

Cod: 2.3005

OPTIMIZATION OF UPLC/MS/MS FOR ANALYSIS OF HBCDS ISOMERS

H. Kim¹, J. Kang¹, H. Yang¹, C. Lee¹, J. Jeon², J. Son², S. Choi²

¹Department of Environment Analysis, Korea Environment Corporation, 42 Hwangyeong-ro, Seo-gu, Incheon, Republic of Korea

²School of Urban and Environmental Engineering, Ulsan National Institute of Science and Technology (UNIST), 50, UNIST-gil, Ulsan 44919, Republic of Korea

Introduction

Hexabromocyclododecanes (HBCDs) are used as a flame retardant. HBCDs are commonly added in expanded or extruded polystyrene foam (EPS/XPS) which are used as building and construction heat insulation. As an additive, there are utilized in home appliances, vehicle seats, latex glues and textile back coatings. HBCDs have also been applied in electrical and electronic applications such as audio-visual equipment, refrigerator linings, and in wire and cable as high-impact polystyrene (HIPS) according to U.S. EPA 2012. 1,535 tons of HBCDs have been consumed in the Republic of Korea in 2015. HBCDs are known as having persistence and long range transport in the environment. Even though the Stockholm Convention's annexes added HBCD in 2013, there is no still standard analysis method at home and aboard. The research about HBCDs monitoring is increasing, but still not sufficient to optimize the analysis of the HBCDs. This study was performed to optimize the peak sensitivity and resolution of the HBCDs isomers; α -HBCD, β -HBCD, γ -HBCD by using liquid chromatography-tandem mass spectrometer; LC/MS/MS.

Materials and methods

This study aimed to establish HBCDs analysis method using standard to analyze environmental media; ambient, river water, sediment and soil. HBCDs were analyzed by UPLC/MS/MS (Waters Xevo TQ-S). The result of recovery ratio were compared using established analysis method based on pretreatment; extraction, clean-up conditions. Also, optimized analysis method was introduced under the result of mass analysis and pretreatment as well as certified analysis method using certified reference material.

Results and discussion

To find optimized extraction solvent, this study used dichloromethane, acetone/n-hexane, dichloromethane/n-hexane for the ambient and soil and dichloromethane for river water samples. The recovery ratio for the every sample shows that around 110% regardless extraction solvent except γ -HBCD for the ambient sample when using acetone/n-hexane. Thus, dichloromethane is recommended for the extraction solvent same as former PBDEs.

Ambient, soil/sediment samples were extracted in a soxhlet during 24 hours using 350mL of dichloromethane and extraction of river water samples was carried out by 3 steps of liquid-liquid extraction using dichloromethane. Multi-layers silica gel column with 2g of sulfuric anhydride, 1g of 2% KOH silica gel, 3g of neutral silica gel, 3g of 44% sulfuric acid impregnated silica gel, 3g of neutral silica gel, 2g of sulfuric anhydride and silica gel/alumina column; 2g of sulfuric anhydride, 5g of alumina, 3g of neutral silica gel, 3g of 44% sulfuric acid impregnated silica gel, 3g of neutral silica gel, 2g of sulfuric anhydride was used for cleanup.

Mass to charge ratio (m/z) for the native HBCD is 640.7 to 79, 81, internal standard; surrogate ¹³C₁₂-HBCDs are 652.8 to 79, 81, internal standard; d18-HBCDs are 657.8 to 79, 81. Optimized cone voltage and collision energy is 25V and 15 eV, respectively.

The resolution and peak sensitivity of analysis of HBCDs using UPLC/MS/MS were optimized when solution A which is methanol/acetonitrile 7:3 (v/v) and solution B which is 10 mM of ammonium acetate in deionized water are used as a mobile phase.

The retention time of HBCDs is all within 15 minutes, the order of retention time is condition 1, 2 and 3. As the ratio of methanol increases, the elution time from the column was shorten. In case of the condition C, however, the peak resolution was decreased even though it has the shortest detection time. So, this study added acetonitrile to methanol as 7:3 (v/v) to improve increasing internal pressure and peak separation resolution.

Acknowledgements

This study was carried out part of Persistent Organic Pollutants Monitoring program under the budget of the Ministry of Environment, Republic of Korea.

References

1. Al-Odaini, N. A., Shim, W. J., Han, G. M., Jang, M., and Hong, S. H., Enrichment of hexabromocyclododecanes in coastal sediments near aquaculture areas and a wastewater treatment plant in a semi-enclosed bay in South Korea, *Science of the Total Environment*, 505, 290-298, 2015
2. Covaci, A., Gerecke, A. C., Law, R. J., Voorspoels, S., Kohler, M., Heeb, N. V., Leslie, H., Allchin, C. R., and Boer, J. D., Hexabromocyclododecanes (HBCDs) in the Environment and Humans: A Review, *Environmental Science & Technology*, 40, 3679-3688, 2006
3. Haukas, M., Hylland, K., Berge, J. A., Nygard, T., and Mariussen, E., Spatial diastereomer patterns of hexabromocyclododecane (HBCD) in a Norwegian fjord, *Science of the Total Environment*, 407, 5907-5913, 2009
4. Hong, J., Gao, S., Chen, L., Han Q., Yu, Z., Peng, P., and Fu, J., Hexabromocyclododecanes in the indoor environment of two cities in South China: their occurrence and implications of human inhalation exposure, *Indoor and Built Environment*, 1-9, 2013
5. Jeong, G. H., Hwang, N. R., Hwang, E. H., Lee, B. C., and Yoon, J. H., Hexabromocyclododecanes in crucian carp and sediment from the major rivers in Korea, *Science of the Total Environment*, 470-471, 1471-1478, 2014
6. Li, H., Gao, Q., Wang, P., Li, Y., Lv, J., Chen, J., Chen, W., Geng, D., Yawei, W., Wang, T., and Jiang, G., Levels and distribution of hexabromocyclododecane (HBCD) in environmental samples near manufacturing facilities in Laizhou Bay area, East China, *Journal of Environmental Monitoring*, 14, 2591-2597, 2012
7. Morris, S., Bersuder, P., Allchin, C. R., Zegers, B., Boon, J. P., Leonards, P. E. G., and Boer, J. D., Determination of the brominated flame retardant, hexabromocyclododecan, in sediments and biota by liquid chromatography- electrospray ionisation mass spectrometry, *Trends in Analytical Chemistry*, 25, 343-349, 2006
8. Son, M. H., Kim, J. C., Shin, E. S., Seo, S. H., and Chang, Y. S., Diastereoisomer- and species-specific distribution of hexabromocyclododecane (HBCD) in fish and marine invertebrates, *Journal of Hazardous Materials*, 300, 114-120, 2015
9. U.S. EPA (2012). Significant New Use Rule for Hexabromocyclododecane and 1,2,5,6,9,10-Hexabromocyclododecane. 77 FR 17386-17394.
10. Yu, Z., Peng, P., Sheng, G., and Fu, J., Determination of hexabromocyclododecane diastereoisomers in air and soil by liquid chromatography-electrospray tandem mass spectrometry, *Journal of Chromatography A*, 1190, 74-79, 2008

Fig 1. The recovery ratio of HBCDs for the environment sample

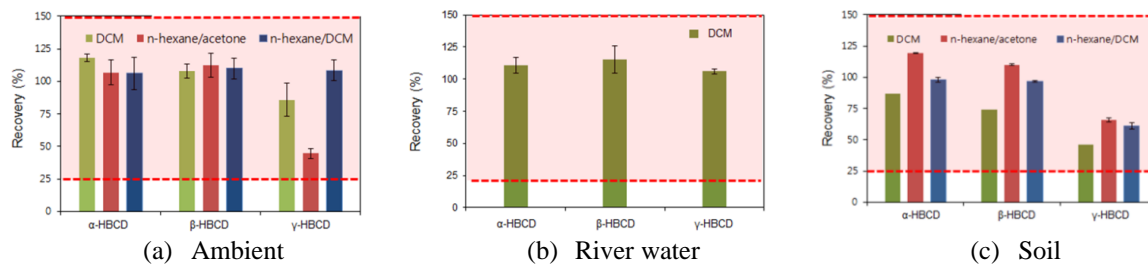


Fig 2. Procedure of HBCDs analysis method

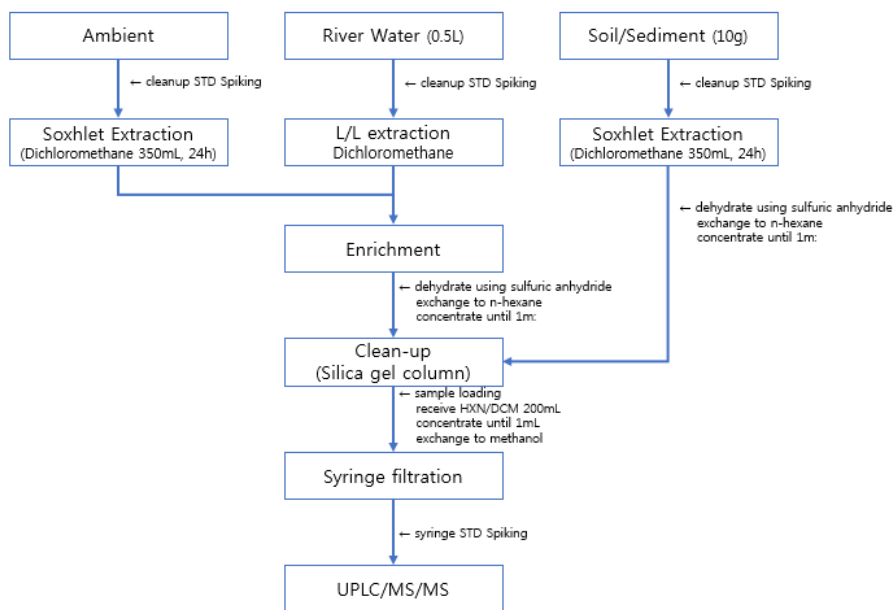


Fig 3. The result of multiple reaction monitoring (MRM) by UPLC/MS/MS

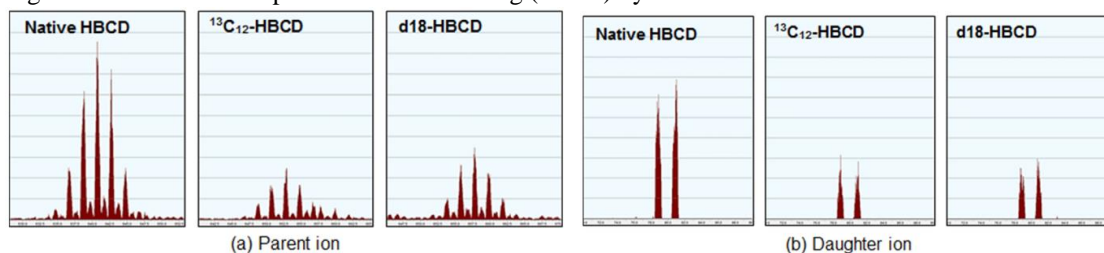


Fig 4. Molecular and ionic clusters of hydrocarbons and spectrum of HBCDs

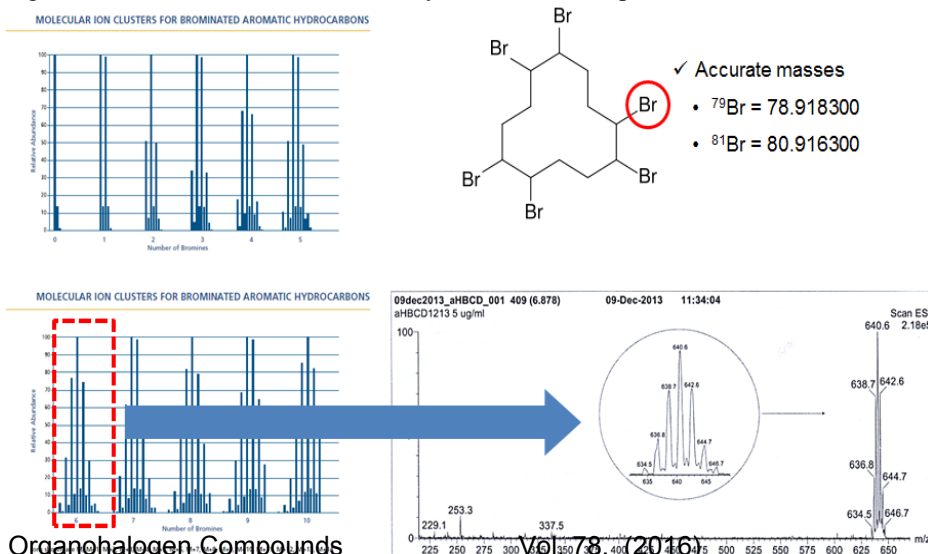


Table 1. Multiple reaction monitoring (MRM) condition of HBCDs using UPLC/MS/MS

Parameter	Native HBCDs	¹³ C ₁₂ -HBCDs	d18-HBCDs
Parent ion (m/z)	640.7	652.8	657.8
Daughter ion (m/z)	79 and 81		
Cone voltage (V)	25		
Collision energy (eV)	15		

Table 2. Mobile phase gradient conditions for UPLC/MS/MS analysis of HBCDs

Time (min)	#1		#2		#3	
	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)
Initial	10	90	50	50	80	20
5	10	90	50	50	80	20
14	95	5	95	5	95	5
20	10	90	50	50	80	20

Table 3. Optimized UPLC/MS/MS for analyzing HBCDs isomers

Analyzer	UPLC (Ultra performance liquid chromatography, Waters)		
Column	Waters Acquity BEH C18 column (2.1 × 100 mm, 1.7 μm)		
Injection	5 μL		
Column temperature	40°C		
Mobile phase	(A) Methanol/ACN (7:3, v/v), (B) 10 mM AA (in DIW)		
Concentration gradient	Time (min)	Methanol (%)	10 mM of Ammonium acetate
	Initial	5	95
	6	20	80
	13	100	0
	15	100	0
	17	5	95
	20	5	95
Total running time	20 min		
Mass analyzer	Waters Xevo TQ-S		
Ion source	Electrospray negative: ESI (-)		
Gas and flow rate	Nitrogen (7 bar), 800 L/h		
Vaporizer temp.	350°C		
Detection method	Multiple Reaction Monitoring (MRM)		