AN ENHANCED LC/MS/MS METHOD FOR THE DETERMINATlON OF TETRABROMOBISPHENOL-A(TBBP-A) IN SOID USING THE QUATTRO PREMIER MASS SPECTROMETER

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Introduction

Tetrabromobisphenol A (TBBP-A) is the most commonly used brominated flame retardant(BFRs) worldwide; over 60,000 tons per year are used, which comprises about 30% of the total amount of brominated flame-retardants [1,2]. It is mainly incorporated as a reactive flame retardant in epoxy resins used in printed circuit boards, but also as an additive in ABS (acrylonitrile–butadiene–styrene) systems[1]. The toxicity of TBBP-A has been studied by in vitro experiments and there are indications for its potency as an endocrine disruptor[3] and immunotoxic compound.[4]. Leakage of the brominated flame-retardants into the environment might occur by evaporation from electronic equipment, as discharges from industry or from wastes at dismantling plants. TBBP-A has been found in sediments[5,6,7], sewage sludge[6,7,8] and indoor air[9]. However, due to the lack of data on its environmental level and temporal trend precludes establishing if the input of TBBP-A is an on-going environmental problem. In China, there are a few of TBBP-A producing plants, especially in JiangSu Province and ShangDong Province. However, almost no studies on the presence of TBBP-A were reported in Chinese environment. The purpose of this research is to develop a simple and rapid method for the determination of TBBP-A level in environmental soil samples.

Materials and Method

All organic solvents were of LC-grade from Merck Ltd. (Merck, USA). All other chemicals were of analytical grade (Beijing, China). Distilled water was purified ‘in house’ using a MilliQ system (Millipore ,MA,USA). Nitrogen gas (99.999%) for drying and for MS were obtained from BABP Corporation (Beijing, China). TBBP-A standard was purchased from Sigma-Aldrich Inc. (St.Louis,MO,USA). $^{13}C_{12}$-TBBP-A standard used for internal standard (ISTD) was purchased from Cambridge Isotope Laboratories Inc. (Andover,MA,USA). Environmental soil samples were obtained outside this TBBP-A producing plant in September 2005 at Shang dong Provinces.

$^{13}C_{12}$-TBBP-A was used as an internal standard to compensate for random errors throughout the whole procedure and for differences between the samples. 400µLof $^{13}C_{12}$-TBBP-A (5µg/mL) was added to the soil sample (30.0g), Soxhlet was used for the extraction of TBBP-A from environmental soil sample. Soil samples were extracted with 200 mL acetone and 50 mL dichloromethane for 22 hours. After the filtrated sample was evaporated by Rotary evaporator, the sample was applied to the preconditioned C-18 SPE cartridge (Alltech 1.5mL, 100mg, USA). The SPE cartridge was washed with 2mL of methanol and 2mL dichloromethane. TBBP-A in the cartridge was eluted with 6mL of methanol, and the eluate was concentrated under nitrogen stream. The final extract is filtered through one 0.45µm syringe filter into a glass HPLC vial. The solution was then placed in the auto-sampler of Waters Acquity UPLC-MS/MS System.

The Waters Micromass Quattro Premier XE Tandem Quadruple Mass Spectrometer with the Acquity UPLC System and MassLynx software delivers a UPLC/MS system. The mobile phase consisted of acetonitrile and water with the following gradient at a constant flow of 0.45 ml/min: initial concentration of acetonitrile at 50% increased gradually to 90% along the first 3 min. A reversed-phase Waters Acquity BEH C18 (2.1×50mm, 1.7 µm) column was used. The column was warmed at 40℃ and the volume of injection was settled at 10μL. The MS operating conditions were summarized in table 1.

Table1. Mass Spectrometer Operation Conditions

Capilary Voltage	3kV
Cone Voltage	50V
Collision Energy	35ev
Source Temperature	120℃
Desolvation Temperature	350℃
Cone Gas Flow	50L/hr
Desolvation Gas Flow	450L/hr
Collision Gas Pressure	Argon at 4e^{-3}m Bar

Results and Discussion

The first step in the development of the method was to establish the mass spectrum of TBBP-A in negative ion mode by infusing a solution of the standard compound. The mass spectrum acquired in full scan (mass range of 100–700) is illustrated in Figure 1. The parent mass was m/z = 542.7 corresponding to the [M-H]$^-$ion. The pseudo-molecular ion [M-H]$^-$ dominates the mass spectrum at m/z = 543 with an isotopic distribution in accordance with the presence of the four bromine atoms on the ion. We acquire mass spectrum in MS/MS mode with the parent ion m/z = 543. Mass spectrum for daughters of 543(ES-) was illustrated in Figure 2. The two daughters ions at m/z = 527.1 and m/z= 447.8 were corresponding to the loss of one methyl group [M-CH_3]$^-$ ion and the subsequent loss of one bromine [M-CH_3-Br]$^-$ respectively. A third daughter ion (M^1)at m/z =290.8 is attributed to the loss of a dibromophenol moiety with a rearrangement of the propyl group to give the 4-isopropylene-2,6-dibromophenol, its isotopic pattern is in accordance with the presence of two bromine atoms. The fourth daughter ion at m/z = 248.9 was corresponding to the loss of [M^1-CH_2-CO]$^-$ or [M^1-C_3H_6]$^-$.]The fifth daughter ion at m/z =168.8 was corresponding to the loss of one bromine atom [M^1-CH_2-CO-Br]$^-$. Thus, the detection of TBBP-A in MS-MS mode with MRM(Multi Reaction Monitoring) is a valuable strategy with daughter ions which are characteristic of the parent molecule.

Recoveries rate for the extraction of TBBP-A in environmental soil samples spiked TBBP-A level from 2.05pg/g to 2.05ng/g was from 30.81 % to 92.52%. The ion chromatogram of soil sample spiked $^{13}C_{12}$-TBBP-A were shown in Figure 3 and Figure 4. Under the above chromatographic and mass spectrometric conditions, TBBP-A extracted from the spiked soil was eluted at a 1.44 min on the reversed-phase Waters Acquity BEH C18 column. We used internal standard method to quantity the concentration in the soil sample. The concentration for soil sample that taken outside the TBBP-A producing plant was 0.12ng/g (wet weight). In 1983, human first found TBBP-A in sediment and soil in Japan, its concentration was 20ng/g (dry weight). Subsequently, TBBP-A has been detected mostly in sewage sludge and sediment[6,7,8] , its concentration ranged from 5 ng/g to 430 ng/g. The release of TBBP-A in the environment may occur through manufacturing, recycling and disposal of various fabrics and materials. The data suggests the TBBP-A has been emitted from this TBBP-A producing plant, the BFRs plant may not apply good measures to prevent BFRs emission into environment.

To our knowledge this is the first method published utilizing SPE and UPLC/MS/MS for determination of phenolic flame-retardant (TBBP-A) in soil sample. The proposed UPLC/MS/MS method for the determination of TBBP-A in soil samples is simpler and more rapid than the previous GC method and HPLC/MS[10], because the derivatization step is not necessary, the sensitivity is improved. That will lead to simple procedure, rapid analysis, and high reproducibility. The full analysis time by UPLC/MS/MS is only 4 minutes. The possibility to extend the method to comprise other brominated flame-retardants such as HBCD will be pursued. Our study demonstrates the effectiveness of Ultra performance liquid chromatography with Micromass Quattro Premier XE MS detection for the quantification of TBBP-A in soil.

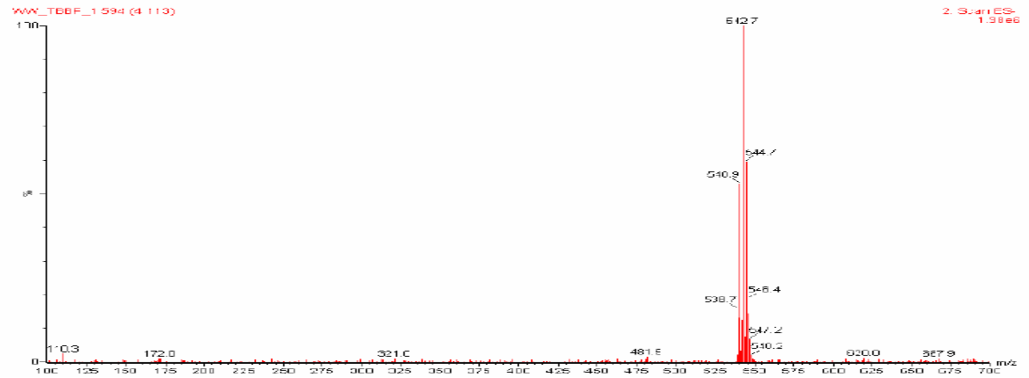

Figure 1. Mass Spectrum of TBBP-A with Full Scan

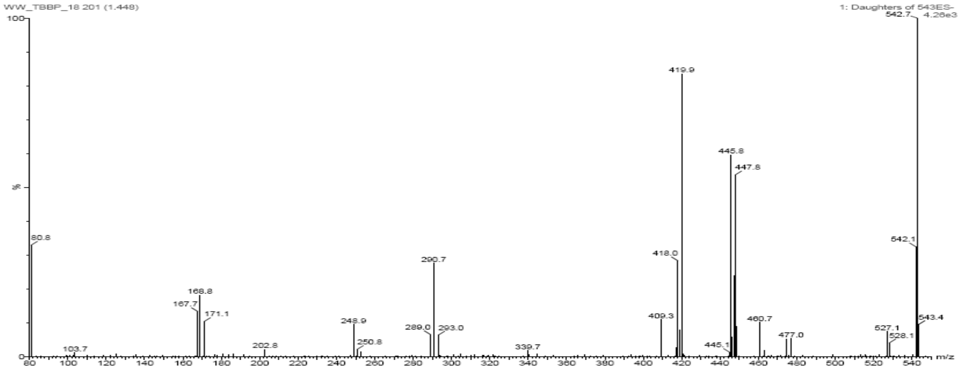

Figure 2. MS/MS Spectrum of TBBP-A with m/z=543 as the parent ion

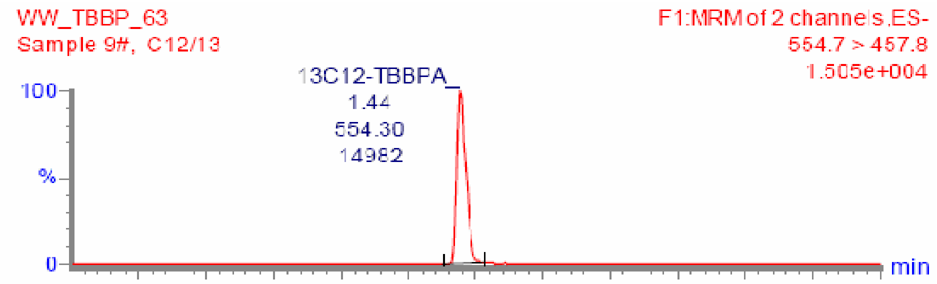

Figure 3. The ion chromatogram of $^{13}C_{12}$-TBBP-A spiked at soil sample

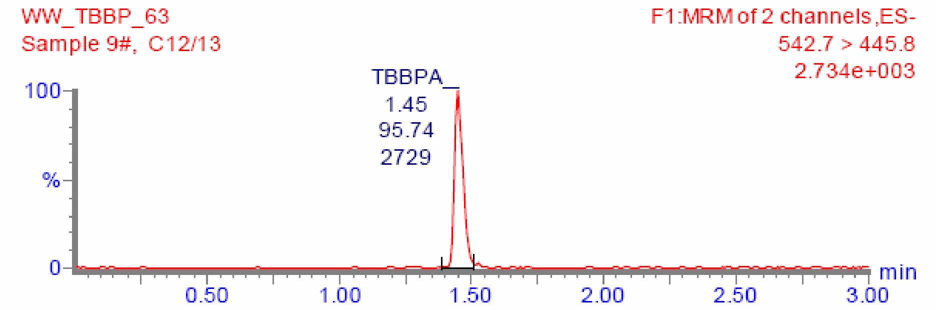

Figure 4. The ion chromatogram of TBBP-A in soil sample

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References

[1] WHO, Environmental Health Criteria 172, World Health Organization, Geneva, Switzerland, 1995.

[2] WHO, Environmental Health Criteria 192, World Health Organization, Geneva, Switzerland, 1997.

[3] I. A. T. M. Meerts, J. J. van Zanden, E. A. C. Luijks, I. van Leewen-Bol, G. Marsh, E. Jakobsson, A. Bergman and A. Brouwer, *Toxicol. Sci* 2000;56, 95.

[4] S. Pullen, R. Boecker and G. Tiegs, *Toxicology* 2003; 184, 11.

[5] I. Watanabe, T. Kashimoto, R. Tatsukawa, *Bull. Environ.Contam. Toxicol* 1983;31:48.

[6] U. Sellstrom , B. Jansson, *Chemosphere*1995;31:3085.

[7]Richard Saint-Louis , Emilien Pelletier,*Analyst* 2004 ;129 :724.

[8] U. Sellstrom , A. Kierkegaard, T. Alsberg, C.Wahlberg, C. deWit, *Organohalogen Compounds,*1999;40:383.

[9] Å . Bergman, C. Ö stman, R. Nybom, A. SjÖdin, H. Carlsson,U. Nilsson, C.A. Wachtmeister, *Organohalogen Compounds,* 1997;33: 414.

[10] Jun Jin, Ying Wang, Hao Peng, Xiaoyan Tang, *Organohalogen Compounds,* 2005;222.