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Analytical Methods

Simultaneous determination of four organotins in food packaging by high-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A simple method based on high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed for the simultaneous determination of organotins—tri-n-butyltin chloride (TBT), triphenyltin chloride (TPT), dibutyltin dichloride (DBT), and diphenyltin dichloride (DPT)—in plastic food packaging. Samples were prepared by ultrasonic extraction with dichloromethane, followed by dissolution in acetonitrile containing 0.1% formic acid, and purification by an MCX column. The extracts were analyzed by LC–MS/MS in multiple reaction monitoring and positive modes with a C18 column; elution was carried out with a gradient of 0.1% formic acid and methanol containing 0.1% formic acid. The limits of detection for TBT, TPT, DBT, and DPT were 0.1, 0.6, 0.8, and 0.3 μg kg^{-1}, respectively. The recovery of organotins in spiked samples ranged from 68% to 113% (relative standard deviation: 0.4–4.2%). The proposed method was successfully employed to identify the target analytes in plastic packaging used for milk and cake.

1. Introduction

Organotin compounds (OTs) are widely used in the transportation, sanitation, agricultural, and food industries; they also find applications in stabilizers, antifouling coatings, pesticides, and germicides (Yi, Leung, Lam, Lee, & Giesy, 2012). However, the widespread application of OTs as biocides in antifouling systems, aquaculture facilities, and agriculture since the 1960s has led to adverse effects on marine and freshwater organisms (Clarke & Smith, 2011). In particular, tri-substituted OTs show the highest toxicity to aquatic life (Qiu, Chan, & Leung, 2011). Previous studies have suggested that OTs might lead to harmful health effects in humans as well, including reproductive and developmental abnormalities, immunosuppression, and some forms of cancer (Antizar-Ladislao, 2008). For example, tri-*n*-butyltin (TBT) and triphenyltin (TPT) can inhibit enzyme activity in ovarian cells at concentrations as low as 2 ng mL^{-1} (Saitoh et al., 2001) and promote the development of prostate cancer cells at 100 nM (Titley-O'Neal, Munkittrick, & MacDonald, 2011; Yamabe, Hoshino, Imura, Suzuki, & Himeno, 2000). In addition, long-term exposure to low OT levels can lead to nuclear anomalies in human red blood cells and DNA mutation (Du, Chadalavada, Chen, & Naidu, 2014; González-Toledo, Compañó, Dolors Prat, & Granados, 2002; Huertas, Morillo, Usero, & Gracia-Manarillo, 2007; Wang et al., 2008). Hence, in September 2008, the International Maritime Organization (IMO) enforced a global ban on the application of OT-based antifouling systems in seagoing vessels (IMO, 2008). OTs have been internationally recognized as persistent toxic substances (PTS). In a recent report published by the Government of the Hong Kong Special Administrative Region, OTs have been highlighted to be among the top seven endocrine-disrupting chemicals of concern in food (Centre for Food Safety [CFS], 2012).

Recent studies have identified OTs in water, crop, and food at concentrations that may be highly toxic to aquatic creatures and carcinogenic to humans (Muenpo, Suwanjarat, & Klepal; 2011; Guðmundsdóttir, Ho, Lam, Svavarsson, & Leung, 2011; Ho & Leung, 2014). Therefore, many countries and organizations have established maximum limit standards for OTs. For example, recent EU directives specify an annual average environmental quality standard (AA-EQS) of 0.0002 μg L^{-1} TBT and a maximum allowable environmental quality standard (MAC-EQS) of 0.0015 μg L^{-1} TBT for all surface waters (Directive, 2000; Directive, 2008). That is, there are very strict requirements for the determination of OT

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levels. However, in Asian countries other than Japan and the Hong Kong Special Administrative Region of China, there is no specific legislation controlling the use of TBT in food. This is probably due, at least in part, to the complex procedures involved in sample preparation, high cost of TBT-sensitive instruments, and trace levels of OT compounds found in seawater, all of which render the analysis of TBT difficult. In the past decades, numerous methods have been developed for the analysis of OT compounds, including gas chromatography–flame photometric detector analysis (GC–FPD), high-performance liquid chromatography (HPLC), atomic absorption spectroscopy (AAS), and inductively coupled plasma-mass spectrometry (ICP-MS) (Camino-Sanchez et al., 2012; David, Jacq, Sandra, Baker, & Klee, 2010; Prieto et al., 2010; Van Hoeck et al., 2009). The main disadvantages of these methods include tedious and time-consuming extraction, and derivatization steps prior to the chromatographic analysis. The use of liquid chromatography with fluorescence detection has also been reported for OT analysis (Carro, González, & Lorenzo, 2013). However, single HPLC is incapable of detecting a large number of OTs, which lack chromophores in their structures. Although ICP-MS is a very sensitive technique, the OT structures cannot be identified and the interference of inorganic tin species cannot be eliminated (Ayanda, Fatoki, Adekola, & Ximba, 2013; Fang, Borggaard, Marcussen, Holm, & Bruun Hansen, 2010; Yamashita, Dozono, Takahashi, & Honda, 2012). Among the detection techniques, tandem mass spectrometry (MS/MS) offers a number of advantages: more selective separation, element specificity, low detection limits, and high sensitivity. However, there few analytical method for identification and quantification of OTs by LC–MS/MS in various samples have been reported.

OTs are well known plasticizers, often used as the main component of plastic products such as rubber, wrapping paper, and food packaging materials. The consequent migration of OTs from the packaging material to the food can pose several health hazards. Variable amounts OTs have been found in foodstuff and beverages, including beans, vegetables, fruits, eggs, milk, meat (Marcic, Lespes, & Potin-Gautier, 2005; Qunfang et al. 2004), and wine (Azenha & Vasconcelos, 2002; Campillo, Viñas, Peñalver, Cacho, & Hernández-Córdoba, 2012). The origin of dietary OTs is considered to be direct or indirect contact between different types of plastic and foodstuff (Rantakokko et al., 2008). Jones-Lepp, Varner, and Hilton (2001) described a new method, in which micro-HPLC–ESI-MS was applied for the detection of dibutyltin (DBT) from water polluted by a potable-water polyvinyl chloride pipe. In the European Union and United States, the use of OTs in plastic food packaging is regulated because of the inherent possibility of these compounds to migrate to the food (Hamasaki, 2013). As the sample preparation procedures for food packaging are more complicated than those used for water samples, a method with higher sensitivity should be developed to detect trace OTs in food packaging. Thus far, an LC–MS/MS method that can simultaneously determine TBT, TPT, DBT, and diphenyltin dichloride (DPT) in food packaging has not been described. Therefore, it is very important to develop a simple, sensitive, selective, and rapid method for the quantitative determination of a large number of OTs in food and in the environment.

The aim of the present study was to evaluate the status of food contamination by OTs and the migratory behavior of these compounds from packaging to foodstuff, and to verify the effectiveness of legal provisions adopted by the Chinese government. In this regard, an accurate, rapid, and highly sensitive analytical method based on HPLC–MS/MS is proposed. Reliable and practical extraction methods for the determination of OTs in complex natural matrices are also of considerable interest. Herein, we developed and optimized liquid–liquid extraction (LLE) combined with solid-phase extraction (SPE) for the extraction of DBT, diphenyltin (DPhT), TBT, and triphenyltin chloride (TPhT) from food packaging samples. In the multiple reaction monitoring (MRM) mode, specific pairs were used as monitoring ions and the background effect was suppressed as compared to that in the selected ion monitoring mode. The parameters for extraction, clean-up, and mass detection were also optimized. Thus, we successfully developed an analytical method that can simultaneously detect the abovementioned four OT compounds in food packaging.

2. Materials and methods

2.1. Chemicals, reagents, and instruments

TBT, TPT, DBT, and DPT were purchased from Sigma–Aldrich (St. Louis, MO, USA). Diphenyltin dichloride was purchased from Acros Organics (USA). Toluene, methanol, chloroform, and methylene chloride were purchased from Beijing Chemical Reagent Co. (China). Deionized water (>18 MΩ cm^{-1} resistance) provided by a MilliQ water (Milli-Q Plus system, Millipore, Bedford, MA, USA) was used for preparing all solutions and standards. All glassware used was rinsed with solvent before use.

2.2. Preparation of standard solutions

Stock solutions of each analyte (1000 μg mL^{-1}) were prepared in methanol and diluted to 5 μg mL^{-1} with methanol prior to storage at −20 °C. Analyte mixtures for working standards and sample fortification were prepared in 0.1% formic acid in water and methanol containing 0.1% formic acid, respectively. All stock solutions and mixtures were stored in the dark at 4 °C and analyzed as soon as possible.

2.3. Sample preparation

Milk or cake packaging was cut into pieces of 0.5 cm × 0.5 cm and 2.0 g were ultrasonically extracted in 10 mL dichloromethane for 25 min. The extractions were evaporated to dryness at 50 °C and residue was re-dissolved in 5 mL of acetonitrile with 0.1% formic acid. After 10 min ultrasonic treatment and concentration to the half volume of the extraction solution, the remaining liquids were purified using a MCX-SPE cartridge as follows: (1) MCX columns were activated sequentially with 2 mL methanol and 3 mL 0.15 mol L^{-1} ammonium hydroxide; (2) 1 ml the extraction liquor was loaded into the column; (3) 2 mL 0.1 mol L^{-1} HCl and 1 mL methanol were sequentially added in the column to wash nonspecific adsorbed impurities; (4) the cartridge was dried under vacuum for about 2 min to remove solvent residues. The analytes retained were eluted with 3 mL 0.3 mol L^{-1} ammonium hydroxide in methanol. The eluent was collected and evaporated to dryness under a stream of nitrogen. It was reconstituted to 1.0 mL with mobile phase. Then the solution was filtered through a 0.45 μm microporous membrane of mixed cellulose ester for LC–MS/MS analysis.

2.4. Instrumentation conditions

LC–tandem MS analyses were conducted on a system consisting of an Agilent 1200 chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an API 2000 mass spectrometer (Applied Biosystems, AB Sciex, Foster City, CA, USA) equipped with a Turbo Ion Spray (ESI) interface.

The OTs were separated by using a reversed-phase analytical column (Agilent Poroshell 120 EC-C_{18} column, 100 × 4.6 mm, 2.7 μm). The column temperature was maintained at 30 °C. The mobile phase used was 0.1% formic acid in water (A) and methanol

Table 1
MS/MS parameters for the determination of four OTs TPT, TBT, DPT and DBT.

Compound	Parent mass (m/z)	Daughter mass (m/z)	Declustering potential (V)	Collision energy (eV)
TPT	350.9	197.0*	30	27
		119.0		86
TBT	291.1	235.1	5	23
		179.1*		14
		122.9		43
DBT	234.9	128.1	25	15
		178.9*		30
		204.2		
DPT	274.1	256.0*	44	63
		179.0		40
		105.8		

* Quantitative ion.

containing 0.1% formic acid (B), and the gradient was set as follows: 95% of solution A for 0.1 min; then, 95% to 0% A over 8 min; and 100% B for 4 min. Finally, the column was equilibrated to the initial conditions for 2 min. The flow rate was 0.2 mL min^{-1}, and the injection volume was 5μL.

The Applied Biosystems API 2000 LC–MS/MS detector was operated at unit resolution in the MRM mode. Depending on the physical and chemical characteristics of the four OTs, the positive mode was further selected to carry out a full scan (Q1 Scan) and identify the parent ions. In the fragment ion scanning mode, the characteristic fragment ion peaks of every compound were determined by changing the voltage conditions. The instrument was equipped with an ESI source. The typical ESI parameters used were as follows: ion spray voltage (IS), 5500 V; atomization air pressure (GS1), 35 psi; auxiliary gas pressure (GS2), 35 psi; curtain gas (CUR), 20 psi; ion source temperature (TEM), 550 °C; entrance potential (EP), 10 V; and collision cell exit potential (CXP), 15 V. The MRM transitions, collision energy (CE), and declustering potential (DP) are summarized in Table 1. System control, data acquisition, and analysis were performed with the AB Sciex Analyst 1.4.2 software (Applied Bioscience).

3. Results and discussion

3.1. Sample pretreatment optimization

The extraction efficiencies of OTs from plastic food packaging with methylene chloride, acetonitrile, hydrochloric acid, methanol and acidified organic solvents were assessed. The extraction efficiency of pure organic solvents was poor, especially for the less polar OTs. We found that the highest recoveries (50–60%) were achieved with acidified organic solvents compared with pure organic solvents (40–50%). Recoveries obtained with pre-extraction of dichloromethane followed by acidified acetonitrile were above 68% of all four OTs higher than with acidified acetonitrile alone. Thus, acetonitrile with 0.1% formic acid and dichloromethane were selected as extraction solvent for further studies.

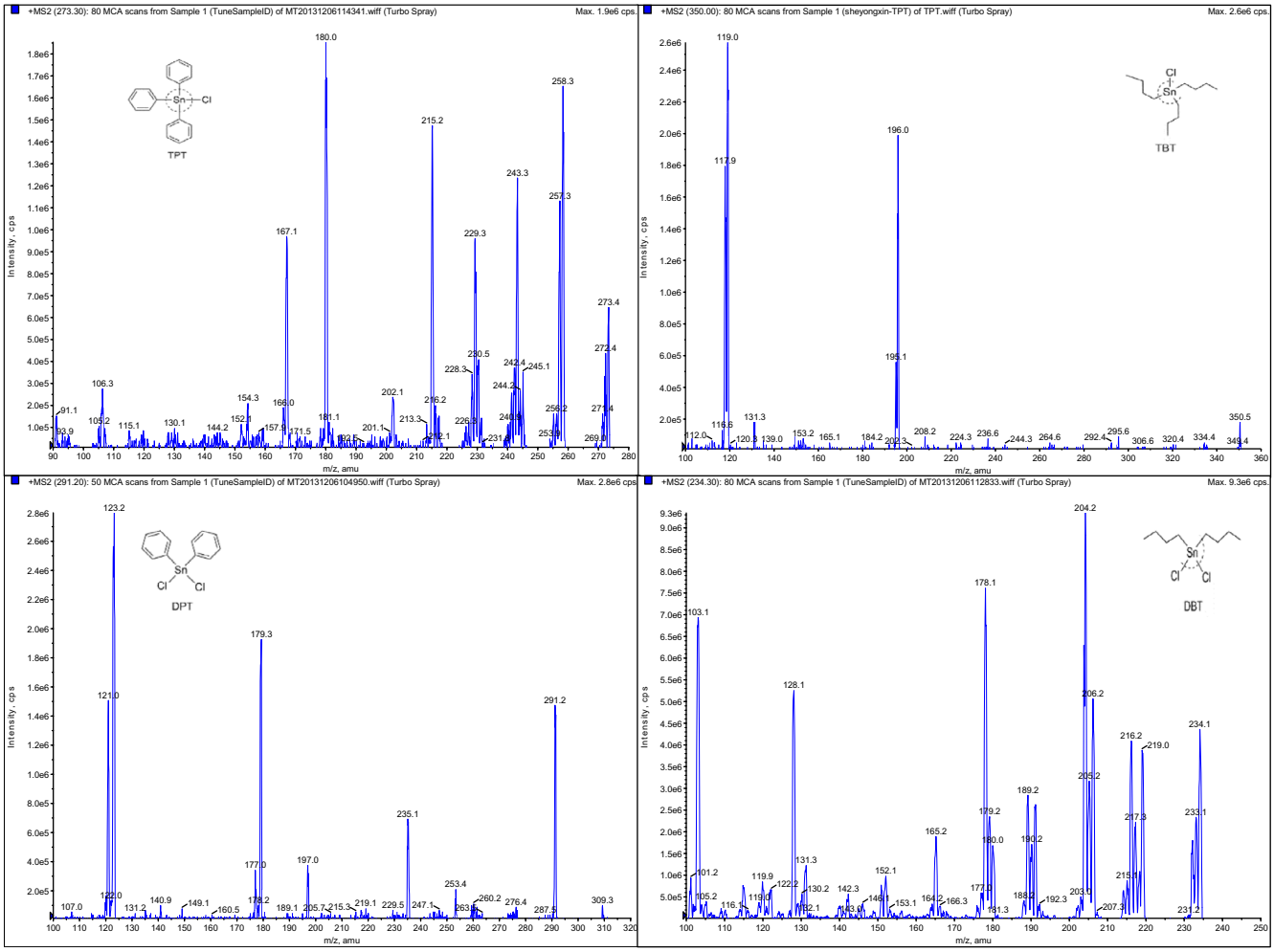

Fig. 1. MS/MS spectra and possible fragmentation mechanisms of four OTs.

HLB, C_{18}, and MCX columns were selected for this study, and various elution solvents with different compositions were assessed for their purification efficiency. The MCX cartridge gave the highest recoveries (>68%) of the four OTs, while the HLB column showed the lowest recovery rates (30–50%). Therefore, the MCX column was selected for SPE in this study. In addition, different elution solvents and their volumes were further optimized for SPE. Herein, 0.1 mol L^{-1} HCl was used for SPE column washes, and 3 mL of a 0.3 mol L^{-1} ammonium in methanol was sufficient to elute most target compounds from the cartridge (recovery rates: 68–113%). Therefore, the sample pretreatment method presented in this study is efficient, shorted and less time consuming.

3.2. Optimization of determination conditions

Since triple quadrupole mass spectrometers can analyze several co-eluting compounds, optimal separation of analytes is not necessary. Since the four OTs had significant differences in their polarities, their retention behaviors on the C_{18} reversed-phase column were also distinct. In order to minimize co-elution of the matrix component and consequent ion suppression, a Waters Surfire C_{18}, an Agilent Poroshell 120 EC-C_{18}, and an Agilent ODS C_{18} were tested. Poroshell EC-C_{18} provided the best peak shapes for these compounds, as well as the best resolution and the shortest chromatographic time; therefore, Poroshell EC-C_{18} was chosen as the most appropriate column for these experiments. Different mobile phase conditions were also studied for optimization of peak shape. As methanol gave better results than did other organic solvents in the present method, a mobile phase composed of 0.1% (v/v) aqueous formic acid solution (A) and methanol containing 0.1% formic acid (B) was chosen for the chromatography. The gradient program described in section 2.4 was used for perfect separation of the four OTs.

In order to optimize the response of the precursor ions, the MS/MS detection method was optimized by individual injections of OTs. Although the $[M+H]^+$, $[M+NH_4]^+$, and $[M+Na]^+$ adducts are generally used in ESI, $[M-Cl]^+$ was selected as the precursor ion for the compounds studied herein. In the MS mode, the most intense signal appeared at m/z 291.1 for TBT, 350.5 for TPT, 273.4 for DPT, and 234.9 for DBT as the parent ion. In the MS/MS mode, the four parent ions underwent fragmentation or rearrangement after entering the secondary mass spectrometer, and a series of ion fragments with different masses were generated. Among these, the characteristic peak at m/z 180 or 179 corresponded to the monobutyltin fragment ion; the peak at m/z 234 or 235 corresponded to the dibutyl OT; the peak at m/z 196.0 represented the phenyl OT; and the peaks at m/z 122 and 119 were characteristic of tin isotopes. Since nucleophiles and electrophiles (e.g., mineral and carboxylic acids) can attack the C–Sn bond and alkali metals induce heterolysis of this bond, the mobile phase was supplemented with 0.1% formic acid to increase the degree of ionization of the organotins. The ESI-MS/MS spectra and possible fragmentation mechanisms for the four OTs under the optimal conditions are shown in Fig. 1. The total ion chromatograms and quantitative ion chromatograms for the four OTs are shown in Fig. 2.

3.3. Method validation

The presence of matrix components in the extract can affect compound ionization during ESI. In this study, method validation was conducted as described in Decision 2002/657/EC. Therefore, quantification of OTs was performed using a matrix-matched calibration curve from fortified blank samples prepared with the same matrix used for the actual samples (Fig. 3). Determination of OTs by HPLC–MS/MS was carried out as described in section 2.4, and method performance was evaluated on the basis of linearity, sensitivity, repeatability, and accuracy.

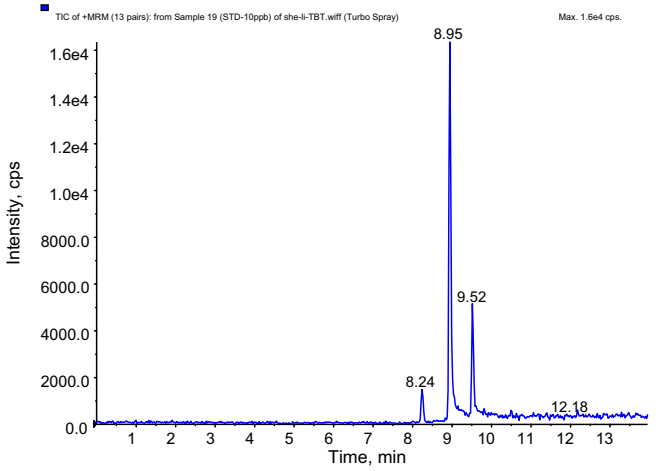

Fig. 2. Total ion chromatogram of four organotin compounds in food packaging samples (samples spiked at 10 μg kg^{-1} for mixed solutions).

To test the linearity of the calibration curve, standard solutions of the OTs (TBT, DBT, TPT, and DPT), in a concentration sequence of 1, 3, 5, 10, 25, and 50 ng mL^{-1}, were added to the blank sample matrices. The linearity of each calibration curve was satisfactory, and the determination coefficients (R^2) were greater than 0.99 for OT concentrations between 1 and 50 μg kg^{-1}. The following linear regression equations were obtained for the four OTs: TBT, $y = 530x + 2.35e+003$, $R^2 = 0.9961$; TPT, $y = 195x + 796$, $R^2 = 0.9966$; DBT, $y = 443x + 1.6e+003$, $R^2 = 0.9934$; DPT, $y = 3.9e+005x + 3.15e+005$, $R^2 = 0.99$. When matrix-matched standards were used for the calibration curve, a recovery rate of 100 ± 2% was obtained for TBT. No matrix effect was observed for the other analytes.

The limits of quantification (LOQs) and limits of detection (LODs) for the method were in accordance with the guidelines issued by international organizations and institutions (Commission Decision 2002/657/EC). To confirm the LODs, 10 blank samples were spiked with OT standards (5 μg kg^{-1}). Then, the LOD for each OT in plastic food packaging samples was determined as the signal corresponding to three times the background noise in each mass chromatogram. The LOQ was calculated from the injection of spiked samples that gave a signal-to-noise ratio of 10. Based on the signal-to-noise ratios, the average LODs obtained in the plastic packaging samples were 0.1, 0.6, 0.3, and 0.8 μg kg^{-1} for TBT, TPT, DBT, and DPT, respectively. The average LOQs were determined to be 0.3, 2.0, 1.0, and 2.5 μg kg^{-1} for TBT, TPT, DBT, and DPT, respectively. The relatively low LODs of the OTs were largely attributable to SPE and mass spectrometric factors. Thus, the LOQs and LODs were satisfactory for assessing OTs in food.

The precision of the method was determined by spiking the blank matrix with the four OTs at three different concentration levels (0.5, 1, and 3 μg kg^{-1}), with six replicate analyses performed at each concentration. The levels detected for these compounds are summarized in Table 2. All the target compounds showed high recovery (68–113%) from various spiked samples, and the relative standard deviation (RSD) at each validation level ranged from 0.4% to 4.2%.

3.4. Analysis of real samples

The combined SPE and HPLC–MS/MS method established in this study was successfully applied for the determination of OT residues in 10 samples, i.e., five plastic packagings each for milk and

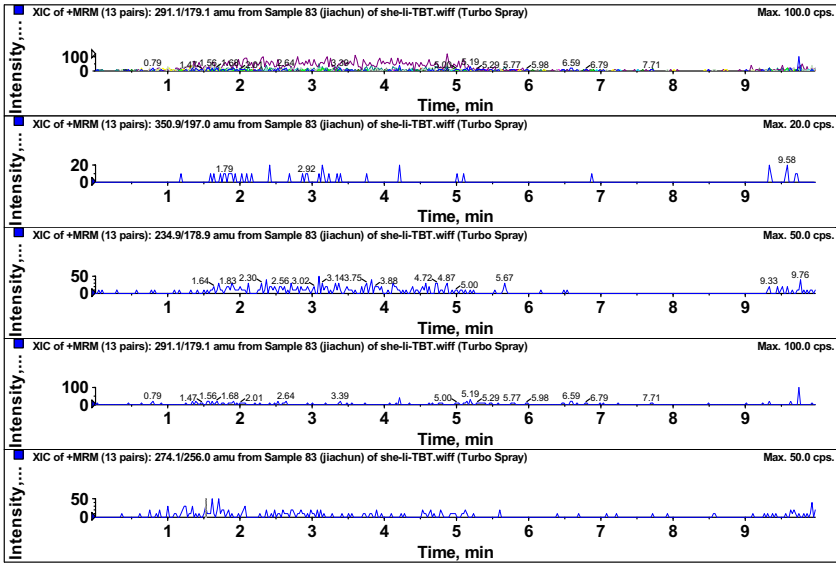

Fig. 3. Chromatogram of four organotin compounds in blank samples.

Table 2
Recovery of four compounds from spiked food packaging samples ($n = 6$).

Compounds	Addition ($\mu g\ kg^{-1}$)	Recovery (%)	RSD (%)
TBT	0.5	70.12–98.56	1.21
	1.0	80.00–102.45	1.07
	3.0	78.10–99.54	1.08
TPT	0.5	74.25–98.54	1.10
	1.0	71.89–88.65	0.40
	3.0	86.44–103.21	1.95
DPT	0.5	89.58–109.54	1.87
	1.0	68.47–100.37	2.08
	3.0	75.56–97.63	1.21
DBT	0.5	68.45–100.89	4.07
	1.0	78.65–99.56	3.98
	3.0	88.41–113.00	4.18

cake, purchased from local supermarkets. TBT was detected at 5.40 $\mu g\ kg^{-1}$ in one milk plastic packaging (Fig. 4), while DPT was detected at 23.45 $\mu g\ kg^{-1}$ in two cake plastic packagings (Fig. 5). These results demonstrated that milk and other packaged foodstuff might be susceptible to OT contamination.

In conclusion, an efficient HPLC–MS/MS method has been developed for the simultaneous determination of TBT, TPT, DBT, and DPT in plastic food packaging. The analytical procedures involve sample extraction by liquid–liquid extraction (LLE) combined with SPE, and detection by HPLC–MS/MS. The proposed method is a convenient tool for detecting traces of OTs in food packaging, and it shows good performance in terms of precision, linearity, LOD, and LOQ. Generally, the simultaneous extraction of many OTs from different packaging samples could be challenging. However, the present approach using based on modified LLE-SPE allows for the

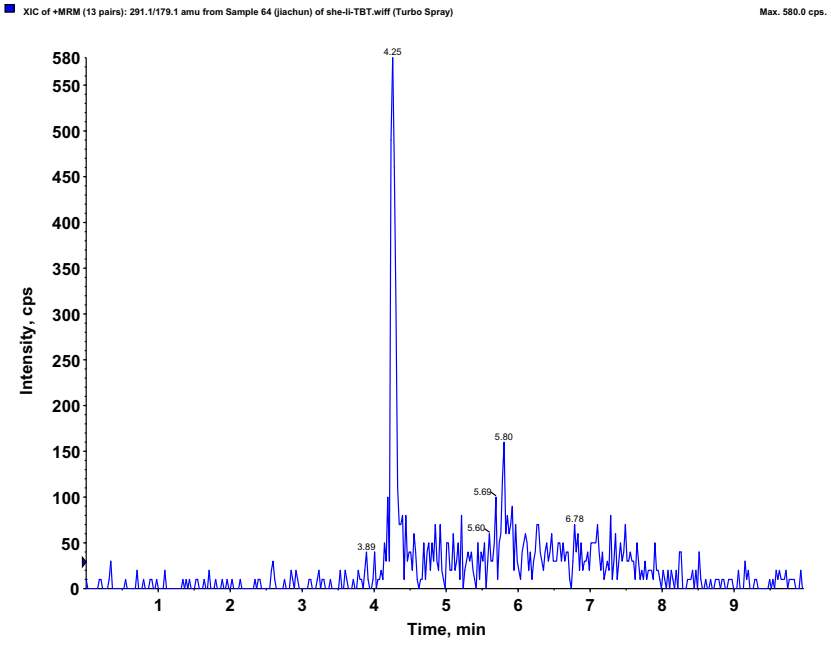

Fig. 4. Chromatogram of TBT detected in milk packaging samples (5.40 $\mu g\ kg^{-1}$).

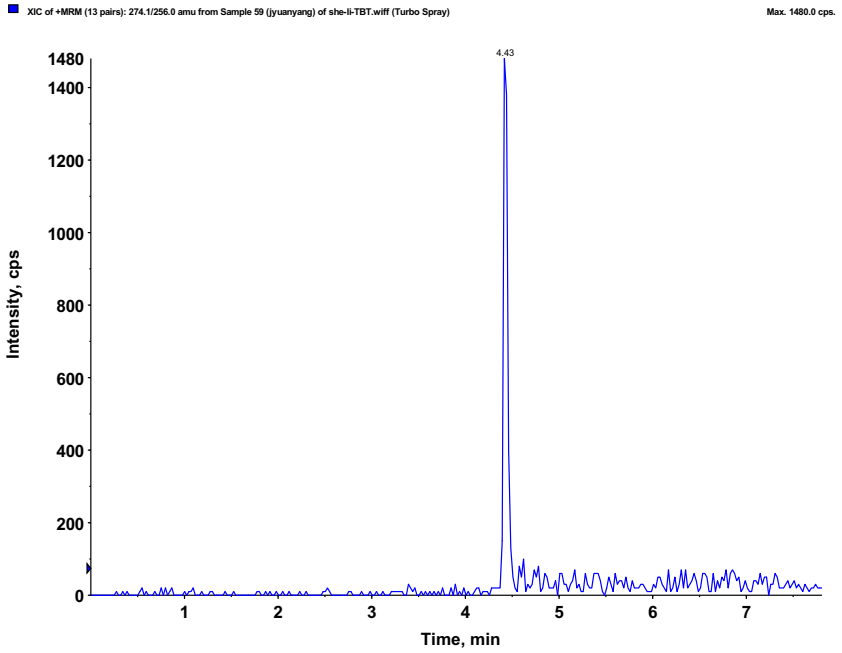

Fig. 5. Chromatogram of DPT detected in milk packaging samples (23.45 g kg^{-1}).

rapid simultaneous extraction of four OTs at a low cost. Hence, the proposed rapid, accurate, and sensitive HPLC–MS/MS method is expected to be a promising technique for the analysis of OTs in food packaging.

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