FAST ANALYSIS OF POLYBROMINATED DIPHENYL ETHERS IN EGGS USING SELECTIVE PRESSURIZED LIQUID EXTRACTION COUPLED WITH AUTOMATED ONLINE GPC-GC/MS

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Abstract

This article reported the applicability of online GPC-GC/MS for the determination of seven predominant polybrominated diphenyl ethers (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183) in eggs to effectively eliminate matrix interference. Method of selective pressurized liquid extraction (SPLE) using acid alumina as fat retainer was run for cleanup of the predominant PBDEs in eggs. It was selected for its advantages with simpler operation, a minimum of time spent on sample handing to get fat free extracts and the less volume of solvent consuming. After concentration, the extract was directly injected into online GPC-GC/MS operated in negative chemical ionization (NCI) mode and fast quantitated on a 15 m column. Recoveries of spiked samples were between 75.1%-102.0% with RSDs (n=3) ranging from 3.69%-11.47% when spiked at levels of 2 and 20 ng/g, dry mass. The limit of detection (LOD) varied from 0.25-0.34 ng/g, dry mass. The method proposed was proved to be rapid, efficient and reliable for the trace determination of PBDEs in eggs.

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as a class of brominated flame retardants in a variety of consumer products such as electronics, furniture, and textiles [1]. They have been recognized as hydrophobic, persistent pollutants that may bioaccumulate through food chains [2,3]. Therefore, as a kind of fatty food, eggs are likely to be a source of PBDEs contamination in humans. Recently, eggs, especially bird eggs, have been successfully used to monitor PBDEs and as sentinels of environmental pollution and as sentinels of human exposure by food [4,5]. In China, although eggs are an important part of the diet for large population, very little information is available for PBDEs in eggs due to lack of accurate, fast analysis method. Thus, to correctly evaluate the dietary intake and health risk of PBDEs, sensitive and accurate method for determination of PBDEs in eggs is needed.

Due to the complex property of the egg samples, a strict pretreatment was conducted. In this study, after optimization sample treatment procedure, the method of SPLE in combing with online GPC-GC/MS was applied and efficiently removed matrix interference. There have been a few works concerning the PBDEs extraction by SPLE in recent studies [6, 7]. However, to the best of our knowledge, there was no report on the analysis of PBDEs in eggs by on-line GPC-GC/MS. Such an approach greatly facilitated sample handing and reduced the workload for routine analysis.

Materials and methods

Freezing dried sample of 0.5 g egg was ground with 10 g activated acid alumina and subjected to PLE(ASE 300 system, Dionex, USA). A 34 ml extraction cell was loaded by inserting a cellulose filter into the cell outlet, followed by 6 g of activated acid alumina. The sample mixture was loaded into the extraction cell on top of activated acid alumina. The residual volume was filled with hydromatrix (Darmstadt, Germany). The extraction conditions were completed through the following sequences. The extraction cell was filled with a hexane/dichloromethane (1+1, v/v) mixture until the pressure reached 1500 psi (1 psi =6.89 Kpa), and heated to 120 ˚C. After an oven heat-up time of 8 min under these conditions, two static extractions of 16 min at constant pressure and temperature were developed. Extracts were concentrated to dryness by rotary evaporator and re-dissolved acetone/cyclohexane mixed solvent (1+4, v/v). After evaporating under high-purity nitrogen, the residue volume was 0.5 mL.

GPC was equipped with a Shodex CLNpak EV-200AC column (2 mm i.d.×150 mm), a SIL-HTC auto-sampler, and a SPD-10 Avp detector. Acetone/cyclohexane mixed solvent (1+4, v/v) was used as the mobile phase of GPC, and the flow rate was set at 0.1 mL/min. GPC elute from 5 min to 7 min was fractionated by the sample loop. The injection volume was 5 μl and the volume of the sample loop was set to 200 μl.

Online GPC-GC/MS (Shimadzu QP 2010 Plus) was equipped with a PTV-2010 large-volume injection (LVI) device. A 15 m×0.25 mm×0.1 μm (VF-5ms) column was used as an analytical column. A 5 m long DB-5 column was applied as pre-column, and a 5 m, 0.53 mm ID deactivated fused silica capillary acted as a retention gap. The capillary column temperature was held at initial temperature of 82 ˚C for 4.5 min, and increased at 27˚C/min up to 310 ˚C where it was held for 3.5 min. The total GC time was less than 14 min for the separation of seven predominant PBDEs. The pressure was also performed in programs. The initial pressure was set at 91.4 Kpa and then immediately increased to 160 Kpa for 3.5 min with a rate of 130 Kpa/min, then resumed to original optimized pressure.

The temperature of the PTV injector was set at 166 ˚C for the initial 4.5 min, and then increased to 280 ˚C at 120˚C/min where it was held for 11 min. The NCI-MS operating conditions were as follows: Methane was used as reagent gas, while the ion source and transfer line temperature were 220 ˚C and 280 ˚C, respectively. The acquisition was performed in selected ion monitoring and the two most abundant isotope peaks from the mass spectra corresponding to m/z 79 and 81([Br^-]) were recorded.

Confirmation criteria for the identification and quantification of PBDEs include the following: (a) retention time for all m/z monitored for a given analyte should maximize simultaneously ±1 s, with S/N ≥3 for each; (b) the ratio between the two monitored ions should be within 20% of the theoretical. Quantification was carried out by internal standard procedure with BDE-77 as internal standard.

Results and discussion

With traditional preparation procedure, samples were extracted by PLE and followed by acidic silica and multilayer column cleanup and subjected to GC/MS on a short column with 7 m length[8]. However, these classical cleanup steps couldn't eliminate matrix interference that lead to incapable of accurate identification and quantification. Peak of interference appeared early and in large signal in all the chromatograms obtained. As can be seen in Fig. 1(a), matrix interference still existed in the chromatogram of egg control spiked with PBDEs congeners at 20 ng/g dry mass. For the removal of matrix interference, tests of increasing the amount of acid silica and the times being used were performed, still yielding unsatisfied results. Moreover, the traditional preparation procedures were labor- and time-consuming.

Finally, the method of SPLE in combing with online GPC-GC/MS was successfully carried out for the analysis of PBDEs in eggs on the basis of our previous work for fish samples. Figure 1(b) presented the chromatogram of 7 PBDEs in eggs spiked at 2 ng/g dry mass. The chromatogram indicated that even at this low level, all the target compounds were determined with no interference from the complex biological matrix.

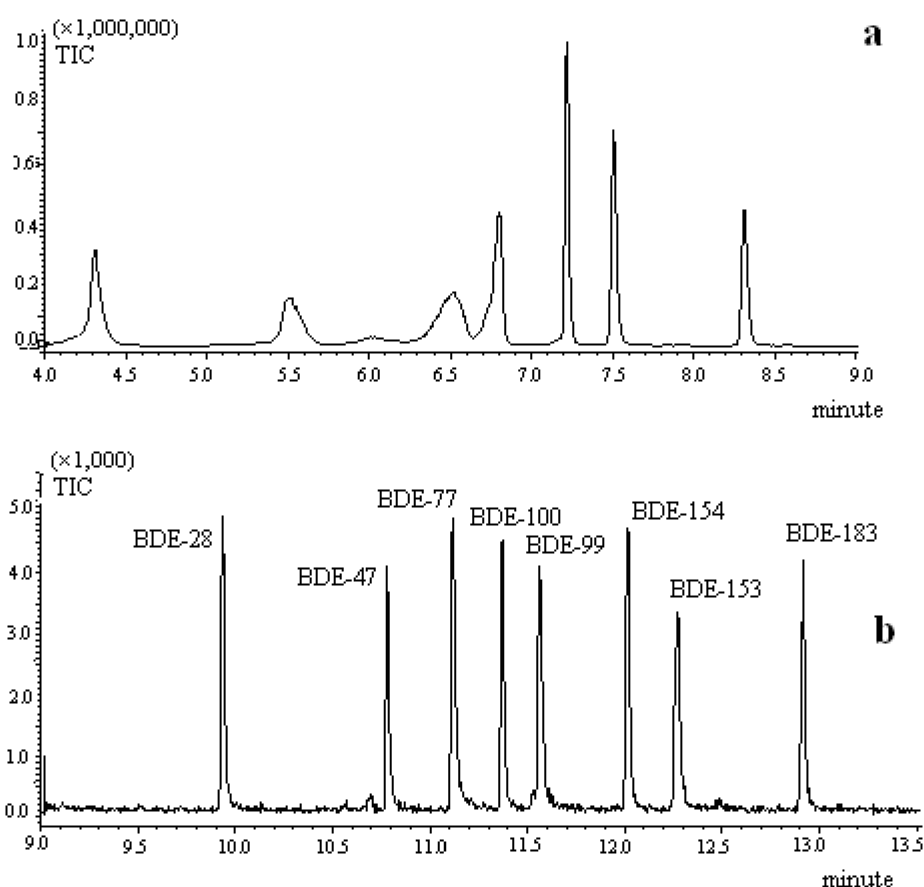

Figure 1. PBDEs chromatograms of spiked egg (a) with cleanup by acidic silica and multilayer column (spiking level 20 ng/g dw) (b) by SPLE and online GPC-GC/MS (spiking level 2 ng/g dw)

Aliquots of 5 μL of PBDEs standard solution(1 ng/mL-100 ng/mL) was injected. Linear calibration curves were plotted by regression of concentration versus the relative peak area of the calibrations standards. Aadequate linearity and higher coefficients were achieved for all the compounds. The LODs of the developed method were based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyses of 0.5 g spiked egg, and on minimal value of signal-to-noise ratio of 3. LODs for the different congeners BDEs ranged from 0.25-0.34 ng/g, dry mass.

Freezing dried sample of 0.5 g egg was employed in the recovery study. The two spiked levels of PBDEs were 2 and 20 ng/g dry mass, respectively. Samples were analyzed separately by the developed methods in this study. Each analysis was performed in triplicate. Recoveries of spiked samples were between 75.1%-102.0% and RSDs (n=3) were ranging from 3.7%-11.5%. Accurate and reliable results indicated that the present method was credible.

Acknowledgements

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