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Development of an analytical method for organotin compounds in fortified flour samples using microwave-assisted extraction and normal-phase HPLC with UV detection

Xiupin Wang [a], Lan Ding [a,*], Huarong Zhang [a], Jianhua Cheng [a], Aimin Yu [a], Hanqi Zhang [a], Li Liu [b], Zhihong Liu [b], Ying Li [b]

[a] *College of Chemistry, Jilin University, Changchun 130012, PR China*
[b] *Shenzhen Entry-Exit Inspection and Quarantine Bureau, Shenzhen 518045, PR China*

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Abstract

The normal high-performance liquid chromatography with UV detection was applied for the determination of tributyltin chloride (TBT), triphenyltin chloride (TPhT), tetraphenyltin (TrPhT), triethyltin chloride (TET) and tetraethyltin (TrET) from flour samples. The separation was performed in the isocratic mode on cyanopropyl column with a mobile phase of hexane–acetonitrile–THF (97/1/2). Under the experimental conditions used, quantitative limit of TBT, TPhT, TrPhT, TET and TrET are 0.95, 0.46, 0.97, 0.75 and 0.96 μg/ml, respectively. Microwave-assisted extraction of organotin (OT) compounds at 100 °C with an extraction time of 3 min was described. The extraction of organotin can be finished in acetic acid–hexane (20/80) medium. The quantitative extraction of five organotin compounds was achieved with recoveries ranging from 88 to 101% R.S.D. 3–8%.

Keywords: Speciation analysis; Organotin compounds; Microwave-assisted extraction; HPLC; Flour

1. Introduction

Organotin compounds (OTs), which have been widely used as fungicides, herbicides, insecticides, bactericides, wood preservatives and polymer stabilizers, have a high toxicity towards organisms. The pollution of crop and food as a consequence of the widespread insufflation of herbicides containing OTs in agriculture has been of great concern. OTs were found in crop and food at concentration levels that may exert sublethal and even lethal effects on organisms and mammals. For instance, butyl- and phenyl-tin compounds, particularly tri-substituted species have been identified as endocrine disruptors at very low concentration [1]. As a result, one of the major applications of OTs was used as the active ingredient in antifouling paints [2], so many studies about sediment and biotic samples have been reported, and people ignored research on food and crop. The concern over the toxicity of OTs in crop and food has led us to devote time to the development of accurate and fast analytical methods for their determinations.

The microwave-assisted extraction (MAE) is based on the non-ionising radiation that causes molecular motion by migration of ions and rotation of dipoles, but does not induce changes in molecular structure. It has advantages of simplicity, lower volumes of organic solvent, reduction of extraction time and increase of sample throughput through extraction of multiple samples compared with other sample preparation techniques such as liquid–liquid extraction, solid-phase extraction [3,4] and supercritical fluid extraction (SFE) [5]. With supercritical fluid extraction (SFE), quantitative recoveries were only achieved for tri- and di-organotin compounds, whereas monobutyltin (MBT) and monophenyltin (MPT) were hardly recovered [6–8]. Early published microwave extraction methods for the determination of organotin compounds were applied to the determination of butyltin and phenyltin compounds. The stability of butyltin and phenyltin compounds in a microwave field was discussed and the analysis of sediments was performed by capillary GC–FPD,

* Corresponding author. Tel.: +86 431 5168399; fax: +86 431 5112355.
E-mail address: analchem@mail.jlu.edu.cn (L. Ding).

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with a complicated derivatization procedure in the literature [9]. Pereiro et al. used MAE–GC–FPD to investigate organotin compounds in biological materials [10]. Microwave-assisted extraction of OTs has been shown to be adequate for the determination of butyltin compounds, but phenyltin compounds are degraded to a large extent during extraction.

There were many methods to determine OTs in environmental samples. The main separation techniques involve gas chromatography (GC) [11,12], high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) [13,14] and supercritical fluid chromatography (SFC) [15,16]. The separation can be coupled to tin selective detection methods, such as atomic absorption spectrometry (AAS) [17,18], atomic emission spectrometry (AES) [19] and a fluorescence spectrometry (FS) [11], flame photometric detection (FPD) [12,20], inductively coupled plasma–mass spectrometry (ICP–MS) [21,22] and electrospray–mass spectrometry (ES–MS) [23,24]. For GC analysis, it is necessary to convert the OTs to more volatile and thermostable derivatives. Previously, the main separation modes of OTs using LC included cation-exchange chromatography [25] and reversed phase chromatography [10]. Cation-exchange chromatography and reversed phase chromatography usually can be used to separate two or three OTs. There were a few papers on the separation of OTs by the use of normal phase HPLC. Praet and co-workers successfully separated seven of OTs by using normal phase chromatography, but they did not apply this method to analyze practical samples [26]. In addition, determination of dialkyltin compounds in tissue sample using normal phase chromatography was furnished by Yu and Arakawa [27].

The aim of this work was to investigate analytical method for organotin compounds in flour samples. The application of the MAE for the extraction of OTs was evaluated and extraction temperatures, extraction solvent and extraction time were examined. The normal-phase-HPLC was applied for the determination of OTs from flour samples and the operation conditions were examined. Because many foods ware prepared from the flour, the flour was used a representative of foods and chosen as the sample matrix in the work.

2. Experimental

2.1. Reagents and instrumentation

Analytical grade methanol, hexane and glacial acetic acids were purchased from Beijing Chemical Factory. HPLC grade methanol, acetonitrile, tetrahydrofuran (THF) and hexane were obtained from Fisher Scientific. Tributyltin chloride (TBT), triphenyltin chloride (TPhT) and tetraphenyltin (TrPhT), triethyltin chloride (TET), tetraethyltin (TrET) were purchased from Alfa Products (Danvers, MA). Individual OT standard stock solutions of 1000 μg/ml were prepared by dissolving the OTs in hexane, respectively. They were kept at 4 °C in dark glass bottles until used. Working standard solutions (10 μg/ml) were prepared weekly by diluting the stock solutions in a hexane. High pure deionized water with 18.2 MΩ/cm was applied. An Agilent Technologies 1100 HPLC system (Palo Alto, CA, USA) was equipped with a vacuum degasser, a quaternary pump, a heated column compartment and an injection loop of 20 μl nominal volume. All stainless-steel parts of the HPLC system that come into contact with the sample were replaced by polyether ether ketone (PEEK) components. The chromatographic column used was ultra cyano column (200 mm × 4.6 mm i.d., Restek, USA). A hexane–acetonitrile–THF (97/1/2, v/v) mixture was used as the mobile phase at a flow rate of 0.6 ml min^{-1}. A CEM MDS-2100 microwave digester equipped with ten Teflon vessels (Michem, NC, China) was used for closed-vessel microwave-assisted extraction of organotin from the flour. ESI–MS was carried out on an Applied Biosystem Q-Trap triple quadrupole mass spectrometer (Applied Biosystems Sciex, Foster City, USA) equipped with electrospray ionization (ESI) source.

2.2. Preparation of spiked sample

Five milliliters of hexane, a certain amount of standard stock solution of OTs and 5 g of edible flour were mixed thoroughly. After an equilibration time of 24 h, the solvent was eliminated with a gentle stream of nitrogen. The concentrations of OTs in the spiked sample, for TBT was 20, 40, 60 μg g^{-1}, for TPhT was 10, 20, 30 μg g^{-1}, for TrPhT was 20, 40, 60 μg g^{-1}, for TET was 15, 25, 40 μg g^{-1}, and for TrET was 20, 40, 60 μg g^{-1}, respectively. Spiked flour sample was left to stand for at least a week. Blank samples were prepared in the same way except that the standard solution containing OT was not added.

2.3. Extraction

Microwave-assisted extraction (MAE) system has the capacity to handle a maximum of 10 reaction vessels at the same time. In one typical heating cycle, seven vessels were used for sample treatment. While the remaining one was used as a means to evaluate the blank. A sample about 0.5 g was put into high-pressure PTFE tubes and 4.5 ml of hexane–acetic acid (80/20, v/v) was then added into it. The samples were placed in the microwave digester and subjected to microwave irradiation at 100 °C for 3 min. The extract from MAE was centrifuged at 12,000 rpm for 10 min to remove residues. An aliquot of the supernatant was put into a test tube and diluted to 8 ml using hexane. The sample solution was filtered through a 0.45-μm membrane and injected into the HPLC–UV system.

3. Results and discussion

3.1. Determination of OTs by HPLC

The LC separation of TBT, TPhT, TrPhT, TET and TrET was carried out in the normal-phase mode on a CN column. In order to optimize the chromatographic separation conditions, the effect of two mobile phases consisting of hexane–acetonitrile–THF and hexane–ethanol mixtures with different ratio was examined as shown in Fig. 1. It can be seen that in the case of hexane–ethanol as mobile phases the retention times of TPhT, TBT and TET decrease with increasing percentage of the ethanol, meanwhile those of TrPhT and

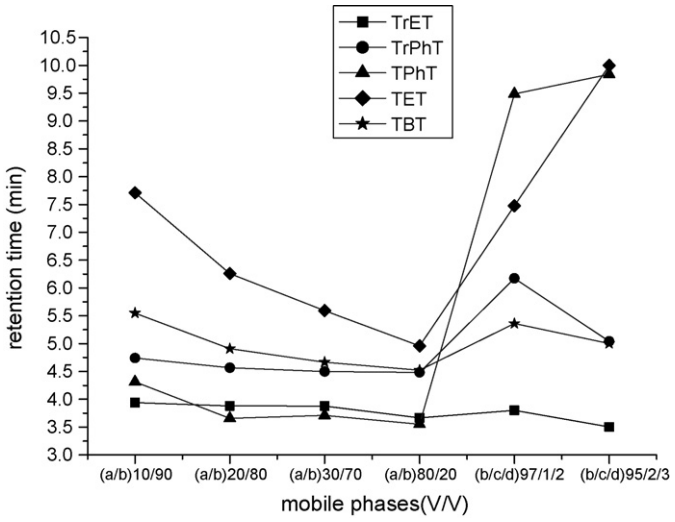

Fig. 1. Retention time of five organotin compounds in different mobile phase. a-C_2H_5OH, b-$CH_3(CH_2)_4CH_3$, c-CH_3CN, d-THF.

TrET are hardly affected. The five organtin compounds cannot be separated at ethanol–hexane ratio (v/v) of 10/90, because the retention times of TPhT, TrPhT and TrET are so close and baseline separation between them cannot be achieved. Praet and co-workers had showed that the separation of the multiple OTs with LC–UV using cyanopropyl-derivatized silica gels column and hexane–acetonitrile–THF (90/4/6) mobile phase could be performed [26]. We attempt to separate organotin compounds according to Praet's method as shown in Fig. 1. A hexane–acetonitrile–THF ratio of (95/2/3 v/v) has given the poor resolution between the five compounds as shown in the last column in Fig. 1. However, a hexane–acetonitrile–THF ratio of (97/1/2, v/v) provided a good baseline separation between the five OTs in less than 10 min as shown in the fifth column in Fig. 1.

Isocratic elution was employed using hexane–acetonitrile–THF (97/1/2, v/v) as the mobile phase at a flow rate of 0.6 ml min^{-1} and the column temperature was kept at 30 °C. The eluent was monitored at 214 nm. Retention times of TrET, TBT, TrPhT, TET and TPhT are about 3.937, 5.329, 5.889, 7.103 and 8.995 min, respectively (Fig. 2). Good linear fit curves ranging from 0.6 to 100 μg ml^{-1} for TBT, TPhT, TrPhT, TET and TrET were obtained. The regression equations and correlation coefficients were TBT: $Y = 22.583X + 39.228$, $R = 0.9931$ ($n = 7$); TPhT: $Y = 32.146\ X + 17.216$, $R = 0.9996$ ($n = 7$); TrPhT: $Y = 41.982X + 54.236$, $R = 0.9983$ ($n = 7$); TET: $Y = 41.472X + 82.754$, $R = 0.9982$ ($n = 7$); TrET: $Y = 23.247X + 42.341$, $R = 0.9984$ ($n = 7$), respectively. The analytical operation can be carried out within 10 min. Compared their retention times with those of standard OT, the organotin compounds can be identified.

To ensure reliable confirmation of identity of the five OTs, two complemental experiments of the liquid chromatography with DAD and MS detection were carried out. The UV spectrograms and MS spectrogram of TPhT obtained were shown in Fig. 3. The TPhT can be confirmed according to the informations shown in Fig. 3. For the other four OTs, the experimental results obtained were similar to that for TPhT.

3.2. Optimization of the extraction procedure

In order to find the suitable extraction solvent for simultaneous extraction of five organotin compounds from one sample, the effects of three kinds of extraction solvents on extraction recoveries of five organotin were studied. The results obtained are shown in Fig. 4. It seems from the Fig. 4 that the solvent HAc-H_2O and HAc-CH_3OH are not suitable for the extraction of TrET, because their recovery are too low and in contrast, HAc-$CH_3(CH_2)_4CH_3$ has definite advantage for extraction of the organotin compounds except for TrPhT. However, use of a higher amount of acetic acid in hexane clearly entails the degradation of TPhT (Fig. 4b). The acetic acid–hexane (20/80), therefore, was chosen as the optimal extraction solvent in all experiments.

Extraction time is important for the extraction of OTs. The effect of the extraction time was studied by exposing the spiked samples to a microwave field at time range between 2 and 10 min for a temperature of 100 °C. Table 1 shows a change in the percentage recovery of TBT, TPhT, TrPhT, TET and TrET

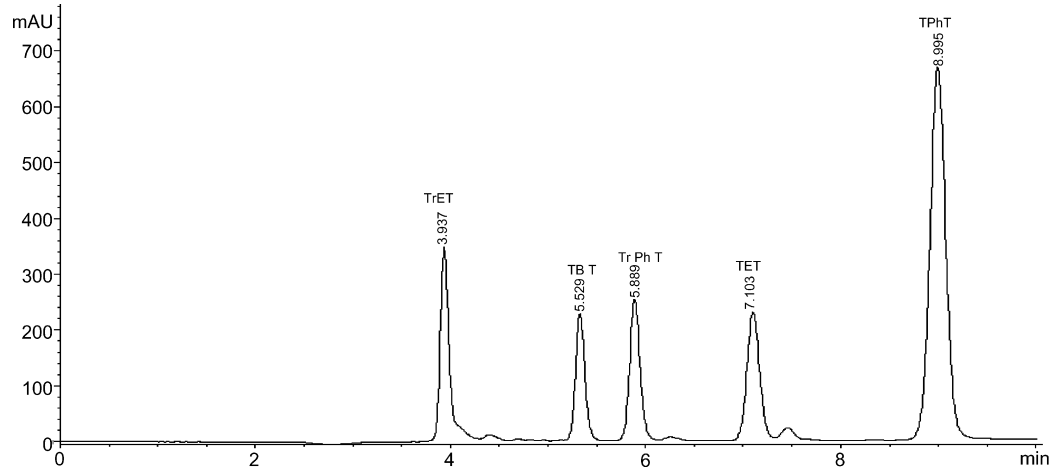

Fig. 2. Normal-phase chromatograms of TPhT, TBT, TrPhT, TET and TrET. Mobile phase: hexane–acetonitrile–THF (97/1/2, v/v). Flow ratio: 0.6 mL min^{-1}.

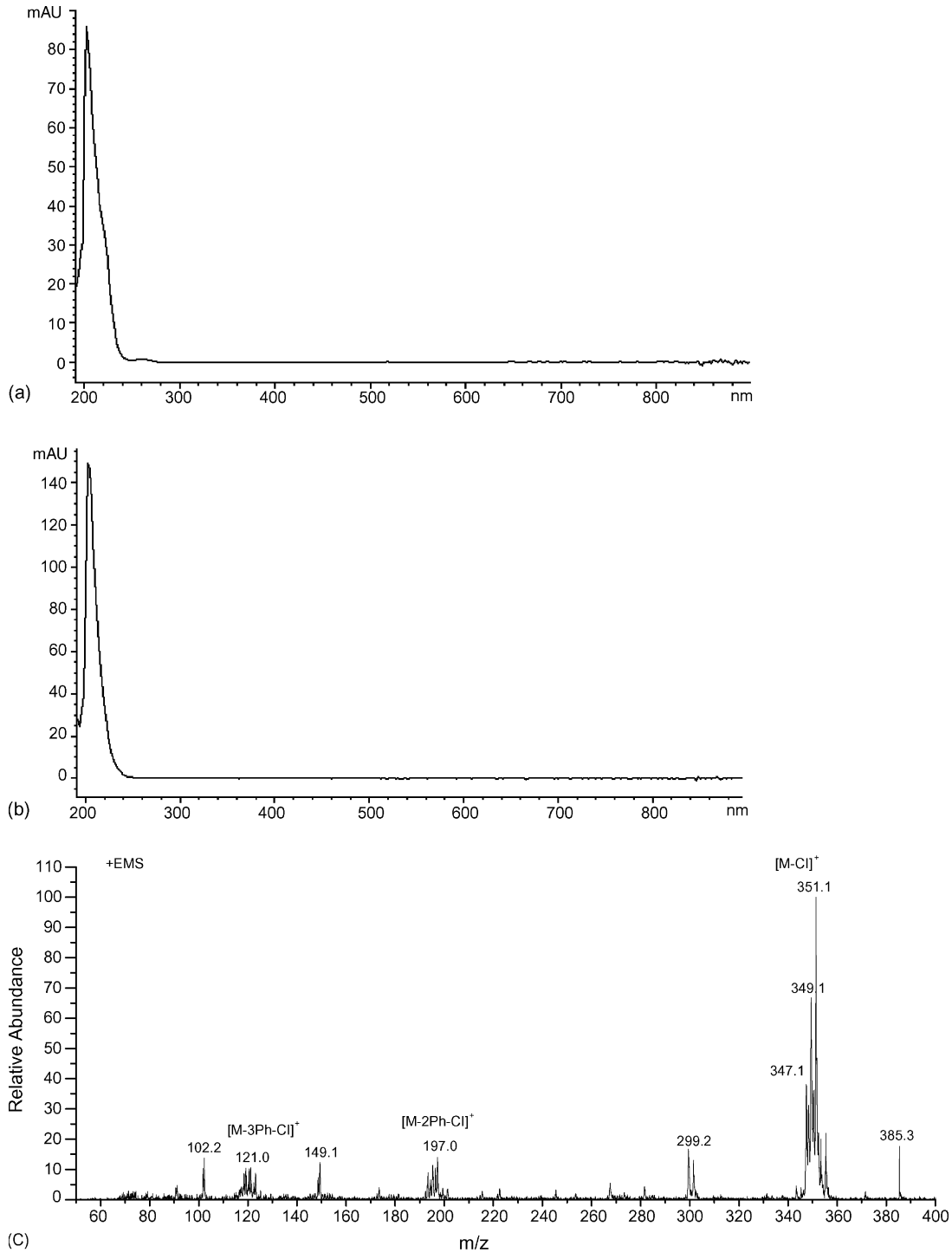

Fig. 3. UV spectrum of standard TPhT (a), UV spectrum of TPhT in extract of flour (b), and mass spectra of TPhT in extract of flour (c).

depending on extraction time. Clearly, the effects of extraction time on the recoveries of all five organotin species are similar. For extraction time from 2 to 3 min the percentage recoveries strongly increase with the increase of extraction time. However, increasing of the extraction time from 3 min to 6 min showed non-significant changes in percentage recovery of OTs (max. 7%), except TrPhT which percentage recovery rapidly decreases after 4 min. When the extraction time is longer than 6 min, the recoveries of all five organotin species decrease with the increase of extraction time. So the optimum extraction time was selected to be 3 min for further experiments.

The extraction temperature controls the energy supplied to the sample. It affects the interactions and the equilibrium rate that control the partition of the analytes between the sample and the solvent. The results in Table 2 show the effect of the extraction temperature on percentage recovering of organotin compounds. The percentage recovery of organotins increase with the increase of extraction temperature from 70 to 100 °C. For all analytes, the

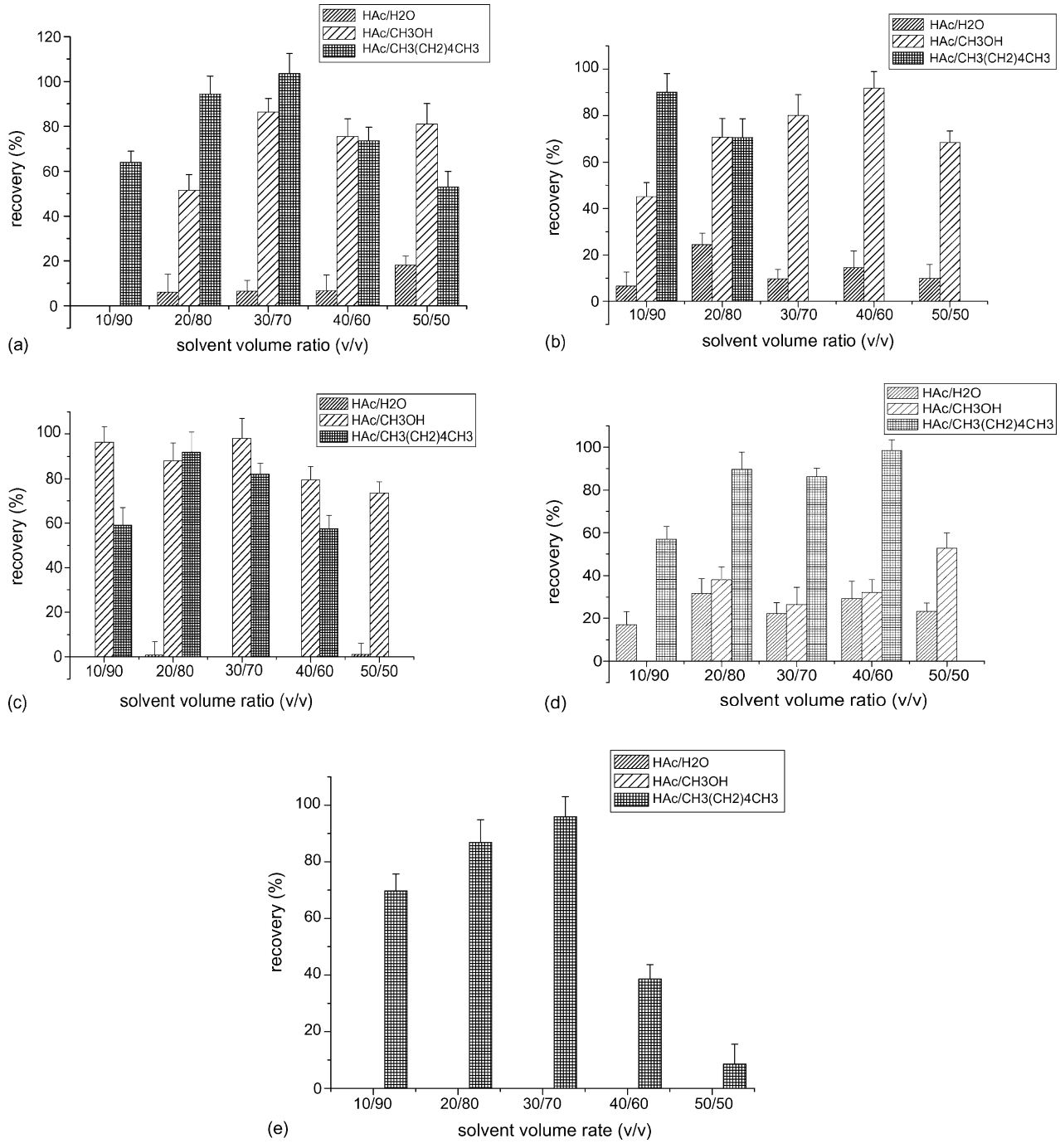

Fig. 4. Effect of three extraction solvents on the recoveries of TBT (a), TPhT (b), TrPhT (c), TET (d), TrET (e). Heating time: 3 min; extraction temperature: 100 °C. Error bars represent standard deviation of three measurements.

Table 1
The effect of MAE time on percentage recoveries of organotins ($n = 3$)

Time (min)	TBT (60 μg/g) Recovery ± R.S.D. (%)	TPhT (30 μg/g) Recovery ± R.S.D. (%)	TrPhT (60 μg/g) Recovery ± R.S.D. (%)	TET (40 μg/g) Recovery ± R.S.D. (%)	TrET (60 μg/g) Recovery ± R.S.D. (%)
2	90 ± 5	90 ± 8	80 ± 5	86 ± 4	83 ± 5
3	93 ± 4	88 ± 5	91 ± 7	94 ± 5	93 ± 5
4	94 ± 8	92 ± 6	93 ± 6	93 ± 3	91 ± 7
5	93 ± 5	91 ± 7	90 ± 5	95 ± 6	90 ± 6
6	92 ± 6	95 ± 8	87 ± 6	96 ± 7	93 ± 5
8	90 ± 8	81 ± 5	80 ± 5	85 ± 5	80 ± 6
10	82 ± 7	83 ± 4	81 ± 3	79 ± 3	70 ± 8

Table 2
The effect of the extraction temperature on percentage recoveries of organotins ($n = 3$)

Temperature (°C)	TBT (60 μg/g) Recovery ± R.S.D. (%)	TPhT (30 μg/g) Recovery ± R.S.D. (%)	TrPhT (60 μg/g) Recovery ± R.S.D. (%)	TET (40 μg/g) Recovery ± R.S.D. (%)	TrET (60 μg/g) Recovery ± R.S.D. (%)
70	85 ± 5	84 ± 8	87 ± 5	89 ± 8	89 ± 6
80	90 ± 8	85 ± 9	87 ± 6	90 ± 5	90 ± 5
90	95 ± 7	90 ± 6	92 ± 5	90 ± 4	91 ± 8
100	101 ± 5	91 ± 7	93 ± 8	89 ± 2	95 ± 3
110	91 ± 6	87 ± 3	86 ± 7	85 ± 6	89 ± 6
120	79 ± 5	74 ± 4	73 ± 6	77 ± 5	78 ± 8

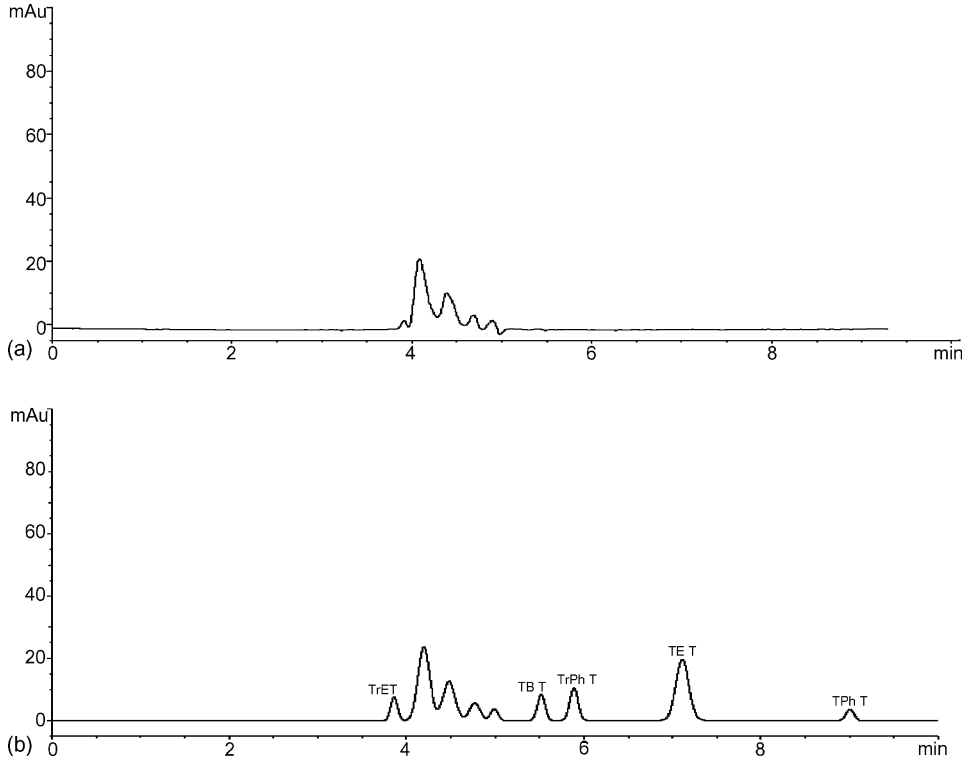

Fig. 5. The chromatograms of the blank flour (a), and spiked sample (b). Mobile phase: hexane–acetonitrile–THF (97/1/2, v/v). Flow ratio: 0.6 mL min^{-1}. The concentrations of TBT, TPhT, TrPhT, TET and TrET in the spiked sample were 20, 10, 20, 15 and 20 μg g^{-1}, respectively.

percentages recovering decrease with the extraction temperature from 100 to 120 °C and this result may be due to the degradation of OTs. The experimental results agree with the Pereiro's observation that TPhT was degraded to DPhT and MPhT and tin [26]. This behaviour is the same for the trisubstituted compounds and tetrasubstituted compounds, which can be easily degraded when the temperature of extraction is higher than 100 °C. So the optimum extraction temperature was selected at 100 °C.

3.3. Analytical performances

The limits of detection (LODs) and the limits of quantitation (LOQs) were calculated using a signal-to-noise ratio of 3 and 10, respectively. The detection limits are 0.31, 0.14, 0.31, 0.22 and 0.31 μg/ml, for TBT, TPhT, TrPhT, TET and TrET, respectively, and the LOQ values were 0.95, 0.46, 0.97, 0.75 and 0.96 μg/ml. Seven replicate determinations on the same day of a standard solution were carried out. For a 1 μg/ml sample, the R.S.Ds. for OTs are between 3 and 5%.

Table 3
Recoveries of five OTs in spiked flour samples ($n = 3$)

Analyte	Added (μg/g)	Found (μg/g)	Recovery ± R.S.D. (%)
TBT	20.0	19.2	96 ± 6
	40.0	37.2	93 ± 3
	60.0	60.6	101 ± 5
TPhT	10.0	9.7	97 ± 4
	20.0	18.4	92 ± 5
	30.0	27.3	91 ± 7
TrPhT	20.0	19.0	95 ± 9
	40.0	36.4	91 ± 4
	60.0	55.8	93 ± 8
TET	15.0	14.6	97 ± 7
	25.0	23.8	95 ± 7
	40.0	35.6	89 ± 2
TrET	20.0	20.0	100 ± 6
	40.0	36.8	92 ± 6
	60.0	57.0	95 ± 3

3.4. Analysis of real sample

The proposed method was applied to the quantitative analysis of five OTs in a real flour samples. As shown in Fig. 5, none of them contained measurable amounts of OTs detected at the sub-$\mu g\,g^{-1}$ level in a real flour samples. To evaluate the accuracy of the method proposed, samples were spiked. The recovery data of three measurements of five OTs from the flour samples are listed in Table 3.

4. Conclusions

To prevent the agrochemical of the organotin from maintenance in the human body through a vegetative chain, it is necessary to establish determination method to determine the organotin compound in food and the flour was chosen as the sample. This study is to present a method for the determination of OTs by MAE normal phase chromatography. The method is sensitive enough to make it suitable for OTs in flour at the low $\mu g\,g^{-1}$ level. The effects of extraction solvent, temperature, and time were investigated. Compared with the conventional methods, the MAE procedure employed provided high extraction efficiency within a short time. The hexane system replaced methanol and water system as extract solvent and the complicated experiment process after MAE can be simplified. The main advantage of normal-phase chromatography is to separate various OTs in very short time (in 10 min). In the work, wheat flour was analysed. The other cereal crop flours, such as rice, corn and barley flour should be analysed by the method proposed in the paper and other food samples also should be analysed by the method with an improvement on chromatographic conditions.

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References

[1] R.J. Maguire, Appl. Organomet. Chem. 1 (1987) 475.
[2] R.J. Huggett, M.A. Unger, P.F. Seligman, A.O. Valkirs, Environ. Sci. Technol. 26 (1992) 232.
[3] E. Magi, M. Di-Carro, P. Rivaro, Appl. Organomet. Chem. 18 (2004) 646.
[4] J. Muñoz, J.R. Baena, M. Gallego, J. Chromatogr. A 1023 (2004) 175.
[5] R.B. Rajendran, H. Tao, T. Nakazato, Analyst 125 (2000) 1757.
[6] Y.K. Chau, F. Yang, M. Brown, Anal. Chim. Acta 304 (1995) 85.
[7] Y. Liu, V. Lopez-Avila, M. Alcaraz, W.F. Beckert, Anal. Chem. 66 (1994) 3788.
[8] Y. Cai, R. Alzaga, J.M. Bayona, Anal. Chem. 66 (1994) 1161.
[9] O. Donard, B. Lalere, F. Martin, Anal. Chem. 67 (1995) 4250.
[10] I.R. Pereiro, V.O. Schmitt, J. Szpunar, Anal. Chem. 68 (1996) 4135.
[11] P. Rodriguez-Gonzalez, J.I.G. Alonso, A. Sanz-Medel, J. Anal. At. Spectrom. 20 (2005) 1076.
[12] M. Leermakers, J. Nuyttens, W. Baeyens, Anal. Bioanal. Chem. 381 (2005) 1272.
[13] L. Guo, F.M. Matysik, P. Gläser, Anal. Bioanal. Chem. 380 (2004) 669.
[14] A.M. Leach, M. Heisterkamp, F.C. Adams, J. Anal. At. Spectrom. 15 (2000) 151.
[15] T. Yamamoto, Y. Noma, A. Yasuhara, J. Chromatogr. A 1017 (2003) 195.
[16] R. Trones, A. Tangen, W. Lund, J. Chromatogr. A 835 (1999) 105.
[17] P. Rychlovsky, P. Cernoch, M. Sklenickova, Anal. Bioanal. Chem. 374 (2002) 955.
[18] D.N. Van, R. Lindberg, W. Frech, J. Anal. At. Spectrom. 20 (2005) 266.
[19] S. Aguerre, C. Pecheyran, G. Lespes, Anal. Bioanal. Chem. 376 (2003) 226.
[20] R. Cassi, I. Tolosa, J. Bartocci, Appl. Organomet. Chem. 16 (2002) 355.
[21] S.J. Kumar, S. Tesfalidet, J.P. Snell, J. Anal. At. Spectrom. 19 (2004) 368.
[22] P. Rodriguez-Gonzalez, J.R. Encinar, J.I.G. Alonso, Anal. Bioanal. Chem. 381 (2005) 380.
[23] J. Wu, Z. Mester, J. Pawliszyn, J. Anal. At. Spectrom. 16 (2001) 159.
[24] E. Gonzalez-Toledo, R. Compano, M.D. Prat, J. Chromatogr. A 946 (2002) 1.
[25] L. Yang, J.W.H. Lam, J. Anal. At. Spectrom. 16 (2001) 724.
[26] A. Praet, C. Dewaele, L. Verdonck, J. Chromatogr. 507 (1990) 427.
[27] T.H. Yu, Y. Arakawa, J. Chromatogr. 258 (1983) 189.