Isomer-Specific Determination Of Hexabromocyclododecane In Abiotic And Biotic Samples By High Performance Liquid Chromatography/Atmospheric Pressure Photoionization Tandem Mass Spectrometry

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Introduction: Hexabromocyclododecane (HBCD) is the third most widely used brominated flame retardant (BFRs) in the world and is considered to be a high-production-volume (HPV) chemical. Global market consumption in 2001 was estimated to be approximately 16,700 tons in 2001.[1] Concentrations of BFRs, including HBCD, are increasing for many environmental compartments [2]. Atmospheric deposition rates of 0.02 to 13 ng m^{-2} d^{-1} in remote areas of the arctic have been reported [3], indicating HBCDs are subject to long-range transport.

Liquid chromatography has been found to be the most suitable for the determination of HBCD as the isomers can not be separated in a gas chromatograph and the analyte is thermally labile. HPLC-APCI/MS has been shown to be a suitable method for the separation and determination of a-,b-, and g-HBCD diasteriomers.[4] Separation of HBCD isomers on reverse phase columns has also been reported by others. [5,6,7]

The use of photoionization (PI) in analytical mass spectrometry is a relatively new technique. Atmospheric pressure photoionization tandem liquid chromatography (HPLC- APPI/MS/MS) is a valuable complement to electrospray (ESI) and atmospheric pressure chemical ionization (APCI) to assist in extending the range of molecules that can effectively be ionized. Common problems for ionization methods such as ESI and APCI include suppression of the desired analyte efficiency by compounds with higher ionization potential or compounds which are present in large excess. This suppression effect is more often experienced with ESI.

An isomer-specific method based on high-performance liquid chromatography / atmospheric pressure photoionization tandem mass spectrometry (HPLC/APPI-MS/MS) in the negative ion mode was developed for the analysis of hexabromocyclododecane (HBCD). When the method was applied to wet deposition samples collected from an Integrated Atmospheric Deposition Network (IADN) site in the Great Lakes basin all three isomers were found in low pg/L concentrations. The method also proved suitable for the analysis of HBCD in skipjack tuna collected from various Asian waters.

Materials and Methods: The a-,b-, and g-HBCD diasteriomers were purchased from Wellington Laboratories (Guelph, ON, Canada). The technical mixture was a gift from Wellington Laboratories. An Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, binary pump and autosampler was used. A Harvard syringe pump was used to deliver the dopant (toluene). An Agilent C_{18}-SB analytical column (150 mm x 2.1 mm i.d., 3.5 um particle size) was used for all samples. A mobile phase of A (water) and B (methanol) at a flow rate of 300 uL/min was used. Separation was achieved using a gradient starting at an initial composition of 30:70 A/B (v/v) and ramped to 100% B in 3 minutes. It was then held for 5 minutes and returned to the starting conditions in 3 minutes and allowed to equilibrate for 10 minutes. The injection volume used was 10 uL.

A Sciex API 2000 triple quadrupole mass spectrometer (MDS Sciex, Concord, ON, Canada) was used in the APPI mode. Infusion experiments utilized the built-in Harvard syringe pump with a flow rate of 10 uL teed into a flow of 300 uL from the HPLC. The Q1 scan range was m/z 100-800. Source parameters were optimized according to the manufacturing instructions. MS/MS detection used MRM conditions for the m/z 640.6$[M–H]^- \rightarrow Br^-$ (m/z 79) reaction with unit resolution on the first and third quadrupoles (Q1 and Q3).

Results and Discussion : To optimize the APPI conditions, different parameters which influence the ionization were investigated. These parameters include the curtain gas flow, the ion transfer voltage, the heater temperature,

the nebulizer and auxiliary gas, and the declustering (DP) and focusing potential (FP). The entrance potential (EP), collision energy (CE), and the collision cell exit potential (CXP) are also optimized.

Optimization of the MS/MS conditions was carried out initially with a technical mixture of HBCD. The technical mixture consists of three prominent diastereoisomers (a-,b-, and g-HBCD), with the g-isomer being the predominant one. A Q1 scan, with a range of m/z 100-800 was performed using a technical mixture concentration of 200 ug/mL in methanol. A flow of 10 uL/min was delivered by the syringe pump, teed into a flow of 300 uL/min of a 50:50 (v/v) solution of methanol:water delivered by an Agilent binary pump. The higher flow rate used for the optimization process is required as the source is a mass dependent detector. This is in contrast to an electrospray source, which is a concentration dependent detector. Figure 1 show's the Q1 scan of technical HBCD (m/z 500-750) obtained as an accumulation of 20 MCA scans. Two dominant clusters are evident. The dominant peak at m/z 640.6 corresponds to the $[M-H]^-$ ion. There is another dominant peak at m/z 720.6 which may corresponds to the $[M-H+Br]^-$ ion. To allow a comparison to published methods using electrospray ionization, the m/z 640.6 ion was used to optimize the MS/MS conditions.

The following conditions were found to provide the optimum signal: curtain gas 10 psi, ion transfer voltage -1150 V, temperature 400°C, nebulizer gas 90 psi, auxiliary gas 60 psi, collision gas 6 psi. The DP and FP were set to -25 V and -360 V respectively. EP, CE, and CXP were -5 V, -30 V and -5 V. The instrument was run in the negative ion mode, and each quadrupole was set to unit mass resolution.

The method was applied to wet-only precipitation samples collected at an IADN site in the Great Lakes Basin. The concentration of HBCD, determined by GC/MS, ranged from n.d. to 35 ng/L and annual wet deposition ranged from 0.36 to 10 ng/m^2/day. Annual variations were observed with peaks in the winter, which are likely the result of the increased scavenging efficiency of snow compared to rain and higher concentrations in the particle phase during the winter. The HBCD isomers were determined in wet-only precipitation sample (Figure 2). The average percent distribution of the a-, b-, and g-HBCD isomers in the samples analyzed were 77, 15, and 8 respectively.

The method was also applied to skipjack tuna samples collected from offshore waters of various regions of the world. Samples were previously analyzed utilizing ESI-tandem MS[7]. The values determined from APPI were in good agreement of those determined previously (< ± 20 %).

Conclusion: An atmospheric pressure photoionization tandem mass spectrometer was evaluated for the determination of hexabromocyclododecane in wet deposition samples and biotic samples. The results show that the technique is appropriate for the determination in the analyte in both these types of environmental samples. Compared to atmospheric pressure electrospray ionization, the method gave levels of detection of approximately ten fold higher. Together the two techniques are complimentary to the analysis of environmental samples for the determination of HBCD. The analysis of the varying ionspray voltage confirmed that this parameter has a significant influence on analyte sensitivity. After establishing the best instrument conditions the method was used to analyze samples. The a-,b-, and g- isomer were determined in selected wet precipitation samples collected from the Great Lakes basin and biota samples collected from offshore waters of various regions in the world.

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References

1. Bromine Science and Environmental Forum, Fact Sheet on HBCD (2003) www.bsef.com

2. Alaee, M., Arias, P., Sjödin, A., Bergman, A. (2003) An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ. Int. 29, 683-689.

3. Remberger, M., Sternbeck, J., Palm, A., Kaj, L., Strömberg, K., Brorström-Lundén, E. (2004) The environmental occurrence of hexabromocyclododecane in Sweden. Chemosphere. 54, 9-21.

4. Heeb, N.V. (1997) Charakterisierung von thermolabilenVerbindungenmittelsKopplung von Flüssigchromatographie und ESI- oder APCI-Massenspektrometrie. EMPA-Report No. 160947, Dübendorf, Switzerland, 1-22.

5. Budakowski, W., and Tomy, G. (2003) Congener-specific analysis of hexabromocyclododecane by high-performance liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun. Mass Spectrom. 17, 1399-1404.

6. Gerecke, A.C., Kohler, M., Zennegg, M., Schmid, P., Heeb, N.V. (2003) Detection of a-isomer dominated HBCD (hexabromocyclododecane) in Swiss fish at levels comparable to PBDEs (polybrominateddiphenyl ethers). Organohalogen Compd. 61, 155-158.

7 Ueno, D., Alaee, M., Marvin, C., Muir, D.C., Macinnis, G., Reiner, E. Crozier, P., Furdui, V.I., Subramanian, A., Fillmann, G., Lam, P., Zheng, G., Muchtar, M., Razak, H., Prudente, M. Chung, K., and Tanabe, S. (submitted). Global pollution monitoring of hexabromocyclododecane (HBCD) using skipjack tuna as a bioindicator. Environ. Sci. Technol.

Figure 1. Q1 mass spectrum (accumulation of 10 MCA scans) of technical HBCD (200 ug/mL in methanol, 10 uL/min teed into a flow of 300 uL/min).

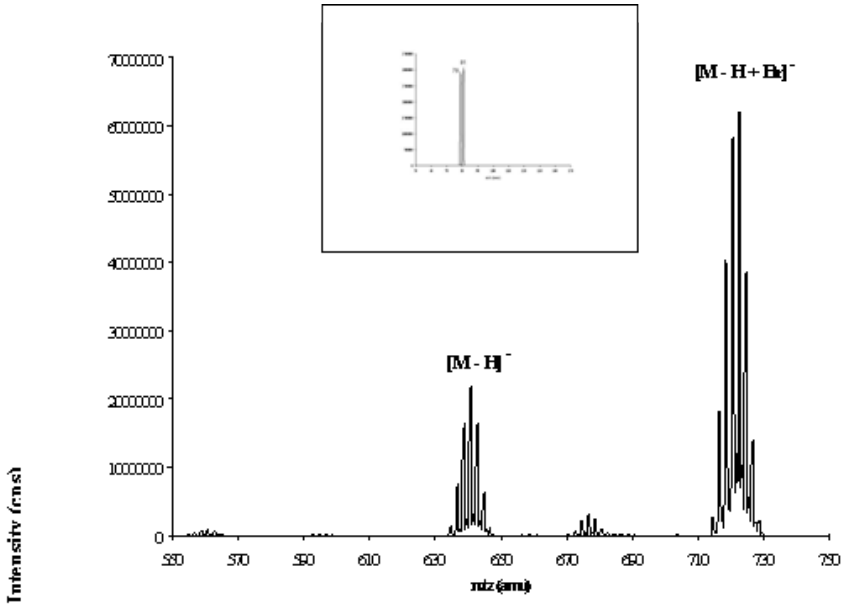
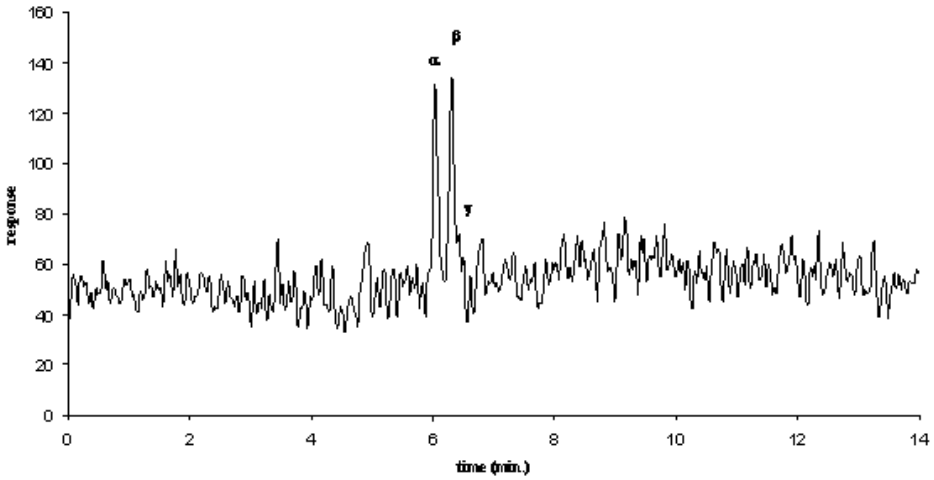


Figure 2. Elution of the a-, b-, and g-, isomers of HBCD in a wet-only precipitation sample.


160
140
120
100
80
60
40
20
0
response
α
β
γ
0
2
4
6
8
10
12
14
time (min.)