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Improvement of Measurement Precision of SPME-GC/MS Determination of Tributyltin Using Isotope Dilution Calibration

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A unique approach was developed to improve the precision of quantification of tributyltin (TBT) in sediments by solid phase microextraction (SPME) using isotope dilution GC/MS. The precision of the analytical technique was initially evaluated using standard calibration solutions. In selective ion monitoring (SIM) mode, the relative standard deviation (RSD) obtained for TBT based on peak area response was 18% ($n = 11$). When an internal standard, tripropyltin (TPrT), was used, the RSD decreased to 12%. A significant improvement in the precision using SPME was noted when a ^{117}Sn-enriched TBT spike was employed; the RSD decreased 4-fold to 3%. Detection limits of 0.2 and 20 ng(Sn) L^{-1} were achieved with SPME sampling and liquid−liquid extraction, respectively. Six analyses were performed for determination of TBT in PACS-2 sediment Certified Reference Material using both standard additions and isotope dilution procedures. For the latter, a ^{117}Sn-enriched TBT spike was used. A concentration of 0.88 ± 0.03 $\mu g\ g^{-1}$ (RSD 3.4%) obtained using standard additions was in good agreement with the certified value of 0.98 ± 0.13 $\mu g\ g^{-1}$ as tin. Concentrations found using isotope dilution were 0.895 ± 0.015 $\mu g\ g^{-1}$ (RSD 1.73%) as tin and 0.874 ± 0.014 $\mu g\ g^{-1}$ (RSD 1.66%) as tin using a liquid−liquid extraction and SPME sampling, respectively. A 2-fold improvement in the precision of TBT concentration measurement using isotope dilution was clearly achieved, demonstrating its superiority in providing more accurate and precise results as compared to the method of standard additions. The isotope dilution technique eliminated the problem of poor reproducibility, which typically plagues SPME.

Organotin compounds have been introduced into the environment mainly through their use as insecticides, fungicides, bactericides, wood preservatives, plastic stabilizers, and biocides in antifouling paints for boats and ships.[1] They have been detected in numerous environmental samples, including water, sediments, biological tissue, and sewage sludge. Their severe toxic effects on aquatic organisms[2] and mammals,[3] including humans, have been observed even at very low concentrations (ng L^{-1}). The high bioaccumulation potential and the suspicion that tributyltin (TBT) is an endocrine disrupter have led to the control of pollution levels in environmental samples. The growing concerns over the toxicity of TBT and its dibutyltin (DBT) and monobutyltin (MBT) degradation products entering the environment have led to a dramatic increase in interest in the development of sensitive, accurate, and rapid analytical methods for their determination.

Various analytical techniques have been developed for the speciation of butyltin compounds, and most of them are based on gas chromatography[4] (GC), owing to its high resolving power and availability of sensitive detectors. In particular, the use of GC in combination with mass spectrometry (MS) is a powerful technique from the point of view of identification and confirmation of the compounds. On the other hand, sample preparation for GC analysis is usually time-consuming, and organic solvents used for their liquid−liquid extraction are toxic. In an effort to simplify sample preparation while retaining the merits of GC, solid-phase microextraction (SPME) was introduced by Pawliszyn and coworkers[5,6] in the early 1990's. Since 1993, following the commercialization of SPME, this sampling technique has gained widespread acceptance as a consequence of its simplicity, low cost, and ease with which analytes can be transferred to the GC column. The drawback noted with this technique is the degraded precision (typically 10% RSD). Haberhauer-Troyer and co-workers explained the poor precision and artifact formation as being the result of degradation of the SPME fiber during repeated usage.[7] Indeed, they observed damage to the coating and physical contamination on the fiber. Because of inconsistency in the interfiber precision, this often made it necessary to employ an internal standard or standard addition methods for quantitation.[8]

More recently, SPME has been widely applied to the determination of organotin compounds and has evolved as an elegant, solvent-free sample extraction technique.[9−27] However, the preci-

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sion of results obtained with this approach is typically in the range of 5 to 15% RSD for organotin compounds.[18,21]

Butyltin determinations generally require several analytical steps, including extraction, derivatization (where GC analysis is involved), separation, and detection, each of which can contribute to the difficulty of analysis, degrading the accuracy and precision of the results.[28,29] To improve the precision of the quantification, isotope dilution techniques can be applied. Isotope dilution mass spectrometry (IDMS) has been widely employed for trace element analysis in a variety of sample matrixes and recently has been considered to be a primary (ratio) method of analysis as a consequence of its high accuracy and precision.[30,31] IDMS addresses means for the correction for species conversion that might occur during sampling, manipulation and even analysis procedures. Unfortunately, its application to species-specific determinations has been limited by the nonavailability of commercial enriched standards.[32] If these are available, a known amount of spike of a known isotopic composition is added to a known amount of sample of known isotopic composition; the isotopic composition of the spike and sample must be different.[33] A number of advantages of isotope dilution (ID) accrue, including enhanced precision and accuracy; matrix effects are accounted for, since quantitation is done by ratio measurements; nonquantitative analyte recovery does not impact on the final results; species alteration during sample workup can be assessed; and an alternative and comparative quantitation strategy is provided.[34,35] Only recently has this approach been applied to the determination of butyltin [34,36−39] and other species, including lead,[40] organolead,[41−44] methylmercury,[33,45−47] Cr(III and VI),[32,48] iodide,[49] methylselenocysteine,[50] and selenite[49,51] using synthesized species-specific spikes. In most of these studies, inductively coupled plasma mass spectrometry (ICPMS) detection has been used and, in only a few cases, with isotope dilution calibration with GC/MS, but never for organotin determinations.

This work was undertaken to evaluate the application of isotope dilution to gas chromatography mass spectrometry (GC/IDMS) for determination of tributyltin using ^{117}Sn-enriched tributyltin chloride. The precision was evaluated for organotin compounds using both absolute peak area and relative peak area (using an internal standard and TBT-enriched isotope quantitation). The ID technique was compared to the standard addition technique. Certified sediment reference material PACS-2 (pacific coastal sediment) was analyzed to verify the accuracy and precision of the method. All experiments were performed using a liquid−liquid extraction, and SPME sampling with both Scan and SIM (selective ion monitoring) mode was used for data acquisition with GC/MS.

EXPERIMENTAL SECTION

Instrumentation. A Hewlett-Packard HP 6890 GC (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada) fitted with a DB-5MS column (Iso-Mass Scientific Inc., Calgary Alberta, Canada) was used for the separation of the butyltins. Detection was achieved with an HP model 5973 mass selective detector (MS). Typical GC/MS operating conditions are presented in Table 1.

A Microdigest model 401 (2.45 GHz, maximum power 300 W) microwave digester (Prolabo, Paris, France) equipped with a TX32 programmer was used for microwave-assisted extraction of butyltins from the sediment. Extractions were performed in open vessels and condenser attached (Prolabo, Paris, France).

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Table 1. Operating Conditions for GC/MS

column	DB-5MS 30 m × 0.25-mm i.d. × 0.10 μm d_f
injection system	split/splitless injector, splitless mode
injector temp	250 °C
oven temp program	50−270 °C at 20 °C/min (temp hold 5 min)
carrier gas; flow rate	helium; 0.9 mL min^{-1}
transfer line temp	290 °C
MS	HP model 5973 mass selective detector
SIM parameters	measured ions: m/z = 232, 235 dwell times: 50 ms for each m/z
MS quad temp	150 °C
MS source temp	230 °C

A manual SPME device, equipped with a fused-silica fiber coated with a 100-μm film of poly(dimethylsiloxane) (Supelco, Bellefonte, PA) was used for sampling of the ethylated butyltins from the headspace above the aqueous solutions.

A 10-μL liquid sampling syringe (Hamilton company, Reno, Nevada, USA) was used for the injection of isooctane samples into the GC/MS.

Reagents. Acetic acid was purified in-house by subboiling distillation of reagent grade feedstock in a quartz still prior to use. Environmental grade ammonium hydroxide was purchased from Anachemia Science (Montreal, Quebec, Canada). OmniSolv methanol (glass-distilled) was purchased from EM Science (Gibbstown, NJ). High purity deionized water (DIW) was obtained from a NanoPure mixed bed ion exchange system fed with reverse osmosis domestic feedwater (Barnstead/Thermolyne Corp, IA). Sodium tetraethylborate solution, 2% (m/v), was prepared daily by dissolving $NaBEt_4$ (Strem, Bischeim, France) in DIW. A 1 mol L^{-1} sodium acetate buffer was prepared by dissolving an appropriate amount of sodium acetate (Fisher Scientific, Nepean, Ontario, Canada) in water and adjusting the pH to 5 with acetic acid.

Monobutyltin trichloride (MBT, 95%), dibutyltin dichloride (DBT, 96.5%), tributyltin chloride (TBT, 96%), and tripropyltin chloride (TPrT, 95%) were purchased from Alfa Products (Danvers, MA). Individual stock solutions of 1000−1500 mg L^{-1} as tin were prepared in methanol and kept refrigerated until used. Working standard solutions of 10 mg L^{-1} for liquid−liquid extraction and 200 μg L^{-1} for SPME sampling were prepared by dilution in methanol.

A ^{117}Sn-enriched TBT stock solution (97% purity) with isotopic composition and uncertainties provided at a nominal concentration of 90.5 μg g^{-1} in methanol was obtained from the Laboratory of the Government Chemist (LGC, Teddington, U.K.). The isotopic composition was stated as follows: ^{112}Sn (0.03%), ^{114}Sn (0.01%), ^{115}Sn (0.05%), ^{116}Sn (7.6%), ^{117}Sn (92.1%), ^{118}Sn (0.20%), ^{119}Sn (0.10%), ^{120}Sn (0.04%), ^{122}Sn (0.01%), and ^{124}Sn (0.01%). Working standard solutions of 0.40 and 10 mg L^{-1} as tin were prepared by volumetric dilution of the stock in methanol. The concentration of the 0.40 mg L^{-1} spike solution, which was used to prepare spiked samples, was verified by reverse spike isotope dilution against the high-purity natural abundance TBT standard.

National Research Council of Canada PACS-2 sediment certified reference material (CRM), certified for mono-, di-, and tributyltin contents, was selected to assess the accuracy of the technique.[52]

(52) http://www.ems.nrc.ca/hissmesspacs.pdf; certification document, 2000.

Because GC techniques require volatile species, derivatization of the analyte was thus necessary.[53] The sample preparation for organotin speciation has drastically been simplified by the introduction of in situ derivatization by Ashby et al.[54−56] Ethylation takes place in the aqueous phase by addition of sodium tetraethylborate ($NaBEt_4$). Derivatization into apolar volatile species and extraction into the organic solvent can take place simultaneously within one handling step. The derivatization reaction can be described as $R_nSn^{(4-n)+} + (4 - n)NaBEt_4 \rightarrow R_nEt_{4-n}Sn + (4 - n)BEt_3 + (4 - n)Na^+$, with R = propyl (Pr), butyl (Bu); n = 1, 2, 3. These reactions are given in detail by Rapsomanikis and coworkers.[57,58]

Sample Preparation of Standard Solutions for Reproducibilty Study. For liquid−liquid extraction experiments, 100 μL of a 10 mg L^{-1} solution of mixed butyltin compounds (MBT, DBT, TBT, and ^{117}Sn TBT) and TPrT added as an internal standard was pipetted into a 50-mL centrifuge tube. A 25-mL volume of acetate buffer (pH = 5), 1 mL of $NaBEt_4$ (2%), and 2 mL of isooctane where then added. After manual shaking for 5 min, the tubes were centrifuged for 10 min, allowing separation of the phases. Then, the isooctane layer was transferred to a 1-mL glass vial and 1 μL of the organic phase was injected onto the head of the column for GC/MS analysis.

For SPME experiments, 100 μL of a 200 μg L^{-1} solution of each of the species (MBT, DBT, TBT, ^{117}Sn TBT and TPrT) was used and mixed with 25 mL of acetate buffer (pH = 5) and 1 mL of $NaBEt_4$ (2%) in an amber glass vial fitted with a PTFE−silicone septum. The solution was stirred with a magnetic stir bar for 15 min, and the organotin derivatized species were sampled from the headspace by means of a PDMS fiber placed in the vortex of the solution. After sampling, the fiber was retracted into the needle of the holder. The SPME fiber was then inserted into the GC injector, where the organotin compounds were thermally desorbed for 1.5 min, allowing for a complete desorption of all organotin adsorbed on the fiber.

Sample Preparation for PACS-2 Sediments Using Standard Addition Calibration. A 0.5-g subsample of PACS-2 sediment, 250 μL of 2 mg L^{-1} TPrT standard solution (used as internal standard), and 10 mL of concentrated acetic acid were placed in a open vessel, and a condenser was attached. Microwave-assisted extraction (MAE) was performed for 3 min at 60% power. After centrifugation, the acidic supernatant was transferred to a sample vial. For the SPME experiments, 200 μL of the acidic phase was pipetted into a glass centrifuge tube along with 25 mL of acetate buffer and 1 mL of 2% $NaBEt_4$. The derivatization and desorption conditions remained the same as described earlier. For the liquid−liquid extraction, 7 mL of the acidic phase was placed in a centrifuge tube along with 1 mL of 2% $NaBEt_4$ and 2 mL of isooctane. The pH was adjusted to 5−6 with 10 mL of ammonium hydroxide (5 N). The derivatization process was identical to that described for liquid−liquid extractions.

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Table 2. Injection Sequence for ID Procedure

1, mass bias drift solution	16, mass bias drift solution
2, unspiked PACS-2	17, spiked sample no. 1
3, unspiked PACS-2	18, reverse ID solution no. 1, stock A
4, natural TBT standard	19, spiked sample no. 2
5, natural TBT standard	20, reverse ID solution no. 2, stock A
6, mass bias drift solution	21, mass bias drift solution
7, enriched spiked TBT standard	22, spiked sample no. 3
8, enriched spiked TBT standard	23, reverse ID solution no. 3, stock A
9, blank no. 1	24, spiked sample no. 4
10, blank no. 2	25, reverse ID solution no. 4, stock B
11, mass bias drift solution	26, mass bias drift solution
12, blank no. 3	27, spiked sample no. 5
13, blank no. 1	28, reverse ID solution no. 5, stock B
14, blank no. 2	29, spiked sample no. 6
15, blank no. 3	30, reverse ID solution no. 6, stock B
	31, mass bias drift solution

For the quantification of organotin compounds in PACS-2 sediment, a standard additions method was used. For each determination, one blank (consisting of TPrT standard solution and concentrated acetic acid) and one PACS-2 sample (no spiking) were prepared as previously described. Additions of two increments of standard solution of the analyte (+X and +2X) to sample aliquots of the same size were prepared. Each organotin standard solution (MBT, DBT and TBT) was added at the beginning of the analytical procedure, as was the TPrT internal standard. For the liquid−liquid extraction, each sample was injected twice onto the GC/MS. Only one such injection (desorption) was made for the SPME experiment.

Sample Preparation for PACS-2 Sediment Using ID. For isotope dilution, nine independent spiking experiments were undertaken to analyze PACS-2 reference material. Three sample blanks were prepared containing 10 mL of concentrated acetic acid and 25 μL of ^{117}Sn TBT (0.40 mg L^{-1}), corresponding to 10% of the amount added to the sediment samples. The procedures applied included MAE and analyte derivatization. Six replicate analyses of PACS-2 spiked with ^{117}Sn TBT were performed. After MAE on 0.5-g subsamples of PACS-2 using 10 mL of concentrated acetic acid and 250 μL of ^{117}Sn TBT (0.40 mg L^{-1}), the acidic supernatant was derivatized as previously described. An unspiked PACS-2 sample (no ^{117}Sn TBT addition) was prepared in the same manner.

To determine an accurate ^{117}Sn TBT solution concentration, which was used for spiking the sediment, reverse isotope dilution (RID) analysis was performed. For this experimental design, six samples were prepared containing 250 μL of natural 2 mg L^{-1} TBT and 250 μL of ^{117}Sn TBT standard at a concentration of 0.40 mg L^{-1}. Two stock solutions of natural TBT standard containing 2 mg L^{-1} were used (stocks A and B). Two sets of data were generated for this analysis. A mass bias drift solution, prepared in the same way, was repeatedly injected at the beginning of the sequence and again after every four samples to monitor mass bias drift.

Unspiked PACS-2 sample, natural TBT, and ^{117}Sn TBT standards and the three blanks were each injected two times. The six spiked PACS-2 samples were then injected. Between injections of spiked PACS-2 sample, the mass bias drift solution was injected. In this way, a total of 31 consecutive injections were performed (Table 2).

This procedure was followed for both liquid−liquid extraction and SPME. For both techniques, each sample was analyzed using both scan and SIM modes. Measurement of the 235/232 peak areas ratios of this sequence allowed the determination of ^{117}Sn TBT standard concentration, the check of mass bias and mass bias drift, and six determinations of TBT in PACS-2 sediment.

Safety Considerations. Organotin compounds are toxic substances, and sodium tetraethylborate is highly flammable. Material Safety Data Sheets must be consulted and essential safety precautions must be employed for all manipulations.

Table 3. Analytical Performance[a]

	LOD (ng(Sn) L^{-1})[b]		
	SPME	liquid−liquid extraction	linearity[c] up to ng(Sn) L^{-1}
MBT	0.2	20	6000
DBT	0.3	80	8000
TBT	0.2	70	4000

[a] Measured in SIM mode. Measured ions: m/z = 232, 235. [b] Limit of detection (n = 10) calculated on the basis of three times the standard deviation of background noise next to the chromatographic peak. [c] Range of concentration exhibiting linear regression slopes.

Table 4. Precision (RSD%) of Liquid−Liquid Extraction and SPME Sampling Using GC/MS in SIM Mode

		SPME	liq−liq extraction
absolute areas[a]	MBT	8	9
	DBT	9	9
	TBT	18	9
relative areas[a]	MBT/TPrT	4	3
	DBT/TPrT	2	2
	TBT/TPrT	12	3
TBT[b]	natural TBT/^{117}Sn TBT	3	3

[a] m/z 235. [b] TBT (m/z 235)/TBT (m/z 232).

RESULTS AND DISCUSSION

Analytical Performance. Initially, the analytical performance of the technique, including limit of detection (LOD), linearity, and precision were determined. These data are summarized in Tables 3 and 4. Purity assessment of butyltin stock solutions was checked in the laboratory and was previously reported.[37]

The precision of the procedure was based on data from the eleven mixed organotin standard solutions (MBT, DBT, TBT, ^{117}Sn TBT, and TPrT), each of which was injected three times for the liquid−liquid extraction and once for SPME in scan and SIM-MS mode. Compared with the liquid−liquid extraction, a 100-fold improvement in sensitivity was obtained with SPME sampling. However, the measurement precision was significantly worse than that achieved with liquid−liquid extraction.

A mass-to-charge ratio of 235 exhibited the best signal/noise ratio used for all compounds. Peak areas for m/z 235 for each compound were measured for all injected samples. The precision was calculated from measurements of both absolute peak areas and relative peak areas (normalized to TPrT internal standard). Areas of extracted ion 232 were measured for ^{117}Sn TBT, and the precision obtained for the ratio between natural TBT (m/z = 235) and ^{117}SnTBT (m/z = 232) was also evaluated. Data are summarized in Table 4.

Signal intensity was higher with SPME, as compared to liquid−liquid sampling. There is a downside to this technique however:

Table 5. Precision Reported by Various Authors for Standard Solutions Using Various Detection Techniques

extraction mode[a]	analytical technique	quantification in	no. replicates	species, RSD (%) MBT	DBT	TBT	ref
HS-SPME	GC-FID	absolute peak areas	5	9	10	9	20
	GC/ICPMS	relative peak areas (/TPrT)	10	5	9	14	21
	GC/MS	absolute peak areas	5	5	7	11	11
	GC-FPD	absolute peak areas	7	4	8	8	17
	GC-rf-(HC)GD-OES	absolute peak areas	5	8	6	9	27
LPh-SPME	GC-FPD	absolute peak areas	6	3	3	5	9
	GC-AED		6	5	4	6	9
	GC-PFPD		6	4	5	7	9
	GC/ICPMS		6	21[b]	17	25	9
				8[c]	9	16	
	GC/ICPAES	not reported	6	5	5	6	10
liq−liq extraction	GC/ICPAES	not reported	6	5	5	5	10

[a] Organotins were derivatized with $NaBEt_4$. LPh, liquid phase; HS, headspace. [b] Uncorrected precision. [c] Corrected precision (using Xe signal as a normalization standard).

it is more sensitive but less reproducible. Indeed, RSDs ranged from 8 to 18% with SPME, whereas ∼9% was obtained with the classical liquid−liquid extraction GC/MS. Contrary to the liquid−liquid extraction technique, the reproducibility evaluated for the SPME-GC/MS procedure is not similar for all species studied. In this last case, the worst precision was obtained for the TBT. In the headspace mode, the analytes ultimately equilibrate between three phases: the fiber coating, the gas phase (headspace), and the aqueous phase (buffer and standard solutions). Tributylethyltin has the lowest volatility compared to the mono- and dibutyltin derivatives. Therefore, for headspace sampling, equilibration between the liquid and gas phases is more difficult to achieve; this results in poorer reproducibility for this species. Similar results were reported by Cardellicchio et al.,[11] who also used SPME-GC/MS.

These high RSDs are much improved when an internal standard, such as TPrT, is used (Table 4). However, even with the internal standard, the RSDs for TBT remain ∼12%. Similar results were obtained by other authors using different extraction mode-detection system techniques, as summarized in Table 5. These results suggest that TPrT is not an adequate internal standard for quantitation of TBT; however, it serves very well for the DBT.

The use of enriched isotope ^{117}Sn TBT considerably improved precision. If the ratio between m/z 235 and 232 for TBT is considered, the RSD improves to ∼4% in scan mode and 3% in SIM mode. Indeed, with the use of the enriched isotope, the reproducibility of the SPME technique is equivalent to the RSD obtained with the liquid−liquid extraction, providing a very sensitive and reproducible means of quantitation of TBT.

Occurrence of memory effect, which may come from the retention of material in the injection port, column, or ion source, and nearby surfaces was checked. Injections of standard solutions of organotin compounds followed by injection of clean solvent have demonstrated for the absence of memory effect.

Quantitation of TBT in PACS-2. *Standard Additions.* The liquid−liquid extraction GC/MS and the SPME-GC/MS techniques were applied to the determination of organotin in PACS-2 sediment reference material using a standard additions technique for quantitation, as described in the Experimental Section. The results obtained are presented in Table 6 and are in good agreement with the certified values for DBT and TBT. The concentration obtained for MBT is somewhat higher than the certified values. The RSDs obtained for six determinations ranged from 3 to 12%.

Table 6. TBT Concentration in PACS-2 Sediment Determined by Standard Additions Technique Using GC/MS in SIM Mode

	μg/g as Sn dry weight MBT	DBT	TBT
SPME	0.58 ± 0.06	1.06 ± 0.07	0.88 ± 0.03
	10.3%	6.6%	3.4%
liquid−liquid extraction	0.61 ± 0.07	1.07 ± 0.09	0.94 ± 0.02
	11.5%	8.4%	2.1%
certified values	0.45 ± 0.05	1.09 ± 0.15	0.98 ± 0.13

Isotope Dilution. ICPMS has often been used as a sensitive and selective elemental detector for speciated ID analysis.

Equation 1 can be used to calculate the analyte concentration in a solid sample using this methodology,

$$C_x = C_y \frac{m_y}{w m_x} \frac{A_y - B_y(k_b R_b)}{B_x (k_b R_b) - A_x} \frac{AW_x}{AW_y} \quad (1)$$

where C_x is the analyte concentration (μg g^{-1}) based on dry mass, C_y is the concentration (μg mL^{-1}) of enriched speciated spike, m_y is the volume (mL) of spike used to prepare the blend solution of sample and spike, m_x is the mass (g) of the sample used, w is the dry weight correction factor, A_y is the abundance of the reference isotope in the spike, B_y is the abundance of the spike isotope in the spike, A_x is the abundance of the reference isotope in the sample, B_x is the abundance of the spike isotope in the sample, R_b is the measured reference/spike isotope ratio in the blend solution of sample and spike, k_b is mass bias correction factor, AW_y is the analyte atomic weight of spike, and AW_x is the analyte atomic weight in the sample or in the standard. As clearly expressed in this equation, only the isotopic ratio in the spiked sample and the mass bias correction factor need to be measured to derive the final analyte concentration. This information can be easily obtained with an elemental detector, such as ICPMS. The mass bias correction factor can be calculated from the expected

ratio (based on natural isotopic abundance of an element) divided by the ratio measured with a natural abundance standard solution.

Although GC/MS has been used for elemental speciation, only a few reports that cite isotope dilution techniques for quantification are available as a result of the complicated molecular spectrum generated. As expected, the measured isotopic pattern of molecular ions would be skewed from that of the naturally occurring elemental tin isotopic pattern due to contributions from organic ligands. All species arising from combinations of various isotopes for the reference and spike ions must be included in calculations for the real abundance of the reference and spike ions to permit eq 1 to be used for the final quantitation. As reported by Barshick et al.,[33] all major species arising from combinations of different isotopes for CH_3HgI^+ were included for the reference ion (m/z 342) and the spike ion (m/z 346) for the quantitation of mercury using isotope dilution. The mass bias correction factor was calculated from the ratio calculated from the major species using natural isotopic abundance divided by the measured ratio 342/346 in a standard.

In this study, a unique approach was developed for TBT determination using isotope dilution GC/MS without using the relative abundance of selected ions. A reverse spike isotope dilution measurement was performed to quantify the concentration of the enriched ^{117}Sn TBT spike solution in order to ensure the quality of the final results. This method was applied to the determination of TBT in PACS-2 to demonstrate accuracy and precision. The final determination of TBT in PACS-2 was performed using GC/MS with isotope dilution and reverse spike isotope dilution techniques. The following equation (which can be derived from eq 1 by rearranging the formula) was used for quantitation of TBT in PACS-2,

$$C_x = C_z \frac{m_y}{w m_x} \frac{m_z}{m'_y} \frac{k_y R_y - k_b R_b}{k_b R_b - k_x R_x} \frac{k'_b R'_b - k_z R_z}{k_y R_y - k'_b R'_b} \frac{\sum(k_{ix} R_{ix})}{\sum(k_{iz} R_{iz})}$$

$$= C_z \frac{m_y}{w m_x} \frac{m_z}{m'_y} \frac{k_y R_y - k_b R_b}{k_b R_b - k_x R_x} \frac{k'_b R'_b - k_z R_z}{k_y R_y - k'_b R'_b} \quad (2)$$

where C_x is the TBT concentration (μg g^{-1}) based on dry mass; C_z is the concentration (μg mL^{-1}) of a natural TBT standard solution; m_y is the volume (mL) of spike used to prepare the blend solution of sample and spike; m_x is the mass (g) of sample used; w is the dry weight correction factor; m_z is the volume (mL) of a natural TBT standard used to prepare the blend solution of spike and standard; m'_y is the volume (mL) of spike used to prepare the blend solution of spike and natural TBT standard; k_y, k_b, k_x, k'_b, and k_z, are the mass bias correction factors for the measured reference/spike ion ratios (235/232) in the enriched spike solution, the blend solution of sample and spike, the unspiked sample, the blend solution of spike and natural TBT standard, and the natural TBT standard solution, respectively; R_y, R_b, R_x, R'_b, and R_z, are the measured reference/spike ion ratios (235/232) in the enriched spike solution, the blend solution of sample and spike, the unspiked sample, the blend solution of spike and natural TBT standard and the natural TBT standard solution, respectively; and $\sum(k_{ix-ix})$ and $\sum(k_{iz\_iz})$ are the sum of the ratios of all isotope abundances to one isotope abundance chosen as reference in the sample and in the standard. Note that $\sum(k_{ix\_ix}) = \sum(k_{iz\_iz})$ for all elements whose isotopic abundance are invariant in nature.

To achieve best accuracy and precision for the ratio measurement, a mass bias drift correction solution (which was prepared the same way as the reverse spike ID sample) was repeatedly introduced to bracket every four sample solutions in a run sequence. Mass bias drift is the observed change in measured isotope ratio over the course of a series of measurements when the true ratio is not changing. The initial mass bias correction factor of k_0 for the 235/232 ratio and the mass bias drift factor of 1.000 ($f_{t=0}$) were used. The mass bias drift correction solution was injected at the beginning of the run sequence, and the 235/232 ratio ($R_{\text{mass-bias-drift-0}}$) was measured. The mass bias drift factor at number t measurement of the mass bias drift solution during the measurement sequence can be obtained from the following equation,

$$f_{t=t} = f_{t=0} \frac{R_{\text{mass-bias-drift-t}}}{R_{\text{mass-bias-drift-0}}} \quad (3)$$

where $R_{\text{mass-bias-drift-}t}$ is the ratio measured at number t time in the mass bias drift correction solution. The mass bias drift correction factors for the bracketed four samples can be calculated using $f_{t=i}$ and $f_{t=i+1}$ assuming a linear drift. During a sequence, the mass bias drift factors obtained were 1.010 $\pm$ 0.004 and 0.990 $\pm$ 0.004 for liquid−liquid extraction and SPME experiments, respectively. It can be assumed that no significant mass bias drift during a run sequence with GC/MS was observed in this study. The initial mass bias correction factor of k_0 for the 235/232 ratio was therefore used for all measured ratios in the samples. Equation 2 can therefore be simplified as follows:

$$C_x = C_z \frac{m_y}{w m_x} \frac{m_z}{m'_y} \frac{k_0 R_y - k_0 R_b}{k_0 R_b - k_0 R_x} \frac{k_0 R'_b - k_0 R_z}{k_0 R_y - k_0 R'_b}$$

$$= C_z \frac{m_y}{w m_x} \frac{m_z}{m'_y} \frac{R_y - R_b}{R_b - R_x} \frac{R'_b - R_z}{R_y - R'_b} \quad (4)$$

These observations suggest that the mass bias drift solution can be injected less frequently to decrease the analysis time.

Equation 4 was used to calculate the TBT concentration (C_x) in the reference sediment PACS-2. Typical chromatograms for PACS-2 sediment and the isotopic pattern are presented in Figures 1 and 2, respectively. As shown in Table 7, TBT concentrations measured in PACS-2 obtained by ID liquid−liquid extraction GC/MS and ID-SPME-GC/MS are in good agreement with the certified value. The results obtained by isotope dilution and standard additions (Table 6) are also in good agreement. The precision of the data was dramatically improved. Such precision is rarely reported for determinations of TBT in PACS-2 sediment CRM with standard additions or isotope dilution using various analytical techniques.

Evidence of the absence of interferences was confirmed for assured accuracy. Isotope dilution measurements were also performed monitoring different masses. Instead of m/z 232 and 235, the TBT concentration (C_x) in the reference sediment PACS-2 was calculated with the pair of ions 204 and 207. TBT concentra-

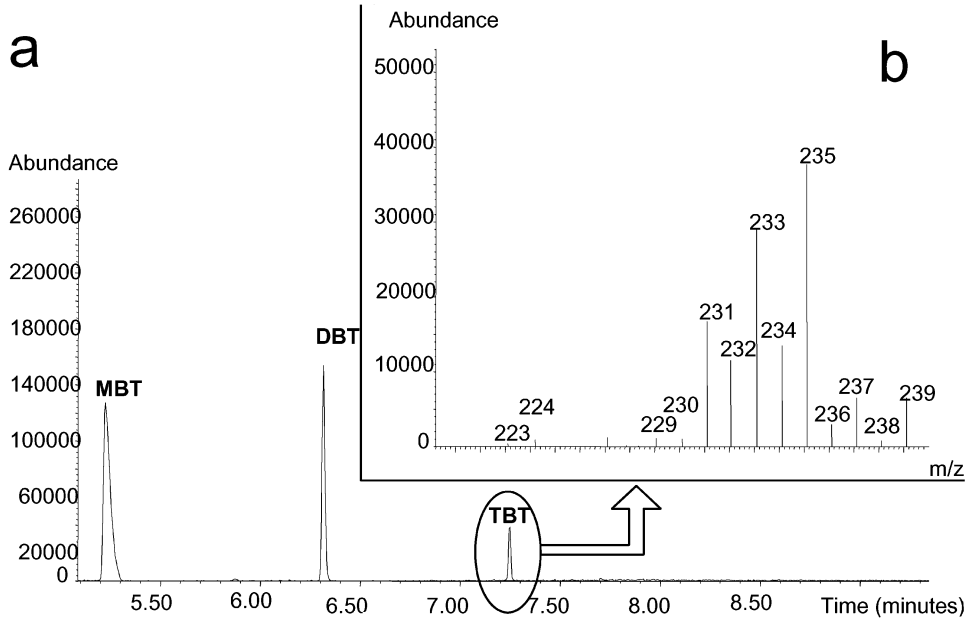

Figure 1. (a) Total ion chromatogram for PACS-2 obtained with SPME sampling using GC/MS in scan mode and (b) isotope pattern of natural TBT.

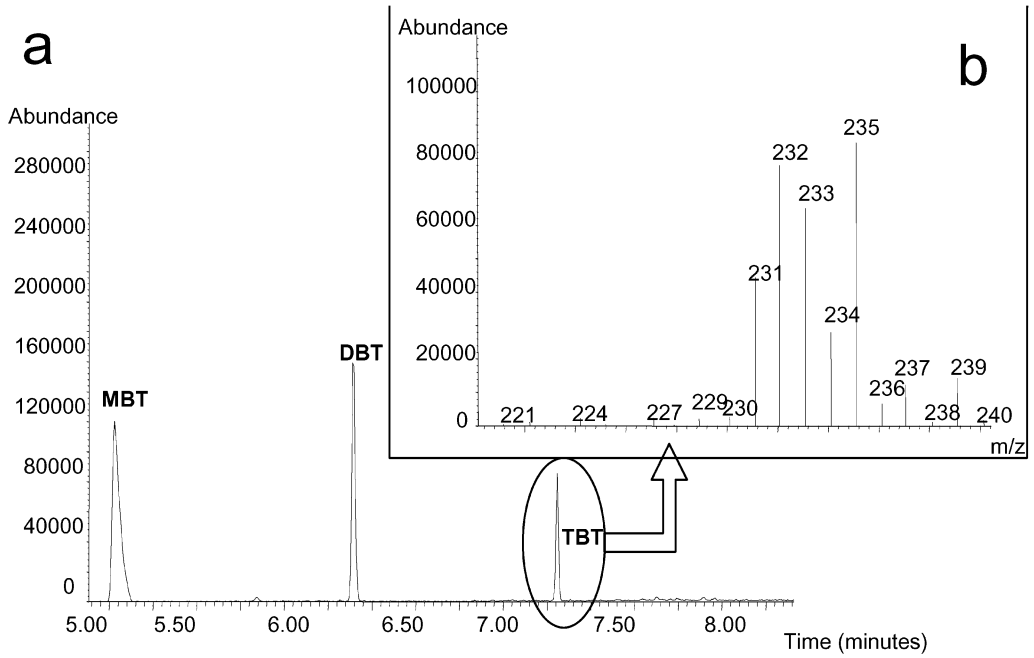

Figure 2. (a) Total ion chromatogram of PACS-2 spiked with ^{117}Sn TBT enriched isotope obtained with SPME sampling using GC/MS in scan mode and (b) isotope pattern of TBT (natural TBT and ^{117}Sn enriched TBT).

tions obtained are in good agreement with the certified value and with those obtained on m/z 232 and 235, but because signal/noise ratios are poorer for this pair of ions, precision is lower.

Ruiz Encinar et al. [34] also performed analysis using ID-GC/ICPMS based on liquid−liquid extraction in hexane, and reported a TBT concentration of $0.86 \pm 0.03\ \mu$g(Sn) g^{-1} with a RSD of 3.5% ($n = 3$) in PACS-2 sediment.

As noted earlier, quantitation of TBT using eq 1, which is based on the relative abundance of isotope compositions of molecular ions, is a difficult task as a result of the complicated calculations, because the contributions of all major species to the chosen reference and spike molecular ions for ID analysis must be considered. It is of interest to compare results obtained using eqs 1 and 2. A software program (Isotope Pattern Calculator v 3.0) developed by Yan [59] can be used to calculate all species contribu-

Table 7. TBT Concentration in PACS-2 Sediment Determined Using ID-GC/MS Technique

	liquid−liquid extraction	SPME	certified value
	μg/g as Sn Dry Weight		
scan mode	0.861 ± 0.025	0.901 ± 0.020	0.98 ± 0.13
	2.88%	2.31%	
SIM mode	0.895 ± 0.015	0.874 ± 0.014	
	1.73%	1.66%	

(59) Yan, J.: http://www.geocities.com/junhuayan/pattern.htm, 2001.

tions for $(C_4H_9)_2SnH^+$ at m/z 235 and 232. Relative abundances of 30.60% for m/z 235 and 8.21% for m/z 232 were obtained in the samples and natural standard based on natural isotopic abundance of Sn, C and H. Relative abundances of 0.052% for m/z 235 and 84.81% for m/z 232 were also calculated in the ^{117}Sn-enriched TBT spike based on the provided Sn isotope composition. The following equation can be used for the calculation of the TBT concentration in PACS-2 using isotope dilution and reverse isotope dilution techniques,

$$C_x = C_z \frac{m_y}{w m_x} \frac{m_z}{m'_y} \frac{A_y - B_y(k_b R_b)}{B_{xz}(k_b R_b) - A_{xz}} \frac{B_{xz}(k'_b R'_b) - A_{xz}}{A_y - B_y(k'_b R'_b)} \quad (5)$$

where A_y and B_y are the relative abundance of ion 235 and ion 232 in the spike solution; A_{xz} and B_{xz} are the relative abundance of ion 235 and ion 232 in the sample or standard.

A mass bias correction factor was obtained from the calculated ratio of 3.727 (30.60%/8.21%) divided by the 235/232 measured ratio in a standard solution. A concentration of 0.874 ± 0.014 μg-(Sn) g^{-1} of TBT in PACS-2 using SPME SIM mode was obtained using eq 5. This result is in a excellent agreement with those obtained using eq 4.

CONCLUSION

The first application of a species specific isotope dilution technique for quantification of TBT by GC/MS provides good accuracy and precision, unsurpassed by other analytical methods, especially with SPME. The mass bias drift is not significant during a run sequence, and the possible quantification of TBT without using the relative abundance of chosen ions provides for a very fast and simple technique, as compared to the standard additions procedure.

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