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Direct Grignard pentylation of organotin-contaminated lard samples followed by capillary gas chromatography with flame photometric detection

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Abstract

During a recent investigation of the organotin-contaminated lard samples, a simple method was developed by direct Grignard pentylation of lard samples and capillary gas chromatography with flame photometric detection using quartz surface-induced tin emission. Using HP-1 capillary column with temperature programming and FPD detector, pentylated tri-, di-, monometyltin, dioctyltin and Sn(IV) can be base-line separated and detected within 20 min. The analysis of pentylated tin compounds by GC–MS confirmed the existence of methyltins and inorganic tin in lard samples, which was agreeable with the results obtained by GC–FPD. The content of organotin compounds was calculated by internal standard method in which methyltripropyltin ($MeSnPr_3$) acted as internal standard. The results showed that these samples were heavily contaminated with mg/g levels of dimethyltin, μg/g levels of tri- and monomethyltin. Among them, one sample contained mg/g level of dioctyltin and one contained a little of inorganic form of Sn(IV). The recoveries of tri-, di- and monomethyltin were 95.7%, 105.5% and 105.7%, respectively.

Keywords: Grignard pentylation; Lard; Organotin compounds

1. Introduction

During the past decades, the usage of organotin compounds in industry and agriculture has markedly increased [1]. The pollution and harmful effects to mankind become more and more serious because of the improper use and management. One disastrous poisoning in the history was known as ''Stalinon'' affair, which happened in France in 1954 and resulted in the death of about 110 people [2]. Stalinon was a proprietary preparation sold in capsules throughout France for the treatment of furuncles and other staphylococcal skin infections, osteomyelitis, anthrax, and acne. In Stalinon, the triethyltin derivative was identified as the toxic contaminant — which resulted in neurological symptoms in many of the afflicted patients. Since then, occasional organotin poisoning affair still occur from careless use in worldwide scope. During this New Year's Days, more than 1000 people in southeast China's Jiangxi province, Longnan and Dingnan county, were poisoned by misusing organotin-contaminated industrial lard as cooking oil, among them, hundreds people were hospitalized and three people died from it. According to the investigation, the

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cause was that the lard they ate was contaminated by organotin compounds. The plastic pails loading industrial lard were perhaps once used as chemical receivers and there remained lots of poisonous organotins. As organotins were oil soluble, they could easily diffuse from the plastic or the wall of the pails and dissolve in the lard. A large scale of this kind of poisoned lard taken would certainly lead to the terrible tragedy.

In this paper, a direct Grignard pentylation method for the preparation of organotin-contaminated lard samples followed by gas chromatography with flame photometric detection was proposed. Because the lard samples contained no compounds with active H^+, such as H_2O, alkylation could be directly realized without the extraction of organotins from the sample matrixes with nonprotic solvent. The proposed method is simple, reliable and can be used for the similar sample matrix.

2. Experimental

2.1. Instrumentation

A GC-9A gas chromatograph (Shimadzu, Japan) was used throughout the experiment. GC operation conditions were as follows: a 25 m×0.32 mm I.D. HP-1 capillary column coated with a film of 0.17 μm was used; the separation was achieved by a temperature program of 50°C (hold for 2 min) to 200°C (hold for 5 min) at 10°C/min; nitrogen (high purity) severed as carrier gas, the column head pressure was 0.26 mPa. A laboratory-made flame photometric detector using quartz surface-induced luminescence (QSIL–FPD) was used, its configuration and analytical figure of merits were described previously [3,4]. The FPD was operated with a hydrogen-rich flame, the flow-rate of hydrogen and air was controlled at 260 ml/min and 90 ml/min. The measurement was carried out by using a 394 nm sulfur interference filter. The temperature of the injector and the detector were set at 220°C and 160°C. Chromatograms were recorded on a SC-1100 PC data processing system.

A QP 5000 GC–MS (Shimadzu, Japan) was used for the identification of methyltin compounds in the contaminated lard. General instrumental operating conditions were given in Table 1.

2.2. Standards, reagents and samples

Trimethyltin chloride (TMT, 98%), dimethyltin dichloride (DMT, 97%) and monomethyltin trichloride (MMT, 97%) were obtained from Aldrich Chem. Co. (USA). Dioctyltin oxide (DOT, pure) was

Table 1
Operation parameters for GC–MS system

GC Parameters:	
Fused silica capillary column	MDN-12 (30 m×0.25 mm I.D.×0.25 μm film)
Injector temperature	260°C
Oven temperature program	initial temp: 50°C; initial time: 3 min; rate: 30°C/min; final temp: 280°C; final time: 9 min
Carrier gas (He) press	52.8 kPa
Split ratio	10
Interface temperature	250°C
MS Scan Parameters:	
Detector volts	1.40 kV
Solvent cut time	2.9 min
Acquisition time	3–15 min
Mass range	40–400
Interval	0.50 s
Threshold	3000
Monitor (TIC and)	spectrum
TIC time scale	10 min
TIC intensity scale	10^6
MC intensity scale	10^6

bought from M&T Chemical Inc. (USA). TMT, DMT and MMT were directly weighed and dissolved in methanol to form a concentration level of 1 mg/ml (as Sn), which were used as the stock solutions. Working standard solution (10 μg/ml) was prepared by diluting the stock solution with cyclohexane just before use. Since it was difficult to dissolve DOT in common organic solvents, the oxide was weighed and dissolved in 12 *M* HCl in a water bath controlled at 40°C. When the white solid completely turned into liquid state, the solution was diluted with methanol to form a concentration of 0.2 mg/ml (as Sn) as a stock solution. Working standard solution (5 μg/ml) was diluted with cyclohexane–acetone (1:1). The method of preparing Sn(IV) standard solution was similar to that of DOT except that the raw material was metal Sn. All of the stock solutions could stand stable at least three months.

The Grignard reagent of *n*-pentylmagnesium bromide (*n*-PeMgBr, 2.0 *M*) were prepared in the laboratory according to the standard synthetic methods [5]. The internal standard monomethyl-tri(*n*-propyl)tin, MeSn(*n*-Pr)$_3$, 2 μg/ml, was obtained by propylation of standard compound $MeSnCl_3$ (10 μg/ml, cyclohexane) [6]. All those alkylated compounds were confirmed by GC–MS.

The contaminated lard samples were collected in Longnan and Dingnan county, from those victim's families. The blank lard was refined from pork fat bought in the retail market.

2.3. Analytical procedure

For the preparation of pentylated standards, 0.10 ml of TMT (10 μg/ml), 0.15 ml of DMT (10 μg/ml), 0.20 ml of MMT (10 μg/ml), 1.0 ml of DOT (5 μg/ml), 0.50 ml of Sn(IV) (10 μg/ml) and 2.0 ml of internal standard $MeSnPr_3$ (2 μg/ml) were added into 0.10 g blank lard oil, then they were dissolved in 0.50 ml cyclohexane and reacted with 1.0 ml of 2.0 *M* (*n*-Pe)MgBr for 15 min under ultrasonic. The excess Grignard reagent was destroyed by carefully dropwise addition of 4 ml of 0.5 *M* H_2SO_4 aqueous solution, followed by an additional wash with 60 ml of de-ionized water. After manually shaken for 2 min, the solution was allowed to stand 5 min for phase separation. The organic layer, containing the compounds of interest, was separated, then dried and purified by anhydrous sodium sulfate (0.2 mg) and florisil (0.8 mg) which had been packed in a glass pipet and pre-washed with 5 ml of cyclohexane [7,8]. The elution with cyclohexane was held on until the volume of the eluted solution was adjusted to 10 ml. 1 μl volume of this sample was used for GC–FPD analysis.

For lard samples, 0.05–0.3 g of lard sample was weighed and dissolved in 10 ml cyclohexane. After 2.0 ml of internal standard $MeSnPr_3$ (2 μg/ml) were added, the rest procedure is similar to the above standard preparation. Suitable amounts of the well processed sample were analyzed by GC–FPD and GC–MS.

3. Results and discussion

3.1. Sample clean-up

Due to the lard samples normally contains no active H^+, such as H_2O, a direct derivatization method by using Grignard reagent can be conducted. After dissolving the lard sample in cyclohexane, suitable amount of Grignard reagent was added to the solution to let the various organotins convert into tetra-substituted ones. We found that sample purification was extremely important after Grignard reaction because of the matrix effects [9,10]. The organic phase contained small amount of dissolved fats and high boiling hydrocarbons, which could cause distort effect at the column and result in peak tailing and negatively affecting the detection sensitivity if it was directly injected into chromatograph. A short Pyrex column (4.5 cm×0.5 cm I.D.) packed with florisil and anhydrous Na_2SO_4 was used for the purification and dryness. Experimental results indicated that by using this column the matrix interference could be eliminated. The determination of the lard samples was carried out through the same process as the standards, which could offset the loss of analytes' adsorption during purification step.

3.2. Selection of derivative group and internal standard

GC methods generally need to include a derivatization step to create volatile and thermal stable

organotin compounds. Alkylation with a variety of Grignard reagents (e.g. methylation, ethylation, propylation, pentylation and hexylation) is the most widely used technique [11]. As the chromatographic column used here for the separation of organotin compounds are basically based on their molecular weight, it was important to choose a suitable derivative alkyl group. Because organotin compounds in natural samples usually contain no *n*-Pr and *n*-Pe groups, here we compared these two groups. From Fig. 1, it could be easily concluded that pentylation was preferred to propylation for the identification and quantitative measurement of methyltin compounds by GC–FPD and GC–MS.

As the structure of internal standard should be similar to that of the object compounds and most of the lard samples contained methyltin compounds which were confirmed below, standard propylated methyltin could act as internal standard which is also different from the pentylated methyltin compounds

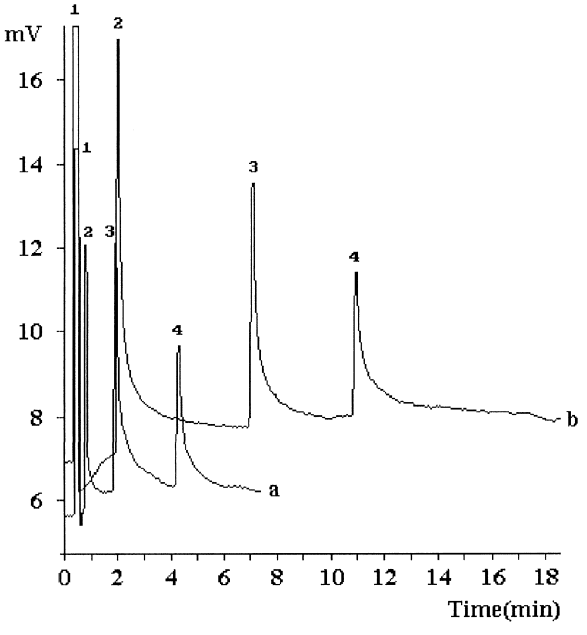

Fig. 1. Comparison of the propylated and pentylated metyltin standards. (a) Propylated metyltin standards. Peaks identified as follows: 1. solvent (1 μl cyclohexane); 2. TMT (0.1 ng as Sn); 3. DMT (0.1 ng) and 4. MMT(0.1 ng). (b) Pentylated metyltin standards. Peaks identified as follows: 1. solvent (1 μl cyclohexane); 2. TMT (0.1 ng as Sn); 3. DMT (0.1 ng) and 4. MMT (0.1 ng).

of the samples. Besides, internal standard's GC peak had better to be located in the middle of all chromatographic peaks, $MeSnPr_3$ was ultimately chosen in the following GC–FPD determination process. From the chromatogram of the five pentylated standard tin compounds after the addition of internal standard (Fig. 2), it was clearly observed that all the peaks were baseline separated.

3.3. Identification of organotin compounds in lard samples

According to the retention time of the standard chromatogram showed in Fig. 3, each compound of interest in the lard samples can be identified. DMT was found in all of the samples, most of the lard samples contain MMT and TMT, only one sample contain DOT and an unknown compound, another sample contain Sn(IV).

The existence of methyltin and inorganic tin compounds in lard samples was further identified by GC–MS. Full scale monitoring with electron impact ionization produced MS spectra of tin compounds characterized by clusters of isotope ion at each fragment was showed in Figs. 3–6. The most significant fragmentation patterns were addressed in these figures. The isotope pattern created by ten tin isotope distributions was particularly useful for recognition of any organotin compound occurring in a sample [12]. Organotin compounds could be identified using the comparison of their mass spectra with the standards'. Excellent matches were obtained in this study. The molecular ion $[M]^{+\cdot}$ was not observed for any of the pentylated tin compounds and the characteristic fragmentation pattern is dominated by successive cleavage of alkyl groups from $[M]^{+\cdot}$ with preferential cleavage of the largest alkyl group accompanied by the formation of $[M-R+1]^+$ or $[M-R-1]^+$ ions as usually observed [13,14]. Identities of m/z ions for spectra shown in the figures were described as below respectively. In the spectrum of $MeSn(n\text{-}Pe)_3$ (Fig. 3), m/z ions of 120, 135, 191, 207 and 276 were the characteristic fragments of this compound which came from the molecular ion's losing one methyl group or one to three pentyl groups. In Fig. 4, m/z ions of 120, 135, 151, 191, 207, 222 and 276 were the special ions belonging to $Me_2Sn(n\text{-}Pe)_2$. In the same way, m/z

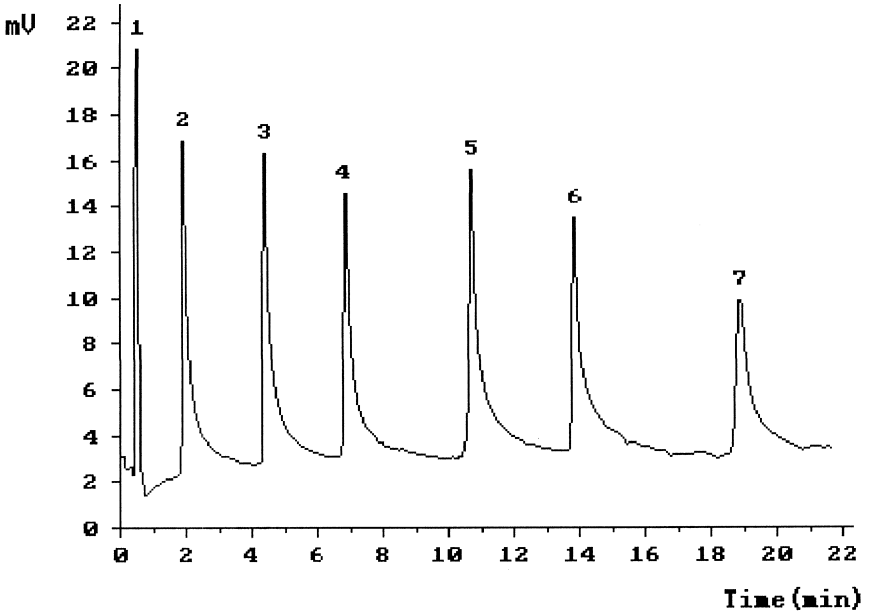

Fig. 2. Chromatogram of all pentylated standards and internal standard. Peak identified as: 1. solvent (1 μl cyclohexane); 2. TMT (0.1 ng as Sn); 3. internal standard (0.2 ng); 4. DMT (0.15 ng); 5. MMT (0.2 ng); 6. Sn(IV) (0.5 ng) and 7. DOT (0.5 ng).

ions of 120, 135, 150, 165, 191, 207 and 221 in Fig. 5 and m/z ions of 121, 191, 262 and 334 in Fig. 6 were the specific ions of $Me_3Sn(n\text{-Pe})$ and $Sn(n\text{-Pe})_4$ respectively. According to the analysis above, the occurrence of methyltin compounds and inorganic tin ion in the lard samples were identified.

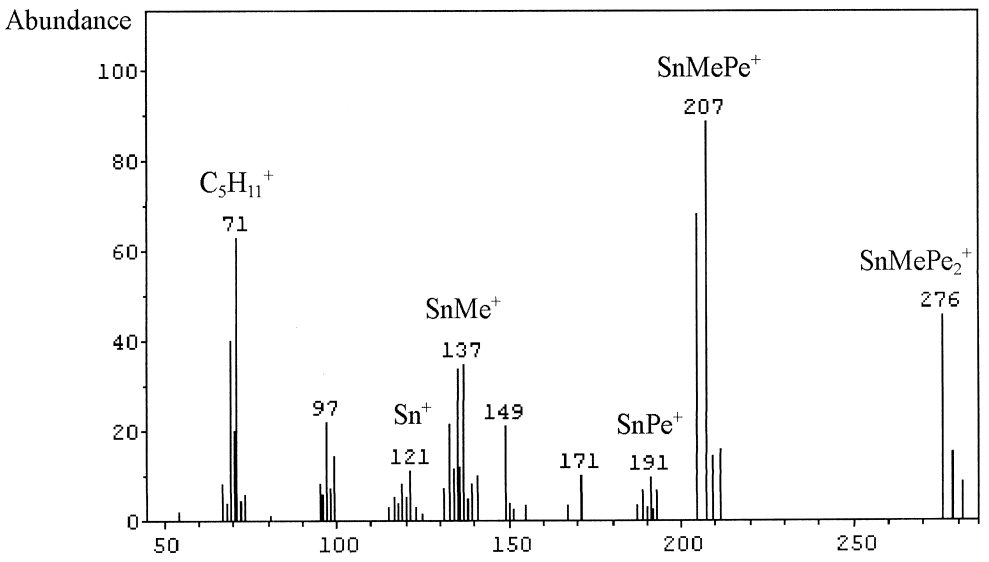

Fig. 3. The EI mass spectra of $MeSn(n\text{-Pe})_3$.

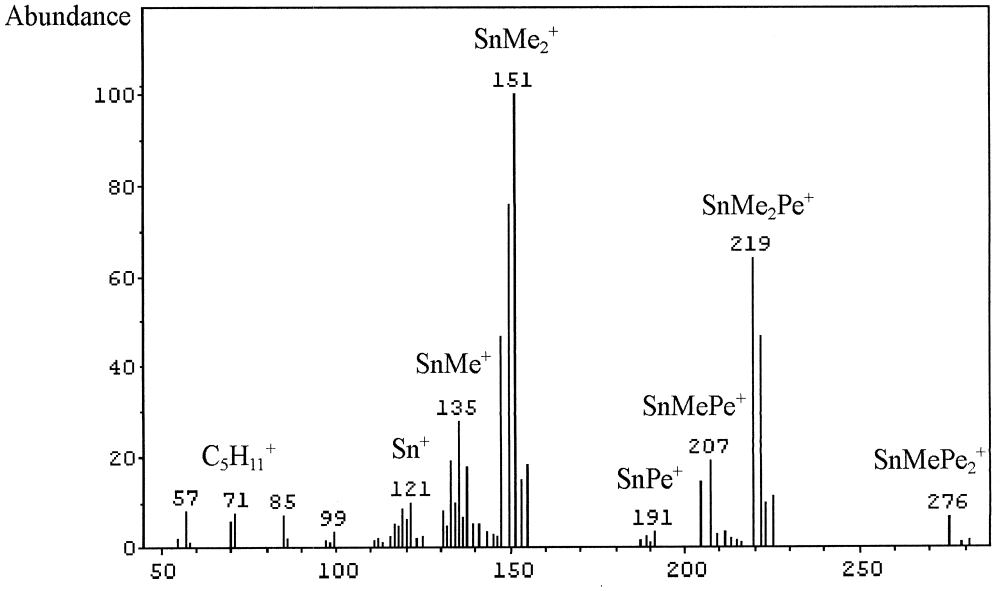

Fig. 4. The EI mass spectra of $Me_2Sn(n\text{-}Pe)_2$.

3.4. Determination of organotins in lard samples

3.4.1. Calibration coefficient

An internal standard quantification strategy was employed to minimize the response variation. As the theory of the internal standard method showed the equation below:

$$C_i/C_s = f_i\, h_i/h_s,$$

we could obtain each calibration coefficient f_i by the other four factors (C_i, C_s, h_i and h_s) according to the corresponding standard compound. The signification of each symbol described as the follows: C_i was the concentration of the target compound and h_i was its peak height; C_s referred to the concentration of

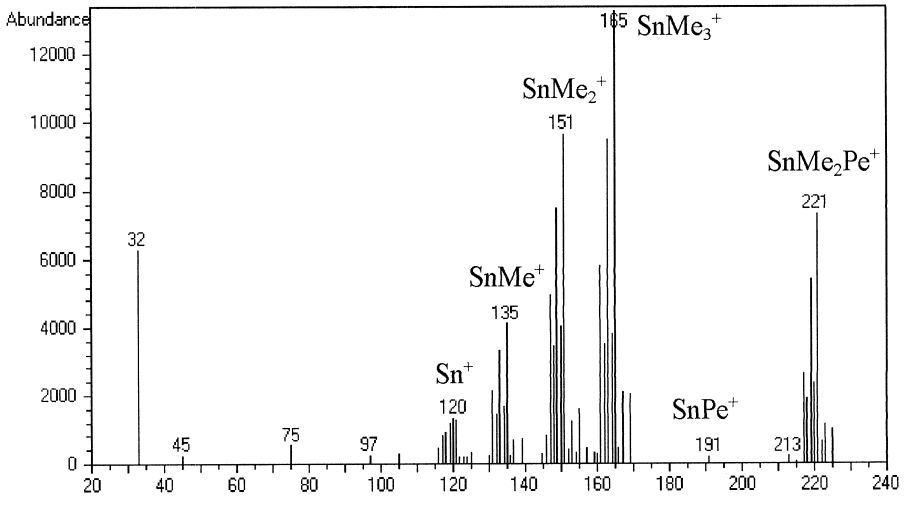

Fig. 5. The EI mass spectra of $Me_3Sn(n\text{-}Pe)$.

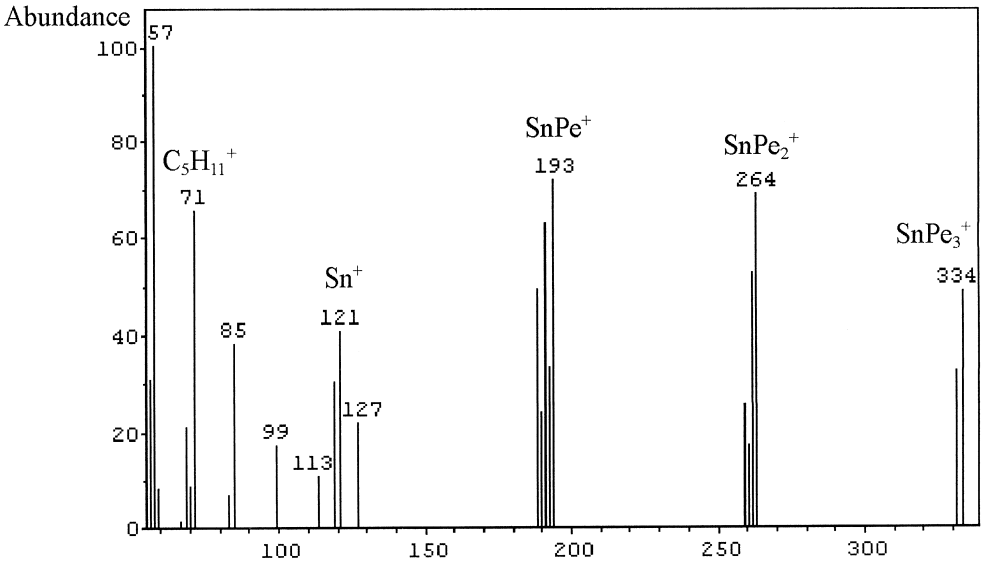

Fig. 6. The EI mass spectra of Sn(*n*-Pe)$_4$.

internal standard and h_s was its peak height; f_i was the calibration coefficient of the target compound relative to internal standard. After five times' repetitive determination of the standard tin compounds and internal standard, each f_i and the relative standard deviation (RSD) were obtained. They were listed in Table 2. It was obvious that there was a much difference between every two f_i, which indicated that the sensitivity of the method to each standard tin compound was different. So it was important to detect the calibration coefficient accurately in order to carry out the measurement of the tin compounds. The data of RSD were all less than 5% which indicated that the detection had good repeatability and was perfect for quantitative analysis.

3.4.2. Sample determination

Using internal standard method, the concentration of each tin compound in the contaminated lard samples was measured by the determination of the peak heights of internal standard and the corresponding target compound. The details were denoted in Table 3, which showed that the samples contained several organotins and their contents were rather high. DMT was clearly the dominating pollutant with concentration levels at mg/g grade. DOT in lard obtained from Longhua oil-shop was high too. The concentrations of the other methyltins were also high enough to cause toxic effect, especially for trimethyltin species which have been demonstrated the highest in toxicity within the series of organotin compounds [15,16]. The presence of organotins in the lard was probable from the plastic pails that had been used to load methyltin related chemicals. The oil-soluble organotin could easily diffuse from the wall of the pails and pollute the lard. As the different amounts of the chemicals remained in different pails, the pollution level was varied for different sample.

Table 2
Calibration coefficient f_i of each standard and its RSD

Standard	MMT	DMT	TMT	Sn(IV)	DOT
f_i	1.5	1.0	0.5	2.6	3.7
RSD%	4.4	4.3	5.0	3.0	3.3

3.5. Determination of spiked recoveries

Dingnan 3 sample was spiked with different amounts of methyltin standard. After the addition of 4 μg (Sn) of internal standard and pentylation, the spiked recoveries of MMT, DMT and TMT were repeated measured for five times by GC–FPD. Two chromatograms' comparison obtained from unspiked

Table 3
Concentrations of organotin compounds in lard samples (μg/g, as Sn)[a]

Sample	MMT	DMT	TMT	Sn(IV)	DOT	Unidentified[b]
Dingnan 1	ND[c]	1300±30	13.6±0.2	ND	ND	ND
Dingnan 2	ND	14.5±0.3	ND	ND	ND	ND
Dingnan 3	211.9±10.0	1160±40.0	2.53±0.15	ND	ND	ND
Dingnan 4	ND	0.13±0.01	0.057±0.001	0.43±0.00	ND	ND
Longnan 1	225.0±7.5	1700±40.0	13.8±0.31	ND	ND	ND
Longnan 2	ND	660±10.0	ND	ND	ND	ND
Epidemic prevention Station	954±30.0	3640±140.0	11.6±0.41	ND	ND	ND
Cook-oil shop	ND	358.7±10.0	ND	ND	2580±250	Detected

[a] Five times replicated measurements.
[b] Unidentified compound.
[c] ND: not detected, less than 0.127 μg Sn/g (3σ) for MMT, 0.083 μg Sn/g for DMT, 0.043 μg Sn/g for TMT, 0.022 μg Sn/g for Sn(IV), and 0.317 μg Sn/g for DOT.

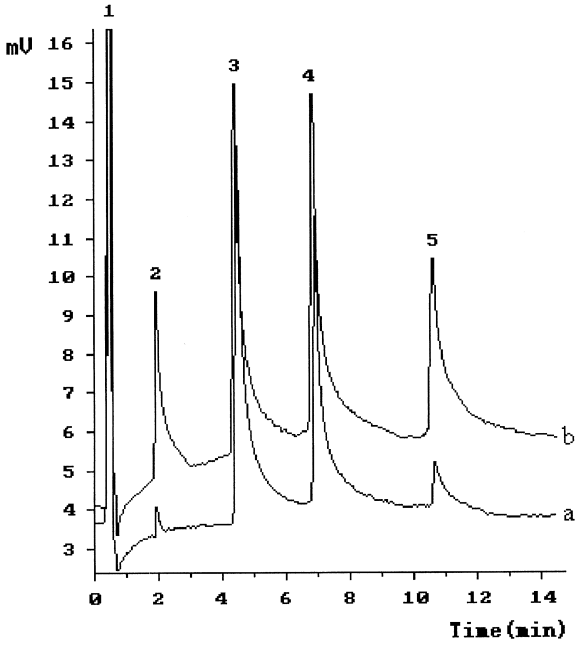

Fig. 7. Two chromatogram comparison between unspiked and spiked Dingnan 3 lard sample. (a) Chromatogram of unspiked dingnan 3 lard ample. Peaks identified and concentrations of each species were measured as follows: 1. solvent (cyclohexane); 2. Me_3SnPe (2.53 mg/g as Sn); 3. $MeSnPr_3$ (IS); 4. Me_2SnPe_2 (1160 mg/g); 5. $MeSnPe_3$ (211.9 mg/g). (b) Chromatogram of Dingnan 3 lard sample spiked with 0.5 mg Me_3SnCl; 50 mg Me_2SnCl_2 and 20 mg $MeSnCl_3$ per gram of the lard, respectively. Peaks identified and concentrations of each species were measured as follows: 1. solvent (cyclohexane); 2. Me_3SnPe (2.90 mg/g as Sn); 3. $MeSnPr_3$ (IS); 4. Me_2SnPe_2 (1276 mg/g); 5. $MeSnPe_3$ (245.1 mg/g).

and spiked Dingnan 3 lard sample was showed in Fig. 7. It further convinced the exact identification of the organotin compounds in the lards. According to the concentration of each methyltin compound in Dingnan 3 sample obtained above and internal standard method, all recoveries of the three compounds were calculated. The recoveries for MMT, DMT and TMT were 106±2%, 106±3% and 96±1%, respectively.

4. Conclusions

Several organotin-contaminated lard samples, which were collected from the terrible incident happened, were analyzed smoothly by GC–FPD and GC–MS. Experimental results showed that the lards were seriously contaminated with several organotin compounds, some concentrations of which were much higher than the amount people could bear. It was enough to disturb the body's metabolism, even to cause death. This tragedy reminded us that we should pay much attention to such poisonous compounds and there should be no time to delay the action taken on the appropriate management of organotins.

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