

Application of a Gas Chromatography/Mass Spectrometric Method for the Determination of Butyltin Compounds in Sediment

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A gas chromatography/mass spectrometric (GC/MS) method has been developed for the determination of trace mono-*n*-butyltin (MBT), di-*n*-butyltin (DBT), and tri-*n*-butyltin (TBT) compounds in sediments. Samples were extracted by 10% acetic acid in methanol containing 0.03% tropolone and were then derivatized for GC/MS analysis. Ethylation by sodium tetraethylborate and phenylation by sodium tetraphenylborate were evaluated as a derivatization reaction of the organotins in sample extract. *n*-Hexane was added into reaction media in the beginning of the reaction for the continuous extraction of derivatized organotins. Ethylation requires less than 2 hours to get proper derivatization yields for MBT, DBT, and TBT altogether and produces relatively low amounts of side reaction products. Compared to ethylation, phenylation requires much longer time but provides relatively lower yield and produces considerable amounts of side reaction products. Therefore, the ethylation reaction was applied for the analysis of organotin compounds in sediment. An isotope dilution mass spectrometric (IDMS) method based on GC/MS has been applied to the accurate determination of DBT compounds in the sediments. The IDMS results from the analyses of sediment samples showed a reasonable repeatability and a good agreement with the values obtained by IDMS based on liquid chromatography/induced coupled plasma/mass spectrometry.

Key Words : GC/MS, Ethylation, IDMS, Butyltin compound

Introduction

The elemental speciation analysis has attracted growing interest in environmental analysis as the toxicity and the mobility of elements have been known to strongly depend on their chemical forms.¹ In this respect, speciation analysis of organotin compounds has a special relevance as tri-*n*-butyltin (TBT), its degrading products such as di-*n*-butyltin (DBT) and mono-*n*-butyltin (MBT), and other inorganic tin species have varying toxicities.^{2,3} Organotins has many industrial and agricultural applications, especially as a selective biocide in marine paints from which the organotins are leached out into water and contaminate marine environment.⁴⁻⁶

Quantitative analytical methods have been developed during the last decade due to the need for the speciation analysis of organometallic compounds^{1,2,7-12} such as organotins, organoleads, and alkylmercuries. Elemental speciation analysis requires a combination of a separation method, usually gas chromatography (GC)¹³⁻¹⁶ or liquid chromatography (LC)^{17,18} with a selective and sensitive detection method. In general GC has been known to have better resolution than LC, but most of organometal species are present as salts and need to be derivatized into volatile and thermally stable forms prior to their separation in GC. Usually GC is coupled with mass spectrometry with

conventional electron impact ionization¹⁸⁻²⁰ or inductively coupled plasma mass spectrometry (ICP/MS),¹⁴⁻²¹ which has been known to have higher sensitivity for metallic species. LC is usually coupled with ICP/MS.^{17,18,22} Currently, GC/MS with electron impact ionization, GC/ICP/MS, and LC/ICP/MS are generally applied for the elemental speciation analysis.¹

In this study, we investigated a GC/MS method for the determination of trace levels of organotins in sediments. We have chosen GC/MS method as the required equipments are in more widespread use than relatively expensive ICP/MS. For the derivatization of the organotin species, Grignard reaction is not considered as a derivatization reaction as the reaction has been known to require strict anhydrous conditions and non-protic solvent, and tends to produce considerable amounts of side reaction products.^{2,16} In this study, we tested an ethylation reaction with sodium tetraethylborate and a phenylation reaction with sodium tetraphenylborate as those reactions can be carried out in an buffered protic solvent media which is known to be an efficient extraction media for organometallic species from sediment.² We also applied the GC/MS method for the accurate determination of DBT in sediments by isotope dilution mass spectrometric (IDMS) techniques.

Experimental Section

Materials. Two duplicate bottles of a test sediment sample and a bottle of PACS-2 sediment reference material were

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provided by the National Research Council of Canada (NRCC). ^{117}Sn -enriched di-*n*-butyltin dichloride (^{117}Sn -DBTCl₂) standard was supplied by the Laboratory of the Government Chemist (LGC), UK, in the form of methanolic solution of 0.36 mmol/kg. Mono-*n*-butyltin trichloride (MBTCl₃), di-*n*-butyltin dichloride (DBTCl₂), tri-*n*-butyltin chloride (TBTCl), tri-*n*-butylphenyltin, sodium acetate, sodium tetraethylborate, sodium tetraphenylborate, and sodium hydroxide were purchased from Aldrich (Wisconsin, USA). *n*-Hexane and methanol were purchased from Burdick and Jackson (Muskegon, MI, USA). A cocktail stock solution containing 1 to 2 mmol/kg levels of MBT, DBT, and TBT in methanol was prepared in this laboratory. For the determination of DBT in sediment, the commercial DBTCl₂ chemical was used as a reference material for the preparation of DBT standard solution. The purity of the reference material was thoroughly evaluated in our institute [see reference 23 for details]. The reagent solutions, 0.1 mol/kg sodium tetraethylborate in 0.1 mol/kg NaOH aqueous solution and 0.1 mol/kg sodium tetraphenylborate in 0.1 mol/kg NaOH aqueous solution, were prepared just before their uses.

Sample Extraction. Details in sample extraction processes are described in reference 23 and only a brief description is given here. Sediments were sampled in 1.0 g unit directly into 100 mL medium-pressure microwave extraction vessels and were subjected to microwave-assisted extraction with 20 mL of an extraction solvent (90% MeOH + 10% AcOH + 0.03% Tropollone) selected based on the extraction recovery test done in reference 23. After centrifugation, 6 mL of the extract was then derivatized as below. The remaining extract was stored in 4 °C refrigerator for LC/ICP/MS analysis, which was carried out in parallel with this study by Inorganic Analysis Group of our institute [see reference 23 for details].

Simulated Sample extract. To test and optimize derivatization conditions, simulated sample extracts were prepared by spiking an appropriate amount of the cocktail stock solution, described above, into aliquots of the extraction solvent.

Derivatization. The sample extract and the simulated sample extract were taken in 6 mL unit for GC/MS analysis. The 6 mL of each extract was transferred to 20 mL screwed-top vial and 6 mL of 1 mol/kg sodium acetate (aqueous) solution was added to adjust pH. 1 mL of the reagent solution was added into the vial, and 2 mL of *n*-hexane was added for the instant extraction of derivatized tin products. The mixture was shaken mechanically for an appropriate period of time at room temperature. Then, *n*-hexane layer was transferred to a glass vial and evaporated to 0.2 mL or an appropriate volume by purging nitrogen gas at room temperature. The extract was stored at 4 °C until it was determined by GC/MS.

Isotope Dilution Mass Spectrometry (IDMS) for DBT Analysis. For the accurate determination of DBT, ^{117}Sn -enriched DBT was used as internal standard. Sediment sample was taken in 1.0 g unit into microwave extraction vessels and were spiked with appropriate amount of the

^{117}Sn -enriched DBT solution so that isotope ratio, $^{120}\text{Sn}/^{117}\text{Sn}$, of DBT in the sample blend to be about 0.9. The exact amounts of sample and the ^{117}Sn -enriched DBT solution taken into each vessel were measured by weighing the vessel before and after adding them. The extraction solvent was then added into the vessel. The contents in the vessel were completely mixed, left for several hours at room temperature for equilibration, and were subjected to the microwave extraction and the derivatization reaction as described above. Two isotope ratio standard solutions with $^{120}\text{Sn}/^{117}\text{Sn}$ ratios of 0.85 and 0.95 were prepared by combining appropriate amounts of ^{117}Sn -enriched DBT solution and the DBT standard solution. $^{120}\text{Sn}/^{117}\text{Sn}$ ratios of DBT in the isotope ratio standards and the sample extract were precisely measured with GC/MS [as discussed below] in selected ion monitoring mode.

GC/MS Analysis. The instrumentation consists of a gas chromatograph (Hewlett Packard 6890) equipped with an automatic liquid sample injector, a double focusing magnetic sector mass spectrometer (Jeol JMS 700). The GC was equipped with a Rtx-5ms column (Restek, 30 m long, 0.25 mm i.d., 0.25 μm film thickness). Helium was used as a carrier gas at the flow rate of 1.0 mL/min. The injection port was kept at 250 °C and operated either in the split mode or in the splitless mode. The interface to the mass spectrometer was maintained at 250 °C. The mass spectrometer was operated under electron impact ionization condition at 70 eV with a source temperature of 250 °C and an ionization current of 0.25 mA. When the mass spectrometer was operated in the scan mode, m/z 50 to 450 range was scanned in 0.6 second. For the IDMS determination of DBT, the mass spectrometer monitored representative ions of the derivatization products of ^{120}Sn -DBT (naturally most abundant isotope) and ^{117}Sn -DBT.

Results and Discussion

Derivatization Study. We used simulated sample extracts containing 1 to 2 mmol/kg levels of MBT, DBT, and TBT to test and optimize the derivatization conditions. Figure 1 and 2 show the total ion chromatograms (TICs) and the mass spectra of derivatized butyltins of the simulated sample extract after 2 hours of ethylation with sodium tetraethylborate and after 2 hours of phenylation with sodium tetraphenylborate, respectively. In the TIC of the ethylated sample, the three butyltin products are dominant. The three butyltins show similar peak heights, indicating that all three have reasonable derivatization yields. In the TIC of the phenylated sample, the organotin peaks are relatively weak compared to those of the ethylated sample, and there are many unexpected peaks whose intensities are comparable with those of tin compounds. The results indicate that the phenylation reaction gives relatively poor derivatization yields to the butyl tins and produces high levels of side reactions. It is also noted that the intensities of the three butyltins in the TICs of phenylated sample decrease dramatically in the order of TBT, DBT, and MBT and the

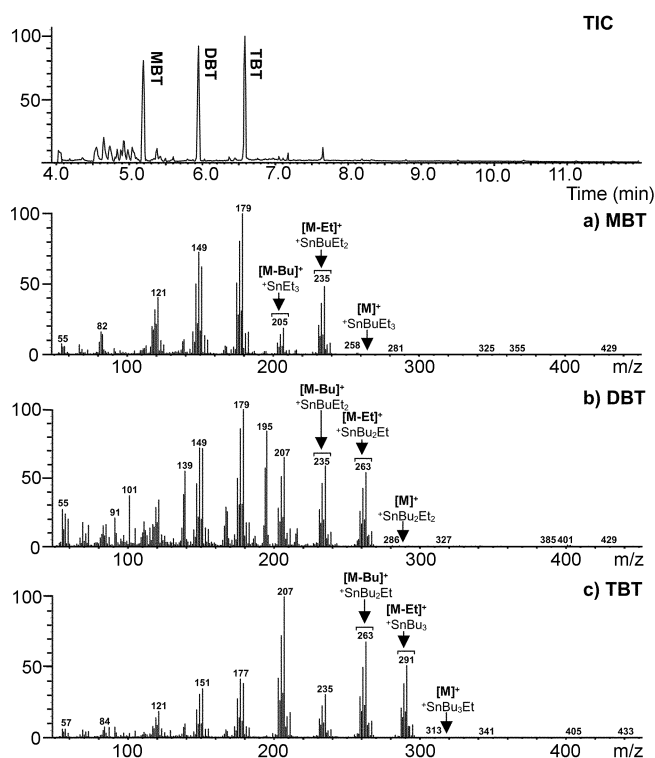


Figure 1. Total ion chromatogram of a simulated sample extract containing MBT, DBT, and TBT after ethylation, and mass spectra of the ethylated MBT, DBT, and TBT. GC oven temperature: 60 °C (1 min.) – 30 °C/min. – 260 °C (10 min.).

peak of MBT is almost undetectable. The recovery, combination of the yield of the derivatization reaction and the extraction recovery of the product into the organic layer, by ethylation reaction of each of the three organotins was tested. We spiked tributylphenyltin into the reaction media as an internal standard. The recoveries of the butyltin compounds for 2, 4, and 8 hours of the ethylation reaction were listed in Table 1. It has to be noted that the recoveries were indirectly calculated by comparing the peak areas of MBT, DBT, and TBT derivatization products in the total ion chromatogram with that of the internal standard under assumption that all the four compounds have similar detection efficiencies in the GC/MS. The results show that ethylation efficiency does not change noticeably by lengthening the reaction time after 2 hours. Therefore, 2 hours of ethylation time was chosen for the rest of this study. The recoveries of the butyltin compounds from 8 hours of phenylation were also estimated and are listed in Table 1. As it was expected above, the phenylation reaction shows considerably lower recoveries than ethylation. Therefore, this study suggests that the ethylation reaction is a choice of priority as a derivatization method for the simultaneous determination of the three butyltins by using GC/MS.

Mass Spectrometric Properties of Derivatized Butyltins.

The mass spectra of ethylated butyltins are shown in Figure 1. The ionic species are denoted as $\text{Sn}^+\text{Bu}_n\text{Et}_m$, where Bu and Et indicate butyl group and ethyl group, respectively. The mass spectra of the ethylated butyltin compounds do not

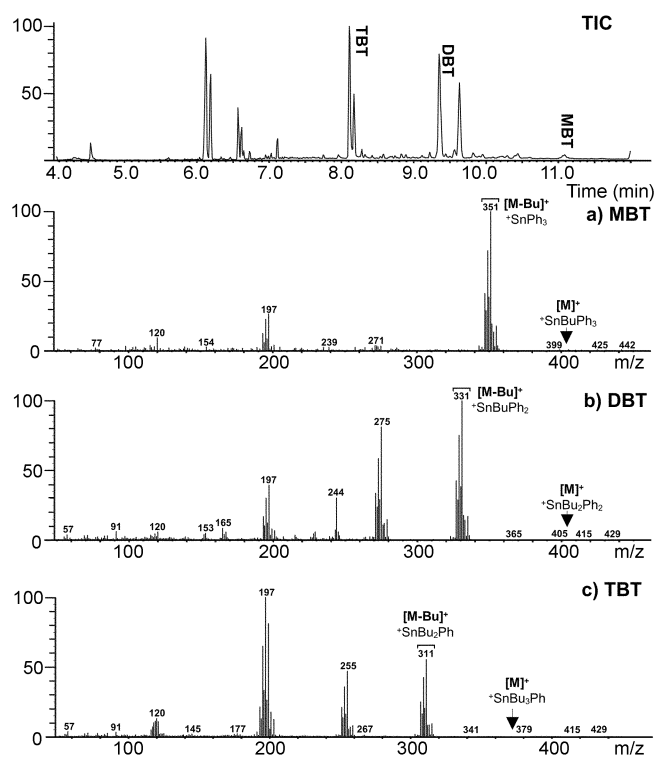


Figure 2. Total ion chromatogram of a simulated sample extract containing MBT, DBT, and TBT after phenylation, and mass spectra of the phenylated MBT, DBT, and TBT. GC oven temperature: 60 °C (1 min.) – 30 °C/min. – 260 °C (10 min.).

show detectable levels of molecular ions. The mass spectra are dominated by the daughter ions that have lost one or more ethyl groups and/or butyl groups such as $[\text{M} - \text{Bu}]^+$, $[\text{M} - \text{Et}]^+$, $[\text{M} - \text{Bu} - \text{Et}]^+$, $[\text{M} - 2\text{Bu}]^+$, $[\text{M} - 2\text{Et}]^+$. Identification of daughter ions were annotated on the mass spectra in Figure 1. Each daughter ion shows a group of peaks representing the natural isotope abundance of the tin element.²⁴ The intensity ratio of $[\text{M} - \text{Bu}]^+$ and $[\text{M} - \text{Et}]^+$ is approximately 1 to 3 in the mass spectra of MBT (derivatized to SnBuEt_3) and 2 to 2 in the mass spectra of DBT (derivatized to SnBu_2Et_2), which match the ratios of the numbers of Bu and Et in the molecular ion. The intensity ratio of $[\text{M} - \text{Bu}]^+$ and $[\text{M} - \text{Et}]^+$ is approximately 3 to 2 in the mass spectra of

Table 1. Recoveries of monobutyltin, dibutyltin, tributyltin from the reaction media

Reaction Time (Hour)	Recovery (%) [*]		
	monobutyltin	dibutyltin	tributyltin
Ethylation reaction with sodium tetraethylborate			
2	72	39	69
4	81	43	75
8	66	35	60
Phenylation reaction with sodium tetraphenylborate			
8	1	2	6

^{*}The expanded relative uncertainty of the recovery is expected to be 10%.

Table 2. GC/MS area ratios of ^{120}Sn -DBT and ^{117}Sn -DBT of independently derivatized aliquots from a single sample

Day	Aliquot No.	Area Ratio (^{120}Sn -DBT and ^{117}Sn -DBT)
1	1	0.7663 ± 0.0011
	2	0.7684 ± 0.0015
2	3	0.7658 ± 0.0012
	4	0.7662 ± 0.0013
3	5	0.7694 ± 0.0014
Mean		0.7672
Standard Deviation		0.0015 (0.2%)

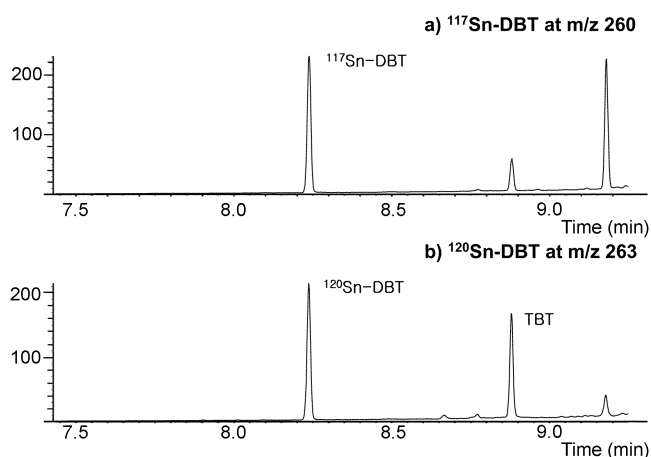
TBT (derivatized to SnBuEt_3), which does not match with the ratio of the numbers of Bu and Et in the molecular ion, but further explanation of the results is not tried. Based on its mass spectrometric properties, $[\text{M} - \text{Et}]^+$ ions at m/z 263 and 260 were chosen for selected ion monitoring of ^{120}Sn -DBT and ^{117}Sn -DBT, respectively, in the ID-GC/MS analysis of DBT in sediment.

The mass spectra of phenylated butyltins are shown in Figure 2. The ionic species are denoted as $\text{Sn}^+\text{Bu}_n\text{Ph}_m$, where Ph indicates phenyl group. The mass spectra of the all three tin compounds do not show detectable levels of molecular ions. The mass spectra are dominated by their daughter ions that have lost one or more butyl groups. The daughter ions that have lost a phenyl group are not detectable. See the mass spectra in Figure 2 for the identification of the daughter ions.

Determination of DBT in Sediment by IDMS. We developed an isotope dilution mass spectrometric method based on GC/MS with ethylation reaction for the determination of DBT. Followings are the experimental details we carried out to evaluate the quality of the method.

We tested if the ethylation reaction fits for the IDMS methods. Any experimental parameter that can cause variation in the ratio of recoveries of ^{120}Sn -DBT and ^{117}Sn -DBT may result in poor repeatability or may lead to bias in the final results if the parameters are not under control. A portion of a simulated sample extract was spiked with an aliquot of the ^{117}Sn -DBT solution. After complete mixing, the sample was then divided to several equal aliquots. For three consecutive days, one or two aliquots per day were subjected to sample preparation with the ethylation reaction. The extracts of the individual aliquots were then analyzed together by GC/MS. The peak area ratio of ^{120}Sn -DBT and ^{117}Sn -DBT in their selected ion chromatogram at m/z 260 and 263 were listed in Table 2. The run-to-run relative standard deviation of the area ratio of each single aliquot was less than 0.2%, indicating that the GC/MS run in SIM mode has a good repeatability. The area ratios showed 0.2% of aliquot-to-aliquot standard deviation, indicating that the derivatization provides reproducible recovery ratio for ^{120}Sn -DBT and ^{117}Sn -DBT.

Figure 3 shows the selected ion chromatograms of ethylated ^{120}Sn -DBT and ^{117}Sn -DBT of the test sediment from NRCC. The chromatograms were dominated by the

**Figure 3.** Ion chromatograms of ethylated ^{120}Sn -DBT and ^{117}Sn -DBT, selectively monitored at m/z 263 and 260 in SIM mode, from NRCC test sediment with a few mmol/kg level of DBT in it. GC oven temperature: 40 °C (3 min.) – 30 °C/min. – 260 °C (10 min.).**Table 3.** Results of the determination of DBT in test sediment from NRCC by ID-GC/MS with ethylation reaction

Sample Source	DBT concentration ($\mu\text{mol/kg}$)
Bottle 1	1.424
	1.415
	1.339
	1.365
Bottle 2	1.467
	1.415
	1.626
	1.407
Mean	1.432
Standard Deviation	0.081 (5.6%)

peaks of the target analytes and showed low background noise and no significant interference peaks from either sample matrix or derivatization byproducts.

The test sediment obtained from NRCC was measured by the proposed ID-GC/MS with the ethylation reaction. Four aliquots from each of two sample bottles went through separate sample preparation processes. All 8 aliquots were then measured together by GC/MS. The results are listed in Table 3. The standard deviation of the multiple measurement results is 5.6% of the mean value, which is better than or similar to the repeatability of currently available ID-LC/ICP/MS. Among the experimental parameters which can cause random effects on the ID-GC/MS results, the GC/MS measurement of the area ratio of ^{120}Sn -DBT and ^{117}Sn -DBT showed less than 0.2% of relative standard deviation for both sample and the isotope ratio standards, indicating that it contribute very little to the standard deviation of the results of multiple sample measurements. According to the measurement protocol we used in this study, the standard deviation of the multiple measurement arouse mostly from the inaccuracies of measuring the amounts of sample taken for analysis and the ^{117}Sn -DBT standard solution spiked into

Table 4. Results of DBT measurements of PACS-2 sediment reference material by ID-GC/MS method with ethylation reaction

Sample	Certified Value by NRCC ^a (μmol/kg)	Results by ID-GC/MS in this study (μmol/kg)
PACS-2	9.18 ± 1.26 ^b	8.59 ± 0.17 ^b

^aValue certified by National Research Council of Canada (NRCC) with ID-LC/ICP/MS. ^bThe numbers after “±” are the expanded uncertainties, with levels of confidence of 95%, of the proceeding values.

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Based on the signal to noise ratio observed in the selected ion chromatograms of the two isotopic analogues of DBT in Figure 3, the detection limit of the IDMS method is estimated to be 0.03 μmol/kg.

Though the repeatability and reproducibility tests proved that the IDMS method has a reasonable quality to be used as a reference method to routine environmental analysis methods, method validation by using well-characterized reference material or by comparison with well-established methods is always desirable. We measured PACS-2 sediment reference material obtained from NRCC. The results obtained by our method were listed in Table 4 in comparison with the value certified by NRCC with their ID-LC/ICP/MS method. Our results show a good agreement with the certified value within their uncertainties, indicating that our method has high degree of equivalence with the previously established ID-LC/ICP/MS method.

Conclusion

Gas chromatography-mass spectrometry (GC/MS) with a derivatization reaction has been developed for the determination of trace butyltin compounds in sediments. The ethylation by sodium tetraethylborate showed reasonable derivatization efficiencies for butyltin compounds while the phenylation reaction by sodium tetraphenylborate requires much longer time, provides relatively lower yield, and produced high amounts of side reaction products. Therefore the ethylation reaction was a prior choice of a derivatization method for the analysis of butyltin compounds in sediments. Isotope dilution mass spectrometry based on the GC/MS method showed an reliability adequate to be used as a reference method for the accurate determination of DBT compounds in the sediments. Based on the results obtained in this study, it is expected the IDMS method can be applicable for the accurate determination of many forms of butyltin species in environmental samples.

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