,2,5,6,9,10-hexabromocyclododecane termed α-, β-, and γ-HBCD. The three diastereomers may be separated by reversed phase HPLC[3,4].

In the technical mixtures, γ-HBCD is dominating (75-89%) while α- and β-HBCD constitute 10-13% and <0.5-12%, respectively[5]. Using HPLC-MS-MS it has been demonstrated that there is a dramatic change of the isomer distribution from the isomer pattern in the technical mixture to a prominent α-isomer in biota[6-8]. The α-isomer is also by far the most abundant HBCD isomer in human breast milk[9].

Each of the diastereomers exists as a pair of enantiomers[10,11]. The stereochemistry of the isomers is illustrated in Figure 1.

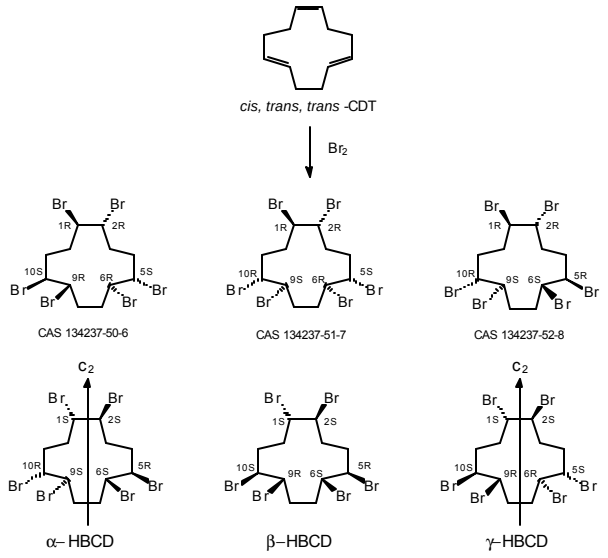

Figure 1. Stereochemical structures of the isomers of HBCD.

The objective of this work was to develop a method for enantiospecific determination of HBCD diastereomers in order to investigate the enantiomeric composition of HBCD in wildlife and humans.

Materials and Methods

LC-MS-MS was performed on a ThermoFinnigan TSQ Quantum equipped with a Surveyor HPLC pump. The MS was used in the ES negative ion mode using SRM for $[M-H]^-$ (m/z 640.6) —— Br^- (m/z 79.0 and 80.7). The collision energy was 17 eV for m/z 79.0 and 21 eV for m/z 80.7.

For achiral chromatography, a Waters Symmetry C18 column (150 x 2.1 i.d. mm) was used. The mobile phase was 60% H_2O/30% CH_3OH/10% ACN for 0.5 min followed by a linear gradient for 3.5 min to CH_3OH/ACN, 1:1 which was held for 13 min. The flow rate was 0.25 mL/min.

For chiral chromatography, a Macherey-Nagel NUCLEODEX beta-PM column (200 x 4.0 mm i.d.) was used. The mobile phase was 40% H_2O/30% ACN /30% CH_3OH for 0.5 min followed by a linear gradient for 7 min to 70% ACN/30% CH_3OH which was held for 14 min. The flow rate was 0.5 mL/min. In both methods, an injection volume of 20 µl was used.

Results and Discussion

The three diastereomers were baseline separated on the C18-column. The retention factors k' for α, β, and γ were 4.6, 4.9 and 5.4, respectively. In Figure 1, the chromatograms are shown for a 1:1:1 mixture of individual diastereomers (A), a technical mixture (B), and the product after thermal treatment of the technical mixture at 180°C for 2 h (C).

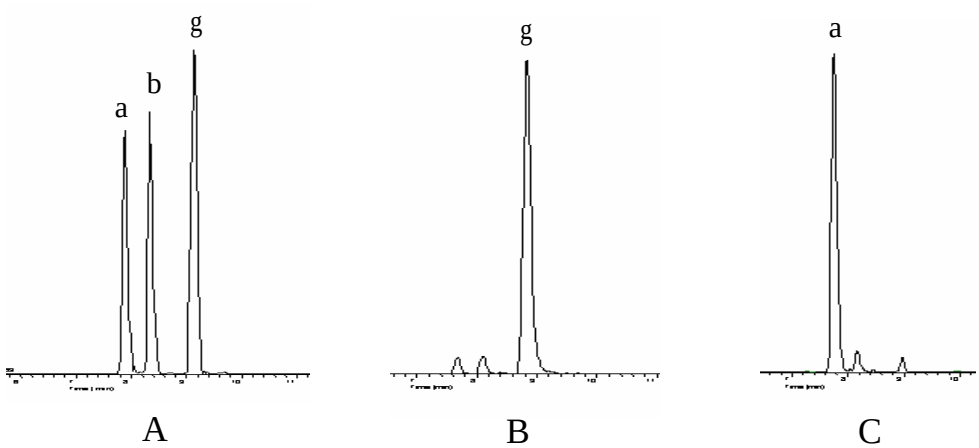

Figure 1. Chromatograms of a standard mixture of individual diastereomers (A), a technical mixture (B) and the technical mixture after thermal treatment (C).

In chromatogram B, the dominance of the γ-isomer in the technical product is clearly demonstrated. Chromatogram C verifies that the α-isomer is thermodynamically favoured comprising 78% of the HBCD isomers after thermal treatment (Peled et al., 1995).

Chromatography of the mixture of the three diastereomers on a chiral β-Cyclodextrin column results in six well separated peaks (Figure 2 A). The retention factors were 3.0 and 3.3 for α-HBCD, 3.1 and 3.4 for the β-HBCD and 3.6 and 4.3 for γ-HBCD. Even when one of the diastereomers was dominating (B and C), the other isomers were still quantifiable with reasonable accuracy.

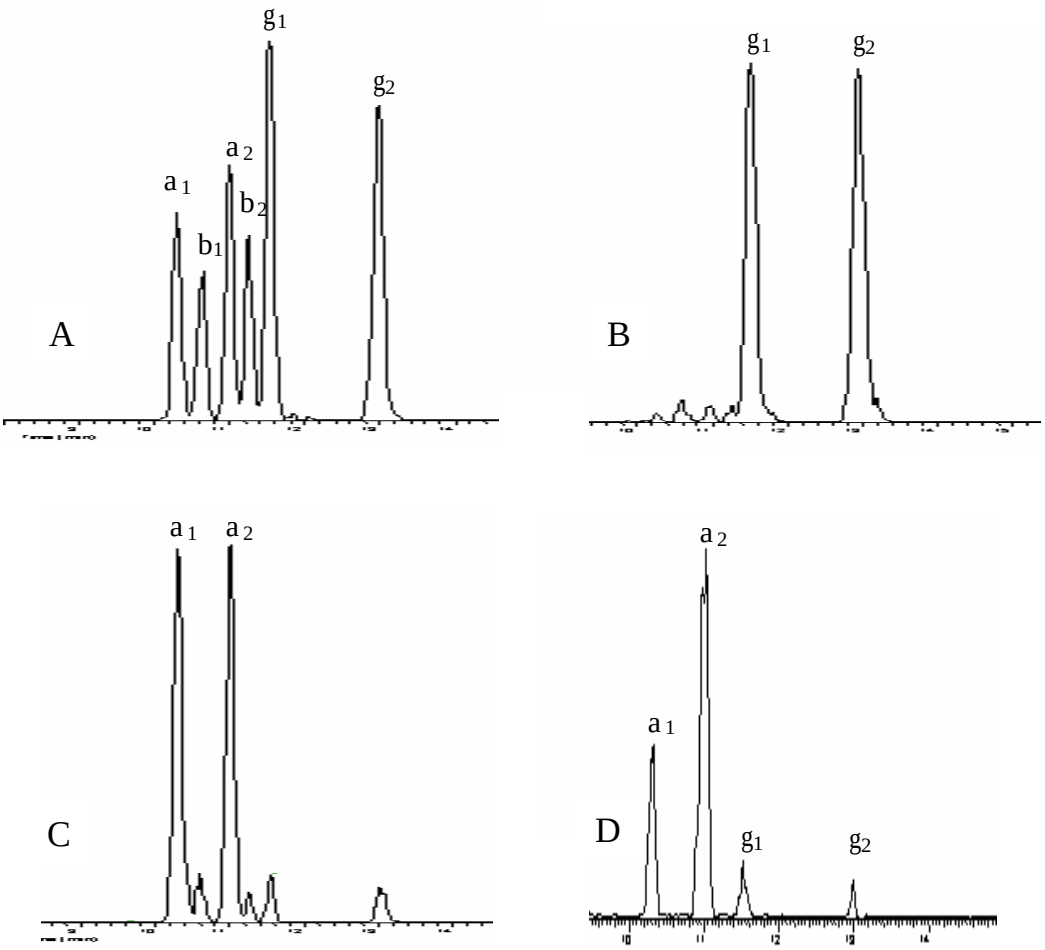

Figure 2. Chromatography on the chiral column of a mixture of individual diastereomers (A), a technical mixture (B), the thermally equilibrated technical mixture (C), and an extract from a composite sample of salmon from the Baltic Sea (D).

Chromatogram D shows the chiral separation of HBCD in an extract of muscle tissue of salmon from the Baltic Sea. The presence of non-racemic α- and γ-HBCD is clearly demonstrated indicating a strongly enantioselective accumulation of HBCD diastereomers. The enantiomer fraction of α_1 is calculated to approximately 0.28 and for γ_1 approximately 0.66.

Conclusion

Using a chiral permethylated β-cyclodextrin HPLC column, the enantiomers of α, β and γ-HBCD can be separated in a single run. We report here, for the first time, on a non-racemic enantiomeric composition of HBCD in biota. The method has great potential for studying enantioselective biotransformations of HBCD in wildlife and humans.

Acknowledgement

This work has been carried out with support from the European Commission, 5th Framework Programme for Research, Technological Development and Demonstration Activities, Quality of Life and Management of Living Resources (QoL), Key Action 4: Environment and Health Contract No: QLK4-CT-2002-00596.

References

1. Alaee, M., Arias, P., Sjödin, A., Bergman, Å., **2003**. *Environ. Int.* 29, 683-689.
2. Barda, H.J., **1985**. Flame retardants. In: Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., vol. A4, chap. 1.4.11.4, VHC, Weinheim, 417-418.
3. Budakowski, W., Tomy, G., **2003**. *Rapid Commun. Mass Spectrom.* 17,1399-1404.
4. Morris, S., Allchin, C.R., Bersuder, P., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Brandsma, S.H., Kruijt, A.W., van der Veen, I., van Hesselingen, J., de Boer, J., **2003**. *Organohalogen Compounds* 60, 436-439.
5. Peled, M., Scharia, R, Sondack, D., **1995**. In: Desmurs, J.R., Gérard, B., Goldstein, M.J. (Eds.). Advances in Organobromine Chemistry II, Elsevier, Amsterdam, The Netherlands, pp. 92-99.
6. Allchin, C.R., Morris, S., **2003**. *Organohalogen Compounds* 61, 41-44.
7. Gerecke, A.C., Kohler, M., Zennegg, M., Schmid, P., Heeb, N.V., **2003**. *Organohalogen Compounds* 61, 155-158.
8. Tomy, G., Budakowski, E., Halldorson, T., Whittle, M., Keir, M., Alaee, M., **2003**. *Organohalogen Compounds* 61, 111-114.
9. Janák, K. Thomsen, C. Becher, G., **2003**. Swedish-Norwegian Meeting in Environmental Chemistry, Geilo, Norway, 16-18 February 2004.
10. Becher, G., **2004**. Manuscript submitted.
11. Gerecke, A.C. **2004**. Personal communication.