DETRRMINATION OF HEXABROMOCYCLODODECANE IN PLANT SAMPLES FROM CHINA LAIZHOU BAY BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY /TANDEM MASS SPECTROMETRY

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Introduction

Environmental contamination engendered by hexabromocyclododecane (HBCD, $C_{12}H_{12}Br_6$) has become a serious concern because of their possible persistence, bioaccumulative property, biological toxicity[1] and possible adverse effects for human and wildlife health. For the further purpose of environmental risk assessment, efficient and rapid method for trace level measurement of HBCD must be developed.

A number of excellent studies have reported analytical methods and levels of HBCD in environmental matrices （e.g. sediment[2-4], soil[5,6], sewage sludge[7], indoor air[8]） and biota matrices (e.g. milk[9], blood[10], eggs[11]). However, to our knowledge, there is no study relating to plant samples. A new method dedicated to trace level measurement of α-、β-and γ-HBCD in plant samples has been developed using Ultra Performance Liquid Chromatography-Electrospray Ion Source-Triple Quadrupole Mass Spectrometry (UPLC-ESI-MS/MS). In this study, α-、β-and γ-HBCD were detected in plant samples collected near a HBCD manufactory. To our knowledge, this is the first study that using UPLC-ESI-MS/MS to detect HBCD diastereomers and this is the first report on HBCD diastereomer specific concentrations in plant samples from China.

Materials and Methods

The sampling sites are situated at Laizhou Bay of Shandong Province in China. Two sample pools were taken at the north and the south sites that approximately 5-10 m away from one HBCD manufactory on July 28, 2007. Each pooled samples were prepared from five to six individual plants in the same species. We only collected the stems and acicular leaves. After sampling, samples were immediately stored in Car Fridge. Then they were transported back to the lab and stored at -18 ℃ until analysis.

Plant samples were extracted by Accelerated Solvent Extraction (ASE) on a Dionex 300 instrument. An accurately 15 g plant samples were weighed after homogenized and then transferred to a 34 mL capacity stainless steel cell. The cells were preheated for 5min and heated to 100℃ in 5min. Samples were extracted with 1:1 DCM/hexane (v/v) at 1500 psi. The flush volume was 75% of the cell capacity over 3 static cycles (5 min $cycle^{-1}$) and each cycles was followed with a 100 s purge process. A 4 times of system rinse progress was adopted between samples to avoid contamination between samples. Extract was collected in 250 mL special vial and then concentrated to about 1-2 mL by rotary evaporation, and was further cleaned with one multilayer silica column (15 mm i.d.) filled from the bottom with 6 g of activated silica, 3 g of silica/H_2SO_4 44% (w/w), 3 g dried Na_2SO_4. The sample was eluted with 45 mL hexane and 65 mL hexane/DCM (1:3). The second fraction which contained the target HBCD was concentrated and solvent exchanged into 750 μL acetonitrile for analysis.

Separation of the α-、β-、γ-HBCD diastereomers was achieved using a Waters UPLC system with automatic injector. Reversed phase liquid chromatography separation was performed on a C_{18} column (2.1×50 mm, 1.7 μm) from Waters. Elution solvents were methanol (A), 90% acetonitrile/water (B). Mobile phase composition (A: B, v/v) was 85:15 and flow rate was set at 0.25 mL min^{-1}. Injected volume was 3 μL. Mass spectrometric data were acquired in negative electrospray ionization (ESI^-) that performed in multiple reactions monitoring mode (MRM). Monitored fragment ions were the m/z 78.9 bromine ion coming from the $[M-H]^-$ > $[Br]^-$ transition. Quantification of α-、β-、γ-HBCD was obtained using MassLynx V4.1 software (Waters) based on the ion signal from the m/z 640.6→m/z 78.9 MRM transition. Capillary voltage was 2.5 kv and cone voltage was 20 v. Source temperature was 120 ℃ and desolvation temperature was 350 ℃. Desolvation gas flow was 550 L hr^{-1}、cone gas flow was 130 L hr^{-1} and collision gas flow was 0.15 mL min^{-1}.

Results and discussion

The calibration curves for the determination of the analytes were obtained depending on the ratio of HBCD standard area to concentration of the standard sample. All r^2 values for the calibration curves (12,60,120,300 and 1000 ng mL^{-1}) for each diastereomers were＞0.99. The instrumental detection limits (IDL) which was obtained based on the ratio of signal to noise (3 times greater) were 2.4 pg for β-, γ-HBCD and for α-HBCD it can be a little more lower because of the better instrumental response. Recoveries of two replicate analyses of spiked blank samples were 63.8% and 60.8% with an average value of 62.3% for ΣHBCD and all three diastereomers in procedure blank were not detected. The limits of detection for each HBCD diastereomers in plant samples were 0.06 ng g^{-1} (w.w).

Analyzes of plant samples were realized using the validated protocol. Example of ion chromatograms for plant sample (MS scan, MRM) and standard sample (MRM) are shown in Fig.1 and mass spectrum (γ-HBCD) for plant sample is shown in Fig.2.

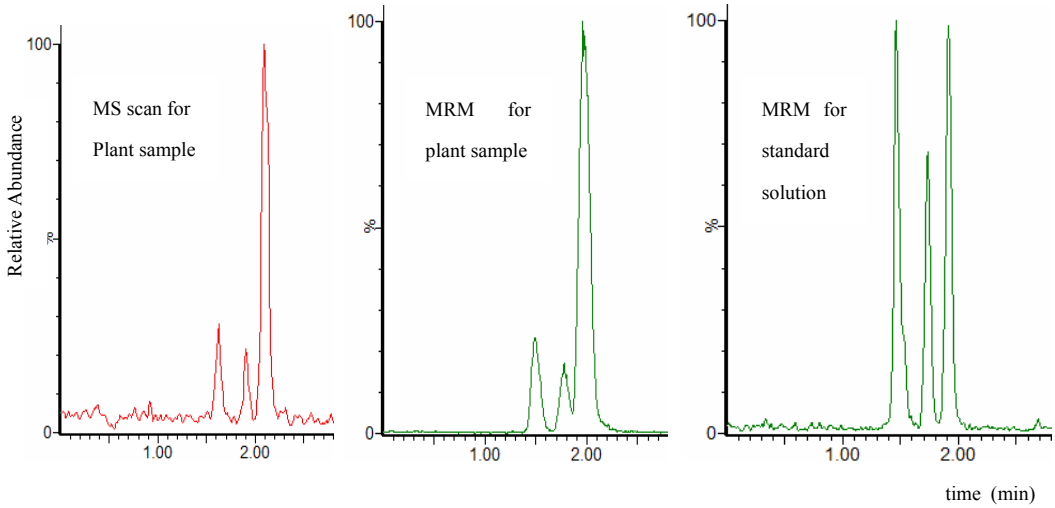

Fig.1 UPLC-ESI-MS/MS chromatograms for plant sample and standard solution (from the left: α-、β-、γ-HBCD)

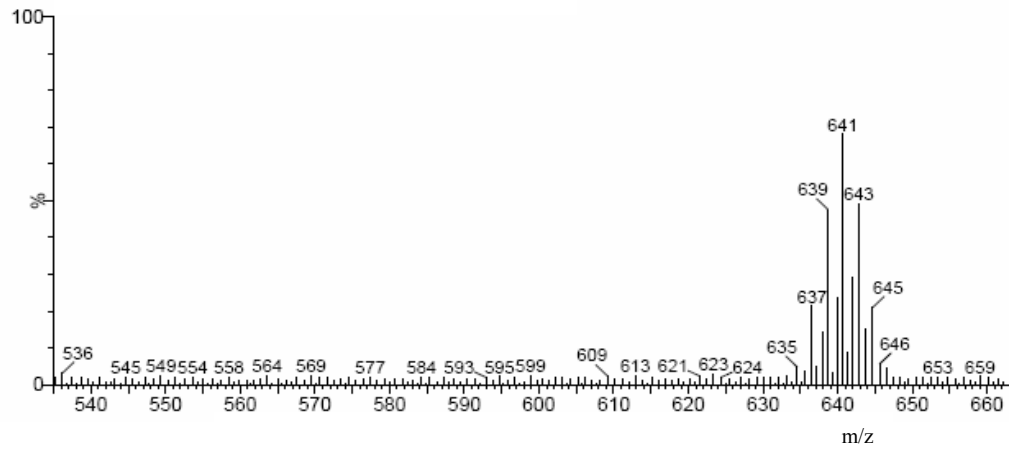

Fig.2 UPLC-ESI-MS/MS spectrum for plant sample (γ-HBCD)

Concentrations of α-、β-、γ-and ΣHBCD in our plant samples are shown in Table 1. Each diastereomers was detected in both samples at a comparable level that indicated the parallel in our analysis. To our knowledge, this is the first report on HBCD in plant samples from China.

Table 1 Concentrations of α-、β-、γ-and ΣHBCD in plant samples

Sampling site	α-HBCD		β-HBCD		γ-HBCD		ΣHBCD
	ng g^{-1} (w.w)	%ΣHBCD	ng g^{-1} (w.w)	%ΣHBCD	ng g^{-1} (w.w)	%ΣHBCD	ng g^{-1} (w.w)
The south site	16.2	24.2%	8.4	12.6%	42.2	63.2%	66.8
The north site	16.2	24.6%	7.8	11.8%	41.9	63.6%	65.9

Using ASE equipment to extract samples can be timesaving. UPLC-ESI-MS/MS can perform very excellent in separation and detection of target compounds because of the rapid procedure and low detection limits. One advantage of our method developed in this study is that the time and sample amount are greatly reduced.

The diastereomer profile of HBCD in our plant samples, which is dominated by γ-HBCD and followed by α- and β-HBCD, is similar with that in HBCD technical mixtures. But compared with the specific individual diastereomer ratio in HBCD technical mixtures (mainly of γ-HBCD (75–89%), α- HBCD (10–13%) and β-HBCD (<0.5–12%))[12], it is apparently that the average ratios of α-/ΣHBCD(24.4%) and β-/ΣHBCD (12.2%) in plant samples have an relatively increase. It was reported that γ-HBCD was the dominated diastereomer in soil samples, but the most abundant diastereomer in air samples was α-HBCD while β-HBCD consistently formed in the smallest amounts[13]. It seems that the ratios in our plant samples are more similar with that in soil samples. This fact may suggest that uptake from soil was the dominated pathway for HBCD in plants. However, Cousins and Mackay[14] suggested that uptake from the soil was important pathway for chemicals with log K_{OW} ＜2.5 and log K_{AW}＜－1. Since the value of log K_{OW} for HBCD is 5.625 at 25℃[15], the uptake from soil should not be the dominate pathway for HBCD in plants. Thus, plants can possibly uptake HBCD either from the air or from soil

through the roots. The diastereomer ratios of HBCD in plants may be contributed to the above pathways.

Acknowledgements

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