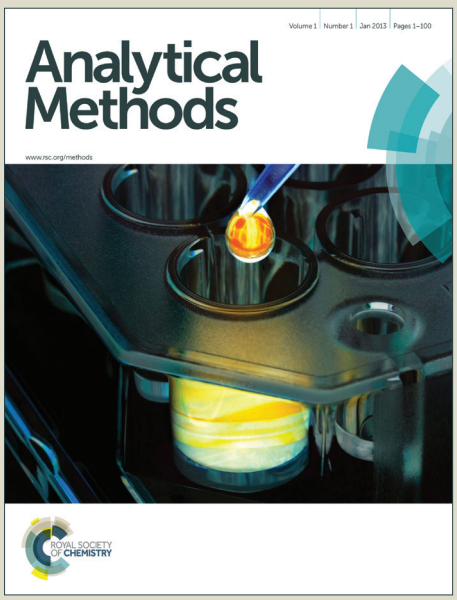
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Low-temperature precipitation for the determination of residual organotin compounds in plant oil using dispersive-solid phase extraction and gas chromatography-mass spectrometry

Yingxia Liu[a,b], Yaqian Ma[a], Lan Guo[b], Aiping Yan[b], Xuejin Mao[a] and Yiqun Wan[a,b*]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, P. R. China

[b] Center of Analysis and Testing, Nanchang University, Nanchang 330047, P. R. China

Corresponding author: Yiqun Wan

Postal address: No.235 Nanjing East Road, Nanchang 330047, Jiangxi, People's Republic of China

Tel.: +86 791 88321370

Fax: +86 791 88321370

E-mail: yqwanoy@sina.com

Abstract:

Most organotin compounds which has been widely used in people's life show serious toxicity effects to human health. In this paper, a simple, low-cost method for the simultaneous determination of four organotins in plant oil samples by gas chromatography-mass spectrometry (GC-MS) has been established for the first time. The method uses dispersive-solid phase extraction (d-SPE) clean-up after a low-temperature precipitation procedure, in situ derivatization with $NaBEt_4$ and liquid-liquid extraction (LLE). The relevant experiment variables influencing the whole results were optimized respectively, the good accuracy and precision were attained under the optimal conditions. The average recoveries obtained for analytes were in the range of 75.6-114.9% with the relative standard deviation (RSD) of 3.9-12.6%, and the limits of detection for each organotin were ranged between 0.19 and 0.33 μg/kg. Finally, these four organotins in different oil samples were detected using this method, which demonstrated the feasibility of our developed method in this study.

Keywords: Organotin compounds; Low-temperature precipitation; Dispersive-solid phase extraction; Plant oil; GC-MS.

1. Introduction

Organotin compounds are widely involved in a lot of human activities both in industrial and agriculture processes, such as pesticides in agricultural crops, fungicides, acaricides, heat and light stabilizers for poly(vinyl chloride) (PVC) plastics, biocides in marine antifouling paints.[1,2] Among these organotins, Tributyltin (TBT) and triphenyltin (TPhT) are mainly used as biocides for protecting vessels, wood preservers or pesticides, monobutyltin (MBT) and dibutyltin (DBT) are commonly employed as stabilizers and catalysts in PVC plastics.[3]

With the extensive use of organotins,[4, 5] it has inflicted great adverse impact on the living environment of people since more than 50 years ago,[6] and resulted in a wide range of threat for human health. According to the relevant researches, the toxicity of organotins increase with the number of organic groups attached to the Sn atom,[7,8] the toxic effects and disorders in the hormonal system of organotins on different kinds of biological species such as mammals and aquatic organisms have been well demonstrated since 1970s.[9,10] In addition, it has been reported that the butyltin compounds have been detected in human liver and blood which imply the direct threat to human health.[11,12] And based on the related immune function researches, a tolerable daily intake content of 0.25 μg TBT/kg bw/day has been developed.[13] Therefore, the determination of organotins need pay particular attention owing to the high toxicity at low concentrations and their widespread commercial use.

After the derivatization, extraction and preconcentration step, different detectors have been coupled with gas chromatography (GC) for organotins speciation,[14] such as microwave induced plasma atomic emission spectrometry (MIP-AES),[15] atomic

emission detector (MIP-AED),[16] atomic absorption spectrometry (AAS),[17] pulsed flame photometric detector (PFPD),[18] flame photometric detector (FPD),[19] inductively coupled plasma-mass spectrometry (ICP-MS),[20] and the high molecule specific mass spectrometry (MS) detector.[21]

The organotins speciation related with the environment samples has been most studied,[22, 23] but researches about organotins in manufactured foods such as alcoholic drinks, honey and fruit juices are still relatively limited, especially edible oils, in spite of quality control of foodstuffs. [3, 24] Considering the application range of organotins, the two most possible paths of human exposure include the direct intake of contaminated food and the indirect exposure from home supplies.[25] There are few researches concerning organotins speciation in edible oil samples. The use of farm chemicals to avoid crops diseases or parasite infection could bring about the raw material contamination of plant oil; meanwhile, according to the process of oil-making and storage with PVC product, the lipophilic organotin maybe presented in the plant oil. Furthermore, the consumption of the plant oil is given priority to edible oil in the global, so developing the analytical methods for quality control of plant oil samples is of great interest.

However, a minimal amount of research has been reported so far on tin speciation in different matrix plant oils. A literature also has reported the determination of organotins in the plant oil samples. [26] Compared with the reported methods where the organotins in edible oils were sensitively analyzed, the oil samples were extracted in low temperature directly in this work rather than cooling the extracts in a dry ice/methanol bath. [26] Another advantage of the present technique was detection method. GC-MS detector for organotins in present method is better than GC-AAS detector in the reported methods.[26] GC-MS was chosen for analyzing

organotins in this work due to its high sensitivity and selectivity, which can be used not only in qualitative analysis but also in quantitative analysis.

In this work, a simple, cost-efficient and effective method was established based on LLE/d-SPE coupled with GC-MS, which was used to analyze and detect organotins in the plant oil samples. The oil sample was added with methanol and kept in freezer (-20 °C) for oil precipitation before going through the derivatization, extraction and clean-up procedure. To the best of our knowledge, the developed method was first applied to monitor the organotins in the plant oil.

2. Materials and methods

2.1 Reagents and Materials

The organotins standards, including monobutyltin trichloride (MBT, 97%), dibutyltin dichloride (DBT, 96%), tributyltin chloride (TBT, 96.5%), and triphenyltin chloride (TPhT, 96%) were purchased from Dr. Ehrenstorfer (Germany). Stock solutions of each organotin dissolved in methanol were 100 μg/mL and stored in the dark at 0-4 °C. Standard working solutions at different concentrations were obtained daily by diluting the stock solutions with methanol. The organic reagents were analytical or chromatographic grade. Acetonitrile, acetone, ethyl acetate, *n*-hexane, acetic acid (HAc), sodium acetate (NaAc) and anhydrous magnesium sulfate ($MgSO_4$) were purchased from Sinopharm Chemical Reagent Limited Company (Shanghai, China). Graphitized carbon black (GCB), primary secondary amine (PSA), neutral aluminum (Alumina N) and florisil which obtained from Beijing Zhenxiang Industrial Foreign Trade Limited Company (Beijing, China) were stored in desiccator before using. The de-ionized water was obtained from Milli-Q system (Millipore, USA). Sodium

tetraethylborate ($NaBEt_4$, 98%) was purchased from Strem Chemicals (USA). The HAc/NaAc buffer solution was prepared by dissolving NaAc in ultrapure water and adjusting the pH to 4.5 by HAc. The 2% (w/v) $NaBEt_4$ solution was prepared daily by dissolving $NaBEt_4$ in methanol before analysis. The plant oil samples were purchased from local markets in China.

2.2 Instrument

An Agilent 6890N series GC equipped with an Agilent 5973I system mass selective detector (MSD) and a 7683 system auto-sampler were used. A HP-5MS (Agilent Technologies, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) fused-silica capillary column was applied to separate the organotins analyte with helium (99.999%) as carrier gas (a constant flow at 1.0 mL/min). GC was manipulated in splitless mode and the injection volume was 1.0 μL and the solvent delay time was 8 min. The injection port temperature was set at 250 °C and the oven temperature was programmed as follows: initially kept at 60 °C for 5 min; increased to 200 °C at 40 °C /min and kept for 1 min; finally increased to 280 °C at 50 °C/min and kept for 20 min. The analysis with MSD was carried out in full scan mode and selected ion monitoring (SIM) mode. The quadrupole analyzer temperatures, ion source and transfer line were operated at 150 °C, 230 °C and 280 °C, respectively. The electron ionization (EI) mass spectrometer was set at 70 eV.

2.3 Analytical parameters

The initial research of organotins with the GC-MS was in full scan mode for getting the characteristic fragment ions and the abundance of each analyte. Each organotin

had formed different characteristic ions depending on the structure as shown in the Fig. 1. The quantitative and qualitative ions of each compound were chosen for the analysis of organotins (shown in Table 1). The total ion chromatogram of the organotins mixed standard solution was illustrated in the Fig. 2.

2.4 Analytical performance

The identification of organotins relied on their different retention times, the abundance ratios of characteristic ions. The retention times of organotins were determined by the standard solutions at 1 μg/mL. The mass ratio scan range was monitored from 100 to 400 m/z to detect the appropriate ions in the SIM mode.

Linearity, limit of detections and correlation coefficients for the detection methodology of organotins were determined by the mixture standard solutions between 0.01 and 1.0 μg/mL. For sample matrix assay, 5.0 g of plant oil sample was fortified at three different addition levels (0.02, 0.1, 0.5 μg/mL) and accuracy and precision were tested. For each concentration level, five replicate tests were performed.

2.5 Sample preparation

5.0 g plant oil samples were taken into 50 ml plastic centrifuge tubes. Each sample was added with 1.0 ml of working mixed standard solution (0.5 μg/mL) for the recovery test, and allowed to stand for half an hour after shaking by a MS2 mini shaker (Guangzhou Yike Lab Technology LTM Co., Guangzhou, China) for 10 min. Then, 10 mL methanol were added and mixed for 10 min with a vortex mixer. For the oil low-temperature precipitation, each centrifuge tube was maintained horizontally in

refrigerator (-20 °C) for 2 h. The supernatant layer of the methanol extract was transferred into the 100 mL separating funnel, and then the substratum was added with 10 ml methanol to extract again in the same way. Finally, the upper layer extract was combined together.

Following that, the derivatization and extraction of sample extracts were performed immediately. The easy, low-cost and precise extract method of LLE was used in this work. 5 mL HAc/NaAc buffer, 1 mL $NaBEt_4$ solution and 20 mL *n*-hexane were added to extract the organotins derivatives for 20 min, and the second extraction was performed in the same way by adding another 20 mL *n*-hexane. Both supernatant were collected in the round-bottomed flask, and evaporated to dryness with a RE-52A rotary vacuum evaporator (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, P. R. China) under a 28 °C water bath, then reconstituted sufficiently with 1 mL of *n*-hexane.

For clean-up step, the reconstituted solution was introduced into a 5 mL micro-centrifuge tube containing 100 mg PSA and 300 mg $MgSO_4$. The tubes were capped tightly and shaken for 2 min with a vortex mixer, and followed by centrifuging at 8000 r/min for 2 min. Subsequently, the upper layer solution of each tube was filtered through a 0.22 μm organic membrane. Finally, the solution was transferred to the vials for GC-MS analysis.

3. Results and discussion

3.1 Optimization of pretreatment conditions

In comparison to the manufactured foodstuffs such as alcoholic drink and fruit juices,

the plant oil contains high amounts of fatty acid and high level of complex matrix. Meanwhile, the liposoluble organotins are hard to separate from the oil. In this study, the analysis and detection of organotins was carried out through the processes of low-temperature precipitation, the in situ derivatization, liquid-liquid extraction (LLE) and the further clean-up with d-SPE. All the trials were made in triplicate.

3.1.1 Optimization of extraction solvent for the freezing extraction

The low-temperature precipitation used in this work was a modification of the method for multiresidue analysis as introduced by Lentza-Rizos et al.[27] Owing to the plant oil can be dissolved in some widespread used solvents such as *n*-hexane, ethyl acetate, dichloromethane and acetone, it is hard to freeze at -20 °C and difficult to form the two-phase separation in these solvents. In laboratory studies, methanol and acetonitrile were chose as the appropriate extraction solvent for the freezing extraction, and there was no obvious difference on the extraction results by making a comparison of the extraction efficiency between them. In order to guarantee the low toxicity of the tests, the methanol as the freezing extraction solvent was employed, which was a common extraction solvent.[28]

3.1.2 Optimization of derivatization conditions

The derivatization conditions of organotins followed a literature procedure[29] with some modifications. Various parameters including the choose of derivatization reagent, the pH of buffer solution and the amount of derivatization reagent $NaBEt_4$ were optimized to improve the extraction derivatization.[10,30,31] The influence of derivatization conditions was investigated by one-factor-experiment at one time. It was found that the 1.0 mL of 2% (w/v) $NaBEt_4$ and a buffer solution of pH 4.5 ensured the quantitative ethylation of organotins s in the plant oil samples and was

used in the following experiment.

3.1.3 Optimization of extraction condition for extracting the derivatives

With the aim of obtaining the best extraction efficiency and the high selectivity, the extraction conditions for extracting the derivatives was optimized based on the sample preparation procedure above. There are some studies reported that several common solvents such as *n*-hexane,[32] dichloromethane[33]and acetone[34] were used for extracting organotins, the extraction efficiency of those three different solvent was compared in this work. In this part, an orthogonal array experimental design was used to get the optimum value of the parameters that affect the extraction yield. The type of organic solvent, extraction solvent volume and extraction time were optimized by a L9 (3^3) orthogonal array design. The factor allocation for the design was shown in Table 2.

A series of sample was fortified with 1.0 mL of working standard solution at 0.5 μg/mL, each level of the experimental trial was made in three replicate measurements, corresponding to a total of 27 tests. The data for the recoveries of four organotins and the average effects (K1, K2 and K3) of each factor at different levels are illustrated in Table 2. The variation ranges of K with the changes of each factor (A, B, C) are 18.3, 3.3 and 2.1, respectively, which implies the influence of different parameters on the experimental results. From the result, we can find the impact of organic solvent is more significant than those of extraction solvent volume and extraction time. It is reported that the process of derivation and extraction could reach equilibrium at about 15 min, and the derivative procedure was completed after 5 min.[35] Based on the obtained results, $A_2B_2C_1$ is shown to be the optimal level, and the experimental

conditions for this work are chose as follows: *n*-hexane as organic extraction solvent, extraction volume at 20 mL and extraction time at 15 min.

3.1.4 Optimization of clean-up conditions

Although most of the plant oil was removed by precipitation, the low content of oil still existed in the extracts which might result in deviation during the qualitative and quantitative detection of organotins. D-SPE clean-up is a kind of fast and cheap sample clean-up technology which possesses the advantage of lower solvent consumption and higher efficiency. So the further clean-up step with d-SPE was performed for getting better results.

The sorbent materials used for d-SPE clean-up tests included GCB, PSA, Alumina N and Florisil. Furthermore 300 mg anhydrous $MgSO_4$ was added to remove the micro quantities of water. The purifying ability of four kind of sorbent materials was evaluated respectively, as shown in the Fig. 3. The GCB, PSA, alumina N and florisil all make a good purification effect for plant oil samples. However, the sorbent materials of GCB, alumina N and florisil increase the recoveries of MBT due to the matrix co-extractants interference. In other words, the other components have the same retention time as MBT in GC chromatogram, which badly affected on quantitative analysis of MBT. Meanwhile, the recoveries of DBT were decreased because of the unexpected absorption by the sorbent materials of GCB, alumina N and florisil. Hence, PSA was selected as the suitable sorbent material for obtaining the lower matrix interferences and better recoveries.

Then the amount of PSA was investigated, and its influence on the recoveries of analyte is summarized in Fig. 4. From this we find the recoveries of four organotins increase when the amount of PSA is increased from 50 mg to 100 mg, but the recoveries of MBT and DBT decrease obviously with further increasing to 200 mg,

and the effect on TPhT and TBT is not obvious. The different influence maybe come from the number of organic groups attached to the Sn, the polarity of different substitution degree of the compounds are different. Hence, in order to maintain the optimal recoveries for all the analytes, 100 mg PSA and 300 mg anhydrous $MgSO_4$ was chosen as the clean-up condition.

3.2 Method validation

The feasibility of using LLE/d-SPE coupled with GC-MS for the determination of organotins in plant oil samples was investigated. Calibration curves were obtained by a series of standard mixtures at 7 concentration levels as follows: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 μg/mL. The correlation coefficients (R^2), limits of detection (LOD) and quantification (LOQ) for the analysis methodology are shown in Table 3. The good linearity of calibration curves are displayed, with the value of R^2 ranging from 0.9919 to 0.9996. LOD and LOQ were obtained using the lowest accessible calibration curve, which was calculated with a signal-to-noise ratio of 3 and 10, respectively. The four organotins could be detected in the range of 0.19-0.33 μg/kg while the determined LOQs were among 0.63-1.10 μg/kg. As there were no available reference materials, accuracy and precision were assessed with oil sample fortified at three different levels of concentration. A mixed standard solution at three levels (0.02, 0.1, 0.5 μg) was added into 5.0 g of sesame oil stored in glass (no organotins founded) obtain three concentrations (4.0, 20, 100 μg/kg), as shown in Table 2, good trueness values are received. The average recoveries for each organotin are ranged from 75.6 to 114.9%, and the RSD for each analyte is lower than 12.6%. On the whole, the developed method was a reliable technique and met the routine analysis requirements for simultaneously screening of organotins in plant oil samples.

3.3 Real sample analysis

The established method was successfully employed to monitor the organotins residue in eight kinds of plant oil samples collected from local markets. As illustrated in Table 4, DBT is detected in 7 samples and TBT is involved in 3 samples among all the analyzed samples which are identified by the ratio of characteristic ions of each analyte. Fig. 5 exhibits the result obtained from oil sample (6#). A literature also has reported the existence of organotins in the plant oil samples.[34] Although studies declared that the application of cyclohexyltins and phenyltins for controlling agricultural pests are available,[36] there is not much about the use of butyltins in agriculture. Besides that, the organotins residual in the plant oil samples also may be from the irrigation water through PVC pipes and the usage of non-food grade PVC materials in processing, storage and transportation facilities. So the present study states the method for analyzing four organotins in the plant oil samples.

4. Concluding remarks

An approach for the simultaneous analysis of four organotins in plant oil samples by LLE/d-SPE coupled with GC-MS was first developed, and the low LODs and good validation parameters were achieved for all the analytes. The analytes were separated from the fat component of plant oil by low-temperature precipitation, then derivatized and extracted, and the further clean-up step with d-SPE was carried out. Simultaneous derivatization and extraction, low overall cost and reliable are the main superiority of this method. The application of the established method to analyze the plant oil samples has demonstrated the existence of organotins in some samples. So far there is short of researches on plant oil samples and still no maximum residue limit (MRL) for

organotins in the plant oil samples. Therefore, establishing the precise method for monitoring the organotins in plant oil samples is significant.

The authors sincerely acknowledge the financial assistance of this work by the National Technology support plan of China (2012BAK17B02), the Natural Science Foundation of China (20965005), the State Key Laboratory of Food Science and Technology in Nanchang University (SKLF-MB-201002), the Natural Science Foundation for Young Scholars of China (201105043).

The authors have declared no conflict of interest.

REFERENCES

1 M. A. Champ and P. F. Seligman (Eds.), *Organotin: Environmental Fate and Effects*, 1996, 1-25.

2 E. Rosenberg, *Handbook of Elemental Speciation II: Species in the Environment, Food, Medicine and Occupational Health*, 2005, **2**,1-788.

3 J. Heroult, M. Bueno, M. Potin-Gautier and G. Lespes, *J. Chromatogr. A,* 2008, **1180**, 122-130.

4 R. D. Oliveira and R. E. Santelli, *Talanta*, 2010, **82**, 9-24.

5 M. Hoch, *Appl. Geochem.,* 2001, **16**, 719-743.

6 A. M. A. Abdallah, *Chemosphere*, 1995, **30**, 707-715.

7 K. E. Appel, *Drug Metab. Rev.,* 2004, **36**, 763-786.

8 K. Fent, *Crit. Rev. Toxicol.,* 1996, **26**, 1-117.

9 B. Antizar-Ladislao, *Environ. Int.,* 2008, **34**, 292-308.

10 E. Magi, C. Liscio and M. Di Carro, *J. Chromatogr. A,* 2008, **1210**, 99-107.

11 K. Kannan, K. Senthilkumar and J. P. Giesy, *Environ. Sci. Technol.,* 1999, **33**, 1776-1779.

12 S. Takahashi, H. Mukai, S. Tanabe, K. Sakayama, T. Miyazaki and H. Masuno, *Environ. Pollut.,* 1999, **106**, 213-218.

13 A. H. Penninks, *Food Addit. Contam.,* 1993, **10**, 351-361.

14 Q. R. Ou and C. W. Whang, *Anal. Bioanal. Chem.,* 2006, **386**, 376-381.

15 J. Szpunar, V. O. Schmitt, R. Lobinski and J. L. Monod, *J. Anal. At. Spectrom.,* 1996, **11**, 193-199.

16 G. Becker, K. Janak, A. Colmsjo and C. Ostman, *J. Chromatogr. A,* 1997, **775**, 295-306.

17 N. S.Thomaidis, F. C. Adams and T. D. Lekkas, *Mikrochim. Acta*, 2001, 136, 137-141.

18 J. Heroult, T. Zuliani, M. Bueno, L. Denaix and G. Lespes, Talanta, 2008, **75**, 486-493.

19 T. Hamasaki, *Talanta,* 2013, **115**, 374-380.

20 J. Cavalheiro, H. Preud'homme, D. Amouroux, E. Tessier and M. Monperrus, *Anal. Bioanal. Chem.,* 2014, **406**, 1253-1258.

21 L. Segovia-Martinez, A. Bouzas-Blanco, P. Campins-Falco and A. Seco-Torrecillas, *Talanta,* 2010, **80**, 1888-1893.

22 R. G. De Waisbaum, C. Rodriguez and N. S. Nudelman, *Environ. Technol.,* 2010, **31**,

1335-1342.

23 M. Flores, M. Bravo, H. Pinochet, P. Maxwell and Z. Mester, *Microchem. J.,* 2011, **98**, 129-134.

24 M. Azenha and M. T. Vasconcelos, *Anal. Chim. Acta,* 2002, **458**, 231-239.

25 M. A. Azenha, R. Evangelista, F. Martel and M. T. Vasconcelos, *Food Chem Toxicol,*. 2008, **46**, 767-773.

26 D. S. Forsyth, D. Weber and K. Dalglish. *Talanta*, 1993, **40**, 299-305.

27 C. Lentza-Rizos, E. J. Avramides and E. Visi, *J. Chromatogr. A,* 2001, **921**, 297-304.

28 M. Abalos, J. M. Bayona, R. Compano, M. Granados, C. Leal and M. D. Prat, *J. Chromatogr. A*, 1997, **788**, 1-49.

29 Y. Q. Wan, Y. Q. Ma and X. J. Mao. *Anal. Lett.* 2012, **45**, 1799-1809.

30 M. Vahcic, R. Milacic and J. Scancar, *Anal. Chim. Acta,* 2011, **694**, 21-30.

31 C. H. Tang and W. H. Wang, *Anal. Chim. Acta,* 2007, **581**, 370-376.

32 J. L. Gomez-Ariza, E. Morales, I. Giraldez, D. Sanchez-Rodas and A. Velasco, *J. Chromatogr. A*, 2001, **938**, 211-224.

33 J. L. Gomez-Ariza, E. Morales, I. Giraldez, R. Beltran and J. A. P. Escobar, *Fresenius J. Anal. Chem.,* 1997, **357**, 1007-1009.

34 K. Kannan, S. Tanabe and R. Tatsukawa, *B Environ. Contam. Tox.,* 1995, **55**, 510-516.

35 T. De Smaele, L. Moens, R. Dams, P. Sandra, J. Van der Eycken and J. Vandyck, *J. Chromatogr. A,* 1998, **793**, 99-106.

36 A. J. Crowe, *Appl. Organomet. Chem.,* 1987, **1**, 143-155.

Table 1 Molecular formula, chemical structure, retention time, characteristic fragment ions used for EI/SIM determination of four organotins.

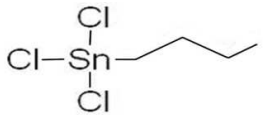
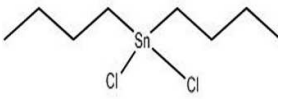
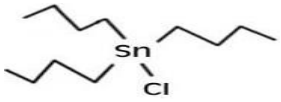
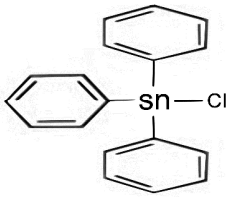
Organotins	Molecular formula	Chemical Structure	Retention time (min)	Characteristic ions（m/z）	Ions used for quantification (m/z)
MBT	$C_4H_9SnCl_3$		8.20	179, 151, 235, 121	179
DBT	$C_8H_{18}SnCl_2$		8.90	179, 207, 151, 263	151
TBT	$C_{12}H_{27}SnCl$		9.53	177, 207,151, 263	177
TPhT	$C_{18}H_{15}SnCl$		12.7	351, 197, 120	351

Table 2 Average recoveries of four organotins obtained from optimization trials by an L9 (3^4) orthogonal array design.

Trial No.	Factor			Recovery (%)				Average recovery
	A[a]	B[b]	C[c]	MBT	DBT	TBT	TPhT	(%)
1	1	1	1	94.3	80.5	102	90	91.7
2	1	2	2	85.1	79.5	105	89.4	89.7
3	1	3	3	85.4	76.6	97.5	86.1	86.4
4	2	1	2	96.6	86.1	128.6	95.8	101.8
5	2	2	3	95	87.5	111.5	104.9	99.7
6	2	3	1	95.8	87.3	111.7	107.4	100.5
7	3	1	3	84.7	84	95.6	63	81.8
8	3	2	1	92.8	87.2	116.6	67	90.9
9	3	3	2	87.8	76.6	120.5	52.5	84.4
K_1	89.3	91.8	93.1					
K_2	104	95.1	92					
K_3	85.7	92.1	91					
Range	18.3	3.3	2.1					
Optimization level	A2	B2	C1					

Ki, mean effect of each factor at level i (i = 1, 2, 3).

[a] Factor A, type of organic solvent: level 1, CH_2Cl_2; level 2, *n*-hexane; level 3, acetone.

[b] Factor B, volume of organic solvent: level 1, 15 mL; level 2, 20 mL; level 3, 25 mL.

[c] Factor C, extraction time: level 1, 15 min; level 2, 20 min; level 3, 25 min.

Table 3 Validation parameters (R^2 of the calibration curve, LOD, LOQ, recoveries at three levels in oils) for four organotins by GC-MS (n=5).

Organotins	R^2	LODs (μg /kg)	LOQs (μg /kg)	Added 4.0 μg/kg		Added 20 μg/kg		Added 100 μg/kg	
				Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
MBT	0.9919	0.33	1.10	75.7	12.6	87.3	3.9	105.8	4.8
DBT	0.9934	0.29	0.96	75.6	7.1	108.9	8.6	99.7	8.0
TBT	0.9996	0.19	0.63	89.8	7.3	114.9	8.1	85.9	5.1
TPhT	0.9906	0.31	1.02	98.6	4.6	91.3	6.6	81.3	6.7

Table 4 Analytical results of four organotins in oil samples (μg/kg, n = 3).

Oil sample	Characteristics (raw material and storage way)	MBT	DBT	TBT	TPhT
1#	peanut oil stored in plastic bottle	ND[a]	2.6 ± 0.28 [b]	ND	ND
2#	nuts blend oil stored in plastic bottle	ND	11.9 ± 0.90	ND	ND
3#	corn oil stored in plastic bottle	ND	9.1 ± 0.51	28.1 ± 2.03	ND
4#	sesame oil stored in glass	ND	ND	ND	ND
5#	blend oil stored in plastic bottle	ND	2.9 ± 0.36	ND	ND
6#	rapeseed oil stored in plastic bottle 1	ND	7.6 ± 0.80	12.6 ± 1.03	ND
7#	rapeseed oil stored in plastic bottle 2	ND	8.4 ± 0.95	ND	ND
8#	sunflower seed oil stored in plastic bottle	ND	12.0 ± 1.05	28.8 ± 2.75	ND

[a] ND, no detected (< LOD)

[b] Data were shown as mean ± SD

List of Figure

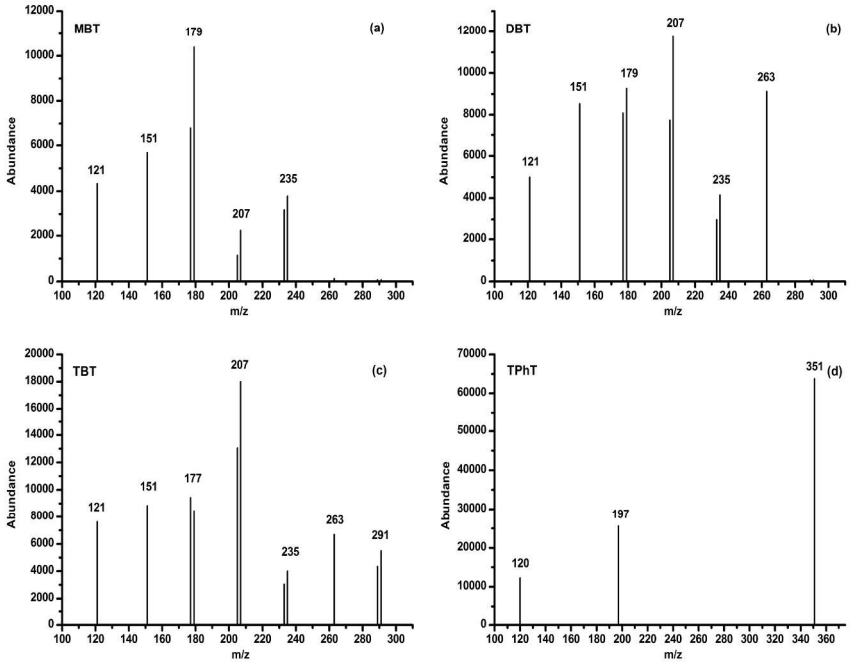
Fig.1 Mass spectrogram of the derivatives of (a) MBT, (b) DBT, (c) TBT, and (d) TPhT produced by electron ionization (EI).

Fig. 2 Total ion chromatogram of organotins standards (0.5 μg/mL): 1. MBT; 2. DBT; 3. TBT; 4. TPhT.

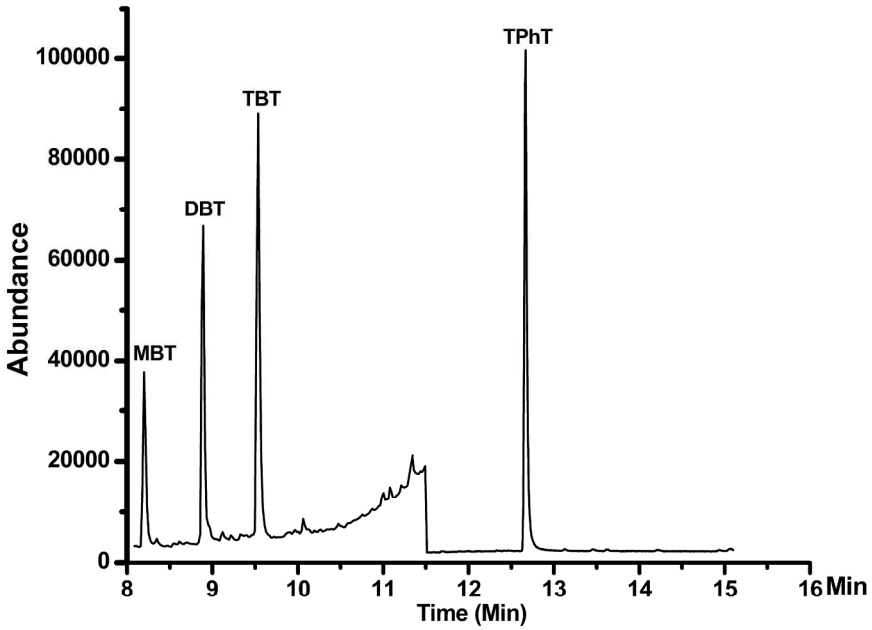
Fig. 3 Chromatograms of oil sample extracts of clean-up with d-SPE using 100 mg PSA (a), 100 mg GCB (b), 100 mg Alumina N (c), and 100 mg Florisil (d).

Fig.4 Recoveries of 4 organotins in oil sample fortified at 0.5 μg/mL with different amounts of PSA for clean-up (n = 3). (1. 50 mg PSA; 2. 100 mg PSA; 3. 150 mg PSA; 4. 200 mg PSA).

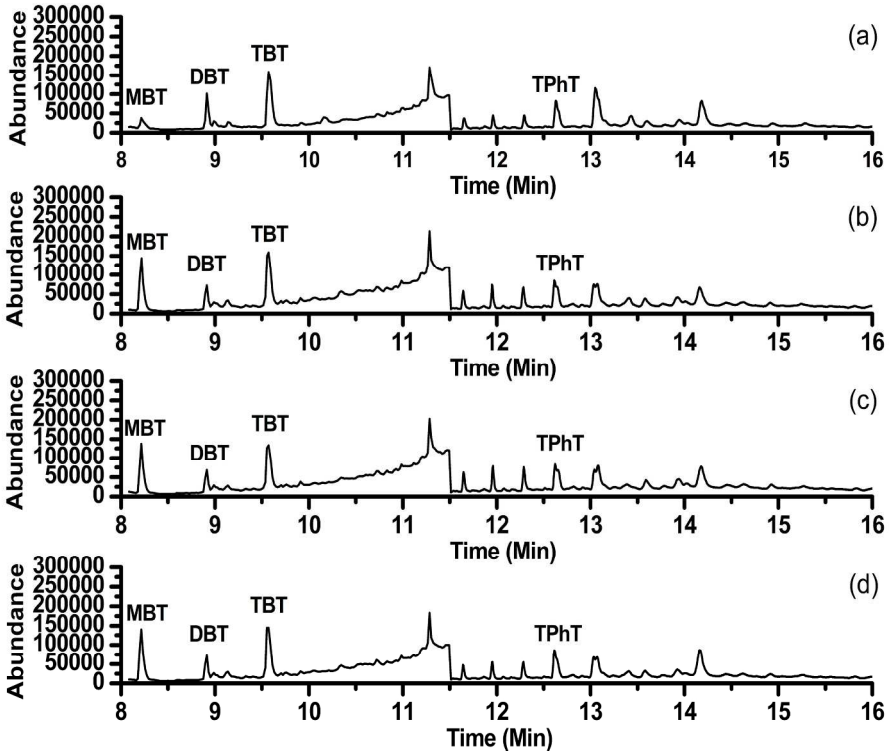
Fig.5 Chromatogram of blank sample extracts exhibiting organotins (the results from sample 6#).



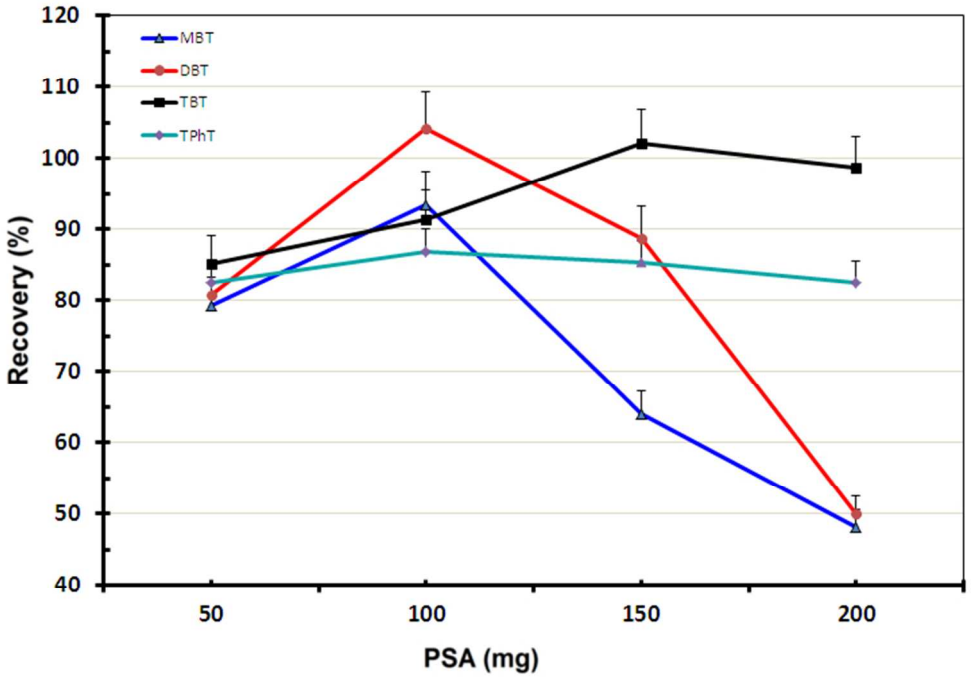
115x89mm (600 x 600 DPI)



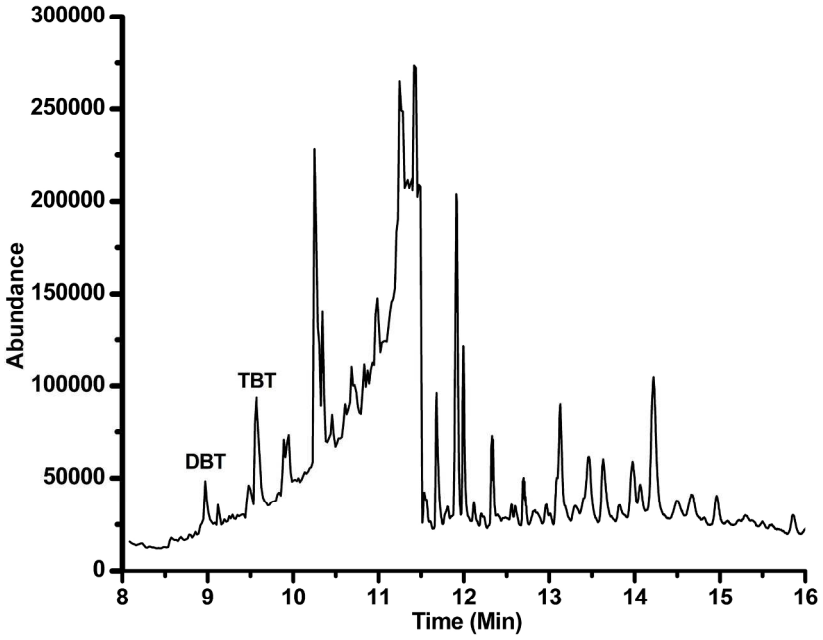
115x89mm (600 x 600 DPI)



115x89mm (600 x 600 DPI)



196x139mm (96 x 96 DPI)



115x89mm (600 x 600 DPI)