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Species-Specific Isotope Dilution-Based Calibration for Trace Element Speciation and Its Combined Uncertainty Evaluation: Determination of Tributyltin in Sediment by HPLC−ICPMS

Lu Yang,* Zoltán Mester, and Ralph E. Sturgeon

Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Ontario, Canada, K1A 0R6

A method is described for the determination of tributyltin (TBT) in NRCC sediment CRM PACS-2 by isotope dilution (ID) analysis using HPLC−ICPMS. Reverse spike ID analysis was performed to determine the accurate concentration of a ^{117}Sn-enriched TBT spike using a well-characterized natural abundance TBT standard. The accuracy of the latter is critical for obtaining reliable results. A unique approach, using hydride generation GC/MS, was developed to quantify the inorganic Sn and dibutyltin impurities in the natural abundance TBT standard. The true natural abundance TBT standard concentration was obtained following correction for these impurities. The total Sn concentration in the natural abundance TBT standard was determined by ID analysis using an enriched inorganic ^{117}Sn following closed vessel mixed-acid digestion. Calibration of the enriched inorganic ^{117}Sn standard was achieved by reverse ID analysis against a natural abundance inorganic tin standard prepared from the high-purity metal. An overall uncertainty associated with the present method was estimated, to which the uncertainties arising from measurement of the natural abundance TBT concentration, from the measurement of the isotope ratio in the spiked sample and in the reverse ID calibration solutions, and from estimation of the extraction efficiency were the main contributors. A concentration of 1.018 ± 0.054 mg kg^{-1} (expanded uncertainty, $k = 2$) as tin was obtained for TBT in PACS-2 using the present method, in excellent agreement with the certified value of 0.98 ± 0.13 mg kg^{-1} (95% confidence interval). A TBT concentration of 0.97 ± 0.11 mg kg^{-1} (expanded uncertainty, $k = 2$) as tin in PACS-2 was determined using the standard additions technique. Much smaller expanded uncertainty was obtained with ID, clearly demonstrating its superiority in providing more accurate and precise results over the method of additions. A detection limit (3σ) of 0.02 mg kg^{-1} for TBT, based on a 0.5-g subsample, was obtained.

Introduction of tributyltin (TBT) into the marine environment stems mainly from its use as an antifouling agent. The toxicity effects of TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), have led to a dramatic increase in interest in the development of accurate and rapid analytical methods for their determination in environmental samples. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are currently two of the most common separation techniques in use. Butyltin determinations generally involve several analytical steps, including extraction, derivatiztion (where GC is used), separation, and detection, each of which can contribute to a difficult analysis, prone to poor accuracy, and precision of results.[1,2] During the past decade, inductively coupled plasma mass spectrometry (ICPMS) has gained popularity as a sensitive and selective detector when coupled to GC[3−6] and HPLC[7−9] for butyltin determination. It is well known that ICPMS possesses high sensitivity, a large dynamic range, and multielement capability. Furthermore, if two interference-free isotopes of a given element can be found, isotope dilution ICPMS (ID ICPMS) can be considered, which generally provides superior accuracy and precision over other calibration strategies, including external calibration and the method of standard additions, because a ratio, rather than an absolute intensity measurement, is used for quantitation of the analyte concentration.[10] Once equilibration is achieved between the endogenous analyte in the sample and the added spike, ID ICPMS is theoretically capable of compensating for any subsequent loss of analyte during sample manipulation, suppression of ion sensitivities by concomitant elements present in the sample matrix, and instrument drift. ID ICPMS is considered to be a primary method of analysis.[11]

* Corresponding author. E-mail: Lu.Yang@nrc.ca.

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Although ID ICPMS has been widely employed for trace element analysis in a wide variety of sample matrixes, its application to species-specific determinations has been limited by the nonavailability of commercial species-specific enriched spikes.[12] If these are available, a number of advantages accrue, including the following: enhanced precision and accuracy in results as the species-specific spike serves as an ideal internal standard; 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. Only recently has this approach been applied to the determination of butyltin[3,5,9] and other species, including organolead,[9,13] methylmercury,[14,15] Cr (VI and III),[12] iodide,[16] methylselenocysteine,[17] and selenite[18] using species-specific synthesized spikes.

Reverse spike isotope dilution analysis is needed to accurately quantify the concentration of the enriched species-specific spike by use of a natural abundance high-purity standard. This approach has been reported by several researchers for butyltin analysis.[3,5,9] In such case, the accuracy of the natural abundance TBT standard is of paramount importance in ensuring accurate results and its concentration must therefore be verified before it can be used as the reverse spike for isotope dilution quantitation of the enriched butyltin standards.

In this study, a method is developed to verify the accuracy of the natural abundance butyltin standard concentrations that are needed for their subsequent use in the reverse spike isotope dilution quantitation of enriched species-specific spikes. The method is applied to the determination of TBT in National Research Council of Canada PACS-2 marine sediment CRM using a coupled HPLC−ICPMS approach.[7] A full combined uncertainty calculation (ISO guide),[19] accounting for all possible sources of uncertainty in the measurement process, is reported. To the best of our knowledge, this is the first report of methodology used to verify the concentration of a natural abundance TBT standard to ensure the accurate determination of TBT using not only isotope dilution and reverse spike isotope dilution techniques but other species-specific calibration strategies as well.

EXPERIMENTAL SECTION

Instrument. A Dionex HPLC model AGP-1 (Dionex, Sunnyvale, CA) and a strong cation exchange column (Whatman SCX, 10 μm, 4.6 mm × 250 mm) were used for butyltin separation. A 0.16 mol L^{-1} ammonium citrate solution at pH 4.8 in a 60:40 methanol/water mixture was used as the mobile phase at a flow rate of 1.20 mL min^{-1}. Samples were injected onto the column using a microinjection valve from a Dionex model LCM liquid chromatography module, equipped with an injection loop of nominal 100-μL volume. The coupling of HPLC to ICPMS was accomplished by directing the eluate from the column to the nebulizer through a 0.3-m length of PEEK tubing (0.254-mm i.d., 1.59-mm o.d.).

The ICPMS used in this work was a Perkin-Elmer Sciex ELAN 6000 (Concord, ON, Canada) equipped with a Gem cross-flow nebulizer. A custom-made quartz sample injector tube (0.9-mm i.d.) was used. A double-pass Ryton spray chamber was mounted outside the torch box and maintained at room temperature. Optimization of the ELAN 6000 and dead time correction were performed daily, as recommended by the manufacturer.

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.

A CEM (Matthews, NC) MDS-2100 microwave digester equipped with Teflon vessels was used for closed-vessel high-pressure digestion of butyltin stock solutions for total Sn determination.

A Hewlett-Packard HP 6890 GC/MS (Agilent Technologies Canada Inc., Mississauga, ON, Canada) fitted with a SGE HT8 50 m × 0.22 mm × 0.28 μL 8% phenyl polycarborane−siloxane column (Iso-Mass Scientific Inc., Calgary, AB, Canada) was used for the separation of inorganic tin and butyltin species impurities in all three butyltin stock solutions using HG-GC/MS.

Reagents and Solutions. Acetic, nitric, and hydrochloric acids were purified in-house by subboiling distillation of reagent grade feedstocks in a quartz still prior to use. Environmental grade ammonium hydroxide was purchased from Anachemia Science (Montreal, PQ, Canada). Certified ACS grade ammonium dibasic citrate was purchased from Fisher Scientific (Nepean, Canada). OmniSolv methanol (glass-distilled) was purchased from EM Science (Gibbstown, NJ). High-purity deionized water (DIW), 18.2 MΩ cm, was obtained from a NanoPure mixed-bed ion exchange system fed with reverse osmosis domestic feedwater (Barnstead/Thermolyne Corp, IA). Sodium tetrahydroborate solution, 0.6% (m/v), was prepared daily by dissolving $NaBH_4$ (Alfa Chemicals Inc., Newburyport, MA) in 0.02 mol L^{-1} NaOH (BDH Inc., Toronto, ON, Canada). High-purity H_2O_2 was purchased from Tama Chemicals Co., Ltd. (Tokyo, Japan).

Monobutyltin trichloride (95%), dibutyltin dichloride (96.5%), and tributyltin chloride (96%) 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 use. Concentrations of these standards were first characterized and then used for reverse spike ID analysis of a ^{117}Sn-enriched TBT spike. The natural abundance TBT working standard solution (2.05 mg L^{-1}) was prepared by diluting the stock solution in a 60:40 (v/v) methanol/water mixture the same day that its concentration verification was undertaken.

A ^{117}Sn-enriched TBT stock (97% purity) with stated isotopic compositions and uncertainties provided at a nominal concentration of 90.5 mg L^{-1} in methanol was obtained from the Laboratory of the Government Chemist (LGC, Teddington, U.K.). A working solution containing 0.35 mg L^{-1} as tin was prepared by diluting the stock in a 60:40 (v/v) methanol/water mixture. The concentration of this spike solution was verified by reverse spike isotope

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dilution against the natural abundance TBT standard solution.

Two independent 1000 mg L^{-1} stock solutions of inorganic Sn were prepared by dissolution of the high-purity metal (99.9999%, Johnson, Matthey & Co. Ltd., London, U.K.) in HCl. Enriched ^{117}Sn isotope was purchased from Isotec Inc. (Miamisburg, OH) as metal, and a stock solution of ~177 mg L^{-1} was prepared by dissolution of this metal in HCl. The concentration of ^{117}Sn spike was verified by reverse spike isotope dilution against the natural abundance high-purity metal solution.

WARNING: Organotin compounds are toxic and must be handled with appropriate personal protection.

The marine sediment CRM PACS-2 (NRCC Ottawa) was used as a test sample for method development.

Calibration Sample Preparation for Total Sn Determination in Butyltin Stocks for ICPMS Analysis. Prior to use, a Gilson Microman M250 piston pipet (Mandel Scientific Co. Ltd., Guelph, ON, Canada) was gravimetrically calibrated using DIW to calculate the accurate delivery volume from the mean of repeated weightings (accounting for DIW density). From previous experience, the uncertainty contribution from volume measurements is usually larger than the uncertainty arising from mass, but the overall uncertainty contributions from dilutions by volume remain insignificant compared to the total combined uncertainty characterizing the overall procedure (as shown later in Table 5). Thus, for simplicity in sample preparation, all dilutions were conducted by volume. This observation was confirmed by repeated weighings of aliquots of methanol as follows. First, the volume was obtained by using the mean value of several repeated weighings of DIW divided by the DIW water density. The repeatability of the pipet (which was needed for uncertainty calculation) was estimated from the calculated standard deviation arising from the weighting of several repeated pipettings of methanol into a beaker containeing 10 mL of DIW (to minimize methanol vaporization during the weighing) on a balance. The uncertainty of pipetting methanol was calculated with use of the following equation:

$$(u_{\text{pipetting}})^2 = (u_{\text{m}})^2 + (u_{\text{d}})^2 + (u_{\text{r}})^2$$

where u_{m} is the standard uncertainty of the mean value of several repeated weights of DIW, u_{d} is the standard uncertainty in the density of DIW, and u_{r} is the repeatability of this pipet for pipetting methanol.

Triplicate 0.10-mL aliquots of each 1000−1500 mg L^{-1} TBT, DBT, and MBT stock were accurately pipetted into individual precleaned Teflon digestion vessels. A suitable amount of the enriched inorganic ^{117}Sn was then added to each vessel. Three process blanks (spiked with 10% of the amount of enriched isotope solution used for the samples) were processed along with the samples. After 4 mL of nitric acid, 2 mL of HCl, and 0.2 mL of H_2O_2 were added, the vessels were capped and digested in a CEM MDS-2100 microwave oven. The heating conditions were as follows: 10 min at a pressure of 20 psi and 40% power, 10 min at 40 psi and 50% power, 10 min at 80 psi and 50% power, 15 min at 100 psi and 60% power, and 25 min at 120 psi and 70% power. After cooling, 0.1-mL volumes of the digested solutions were transferred to precleaned polyethylene screw-capped bottles and diluted to 25 mL with 2% HCl.

Table 1. ICPMS Operating Conditions

rf power	1100 W (1600 W for HPLC−ICPMS system)
plasma Ar gas flow rate	15.0 L min^{-1}
auxiliary Ar gas flow rate	1.0 L min^{-1}
nebulizer Ar gas flow rate	0.35 L min^{-1}
sampler cone (nickel)	1.00 mm
skimmer cone (nickel)	0.88 mm
lens voltage	7.50 V
scanning mode	peak hopping
points per peak	1
dwell time	100 ms
sweeps per reading	1
readings per replicate	2000
number of replicates	1
dead time	55 ± 6 ns ($k = 2$)

Calibration of the 177 mg L^{-1} ^{117}Sn-enriched tin spike was achieved by reverse spike isotope dilution analysis. This experiment was performed at the same time as the butyltin stocks were digested. Six replicate blends were prepared by accurately pipetting 0.20-mL volumes of 177 mg L^{-1} ^{117}Sn spike solution into precleaned polyethylene screw-capped bottles. Aliquots of 0.18 mL of the first 1000 mg L^{-1} natural abundance Sn stock solution were added to the first three bottles; this was repeated for the other three bottles using the second 1000 mg L^{-1} Sn stock solution. The contents of each bottle were then diluted with 15 mL of 2% HCl. Prior to analysis by ICPMS, these six reverse spike isotope dilution calibration samples were further diluted 150-fold with 2% HCl. A mass bias drift correction solution was prepared by dilution of 1 mL of the first reverse spike ID calibration sample into 150 mL of 2% HCl.

The digested spiked samples and the six reverse spike ID calibration samples were analyzed by ICPMS on the same day, following a measurement sequence approach similar to that reported by Watters et al.[20] wherein a spiked sample and reverse spike isotope dilution sample were bracketed between mass bias drift correction solutions for best accuracy and precision. The ELAN 6000 ICPMS was optimized daily for nebulizer gas flow and lens voltage in order to achieve the best signal-to-background ratio for tin. Detector dead time was also determined according to the procedure recommended by the manufacturer. The ICPMS operating conditions used in this study are summarized in Table 1.

Calibration Sample Preparation and Procedure for Purity Assessment of Butyltin Stock Solutions Using Hydride Generation (HG)-GC/MS. Aliquots of the 1000−1500 mg L^{-1} stocks of natural abundance TBT, DBT, MBT, and Sn were diluted 10-fold in 50:50 (v/v) methanol/water. Triplicate samples of each working solution were then prepared and analyzed individually. In brief, 0.5 mL of each 100−150 mg L^{-1} Sn (or MBT, DBT, and TBT) working solution was accurately pipetted into a 15-mL vial. After 1 mL of 0.6 mol L^{-1} HCl was added, the vial was capped with a septum. A gas sampling syringe with nominal volume of 10 μL was inserted into the vial through the septum. A 1-mL volume of a 1% $NaBH_4$ in 0.02 mol L^{-1} NaOH solution was injected into the vial through the septum, and the hydrides so formed accumulated in the headspace from which they were sampled by

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Table 2. GC/MS Operating Conditions

injection mode	splitless
injection volume	10 μL
injector temperature	180 °C
column	HT8 (50 m × 0.22 mm × 0.28 μL)
carrier gas	He at 1.2 mL min^{-1}
oven program	30 °C (2 min) to 250 °C at 30 °C min^{-1}
transfer line temperature	250 °C

gas syringe. The sampled hydrides were immediately injected onto the GC column for separation and detection. The GC/MS program used in this study is summarized in Table 2. Quantitation was achieved by one-point external calibration using a working standard solution.

Sediment Sample Preparation and Procedure for HPLC−ICPMS Analysis. The sediment extraction procedure used in this study was described earlier.[7] Three process blanks, triplicate samples of PACS-2, and six reverse spike isotope dilution calibration samples were prepared at the same time. In brief, 0.5 g of PACS-2 spiked with 0.400 mL of ^{117}Sn-enriched TBT solution and 5 mL of acetic acid were heated in a Prolabo microwave digester at 60% power for 3 min. The contents were centrifuged at 2000 rpm for 5 min. A 1.00-mL volume of the supernatant was transferred to a reaction vial, 1 mL of DIW added, and the mixture adjusted to pH 5−6 with 1.2 mL of ammonium hydroxide. The contents were then buffered with 0.8 mL of ammonium citrate (2 mol L^{-1}) and diluted to 10 mL with methanol. Three process blanks were prepared in the same way. Six reverse spike isotope dilution calibration samples were also prepared as described below. A 0.150-mL volume of ^{117}Sn-enriched TBT spike solution and 0.250 mL of 2.05 mg L^{-1} natural abundance TBT solution were accurately pipetted into a vial. After 1 mL of acetic acid and 1 mL of DIW were added, the pH was adjusted to 5−6 with 1.2 mL of ammonium hydroxide and the mixture then buffered with 0.8 mL of ammonium citrate (2 mol L^{-1}) and diluted to 10 mL with methanol. A mass bias correction solution was prepared the same way without microwave heating using 0.50 mL of 2.05 mg L^{-1} natural abundance TBT standard. An uncertainty associated with the mass bias correction factor was also calculated.

A spike recovery experiment was conducted to elucidate the extraction efficiency of the method used. Sample preparation followed the procedure reported earlier.[7] In brief, four replicate spiked samples were prepared by addition of a 0.500-mL volume of 2.05 mg L^{-1} natural abundance TBT standard to each 0.5 g of sediment subsample. The ^{117}Sn-enriched material could have been used to advantage here, but a decision was made to conserve the limited amount of ^{117}Sn-enriched species available. For the unspiked sample, to match the matrix, a volume containing 0.500 mL of 60:40 methanol/water was added. After 5 mL of acetic acid was added, the sample was heated in the Prolabo microwave digester at 60% power for 3 min. The contents were transferred to centrifuge tubes and centrifuged at 2000 rpm for 5 min. A 1.00-mL volume of the supernatant was transferred to a reaction vial. A 1-mL volume of DIW was added, and the mixture was adjusted to pH 5−6 with 1.2 mL of ammonium hydroxide. The contents were then buffered by addition of 0.8 mL of ammonium citrate (2 mol L^{-1}) and diluted to 10 mL with methanol. A matrix matched calibration standard was also prepared by adding 0.0909 mL of 2.05 mg L^{-1} natural abundance TBT standard to 1 mL of supernatant from the unspiked sample digest. Samples were then delivered to the HPLC−ICPMS for analysis.

The HPLC was coupled to the ELAN 6000 after optimization of the instrument. Following injection of the sample (100 μL) onto the HPLC column, data acquisition for the Elan 6000 was manually triggered. Both ^{117}Sn and ^{118}Sn were monitored. The mass bias solution was injected between samples to permit calculation of a mass bias correction factor. At the end of the chromatographic run, the acquired data were transferred to an off-line computer for further processing using in-house software to yield both peak height and peak area information. In this work, only peak areas were used to generate ^{118}Sn/^{117}Sn ratios, from which the analyte concentration in the sediment was calculated.

RESULTS AND DISCUSSION

ID MS has been identified as a primary (ratio) method of measurement[11] as it is a method within the SI having the highest metrological qualities whose model (mathematical equation) and realization are completely described and understood in terms of SI units. In practice, this can only be achieved if its limitations are thoroughly recognized and appropriate actions are taken to minimize or eliminate all sources of error. In the present study, contamination control is effected by conducting all sample manipulations under cleanroom conditions. Equilibration between the added spike and the endogenous analyte in the sample has been investigated by serial spike experiments (as shown latter), and most significantly, attention has been devoted to elucidation of the true concentration of the species-specific enriched spike to achieve full traceability of the final result to the SI via calibration against a gravimetrically prepared high-purity metal standard.

Characterization of Natural Abundance TBT Stock. Earlier studies in our laboratory revealed that up to 30% loss of TBT could occur from a 100 mg L^{-1} standard solution kept in an open vial at 50 °C for 1 h. No detectable loss was found for DBT and MBT standard solutions under these same conditions. Loss of TBT from acidified solutions (HCl and acetate) was also reported by Mester et al.,[21,22] even at room temperature. These observations confirm that TBT is a very volatile compound and care must be taken during preparation and storage of its solutions and samples to minimize possible loss and consequent errors. Experiments were therefore designed to obtain the true concentration of natural abundance TBT stock solution, which was used for reverse spike isotope dilution determination of the concentration of a ^{117}Sn-enriched TBT spike. The entire process is outlined in the flowchart illustrated in Figure 1. Step 1 employed reverse spike isotope dilution ICPMS to determine the concentration of the enriched ^{117}Sn spike using a natural abundance Sn stock prepared from the high-purity metal. In step 2, total inorganic Sn concentrations in the digested natural abundance TBT, DBT, and MBT stocks were quantified by isotope dilution ICPMS analysis using the enriched ^{117}Sn spike. Step 3 quantified the inorganic Sn impurity in the natural abundance MBT stock by HG-GC/MS. Step 4 calculated the true MBT concentration in the natural abundance MBT stock. Step 5 quantified the MBT impurity in the natural abundance DBT stock by HG-GC/MS. Step 6 obtained the true

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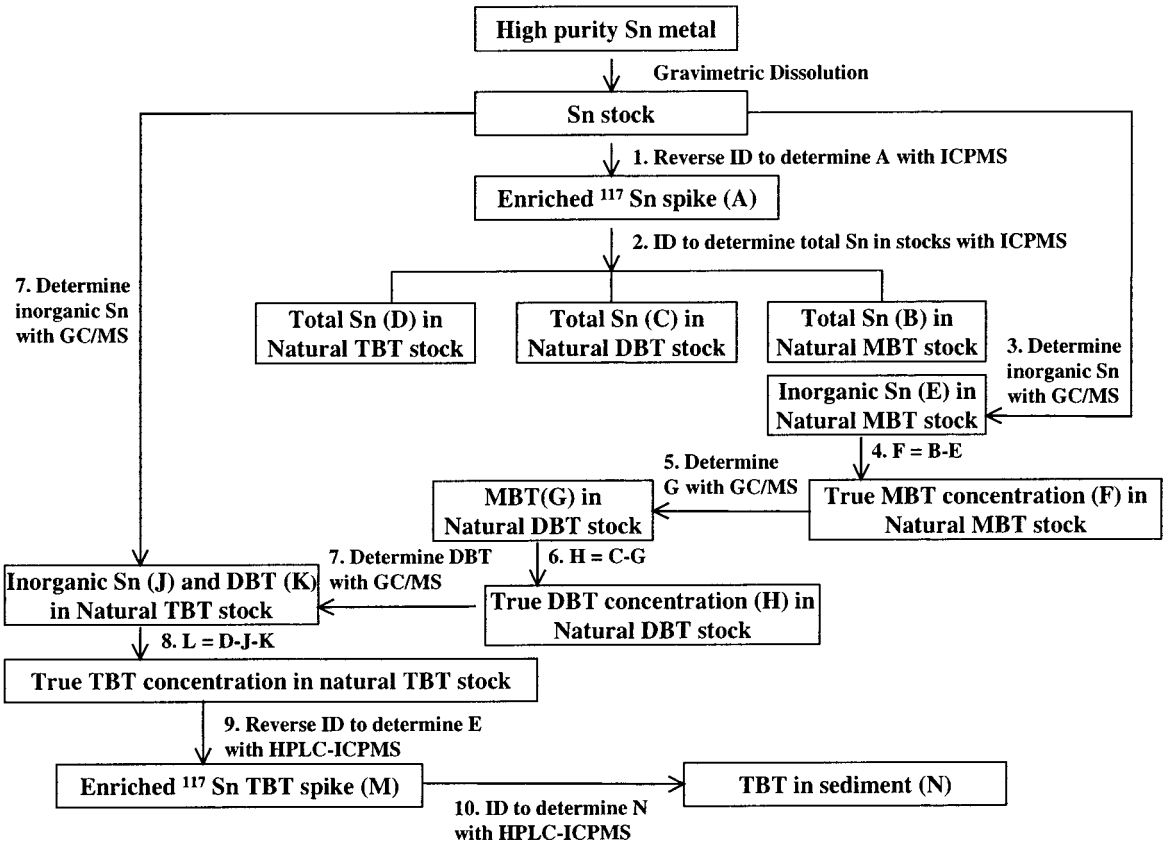

Figure 1. Flowchart for isotope dilution and reverse spike isotope dilution for determination of TBT in PACS-2 sediment.

Table 3. Characterization of Natural Butyltin Stocks (mg L^{-1} as Sn)[a]

	TBT stock	DBT stock	MBT stock
total Sn measured	1523 ± 30	1259 ± 32	1087 ± 29
inorganic Sn determined	0.95 ± 0.14	<0.5	13.8 ± 1.7
MBT determined	<0.5	2.9 ± 1.6	
DBT determined	34 ± 40		<0.5
TBT determined		<0.5	<0.5
corrected butyltin conc	1488 ± 51	1256 ± 33	1073 ± 29

[a] Results are reported with expanded uncertainty, $k = 2$.

DBT concentration in the natural abundance DBT stock. Step 7 quantified inorganic Sn and DBT impurities found in the natural abundance TBT stock by HG-GC/MS. Step 8 calculated the true TBT concentration in the natural abundance TBT stock. Step 9 employed reverse spike isotope dilution HPLC−ICPMS analysis to determine the concentration of an enriched ^{117}Sn-TBT spike using the natural abundance TBT stock. Step 10 measured the TBT concentration in sediment subsamples by isotope dilution HPLC−ICPMS using the enriched ^{117}Sn-TBT spike. As evident from the above, the accuracy of the final TBT determination in the sediment is traceable to an accurate gravimetrically prepared natural abundance Sn stock solution. It is noteworthy that all responses were ultimately based on inorganic tin and when individual organotin compounds were quantitated, a matched species standard was used for calibration to eliminate variable responses from the hydride forms of these species. This flowchart is not only useful for TBT determination in marine sediment CRM PACS-2 but is generically applicable to all species-specific ID-MS. To achieve the most accurate results, measurements with natural TBT stock solution (determination steps 2−7) and those for TBT determination in PACS-2 (step 10) were conducted as close as temporally possible to minimize any unforeseen change in the concentration of TBT stock solution after its quantitation. Once the characterization of natural abundance TBT stock (steps 1−7) is established and its associated uncertainty calculated, these data (shown in Table 3) can be used in future studies without having to repeat these determination steps.

The following equation was used to quantify total Sn concentration (step 2 in the flowchart) in the TBT, DBT, and MBT stocks using isotope dilution and reverse spike isotope dilution techniques:

$$C = C_z \frac{m_y}{m_x} \frac{m_z}{m'_y} \frac{A_y - B_y R_n}{B_x R_n - A_x} \frac{B_z R'_n - A_z}{A_y - B_y R'_n} \frac{\mathrm{AW}_x}{\mathrm{AW}_z} - C_b \qquad (1)$$

where C is the blank-corrected total Sn concentration (mg L^{-1}) in the stocks; C_z is the concentration of primary assay Sn standard (mg L^{-1}); m_y is the volume (L) of spike used to prepare the blend solution of sample and spike; m_x is the volume (L) of butyltin stock used; m_z is the volume (L) of primary assay Sn standard; m'_y is the volume (L) of spike used to prepare the blend solution of spike and primary assay Sn standard solution; A_y is the abundance of the reference isotope (^{118}Sn) in the spike; B_y is the abundance of the spike isotope (^{117}Sn) 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; A_z is the abundance of the reference isotope

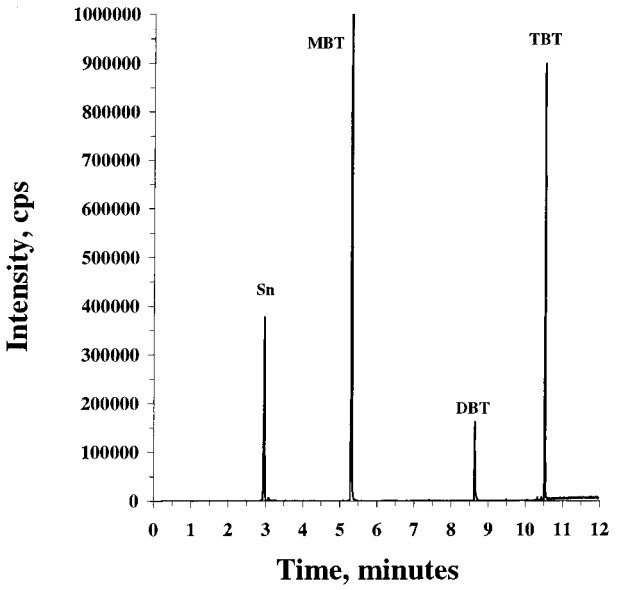

Figure 2. Chromatogram of inorganic Sn, MBT, DBT, and TBT hydrides obtained from a mixed standard solution (0.5 mL each of 100 mg L^{-1}) using HG-GCMS, 10-μL gas injection.

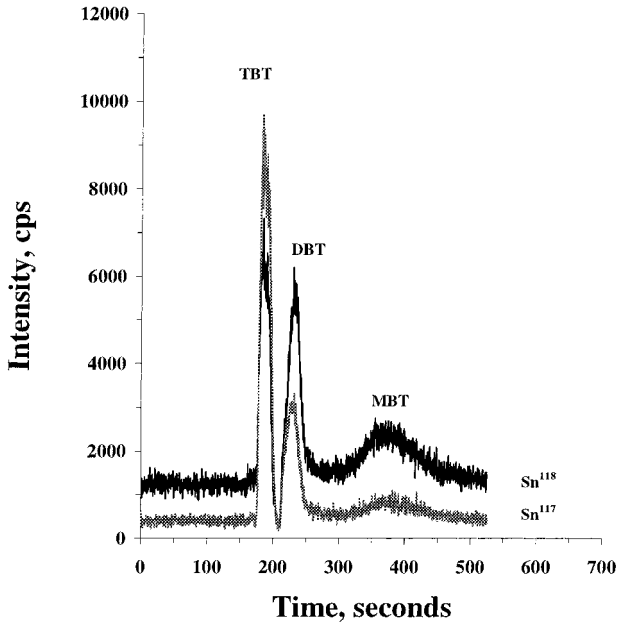

Figure 3. Chromatogram of butyltins in extract of ^{117}Sn-TBT spiked PACS-2. Optimum elution conditions using 0.16 mol L^{-1} ammonium citrate in a 60:40 methanol/water at pH 4.8 as carrier using HPLC−ICPMS; 100-μL injection.

in the primary assay standard; B_z is the abundance of the spike isotope in the primary assay standard; AW_x is the atomic weight of analyte in the sample; AW_z is the atomic weight of the Sn standard; R_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike; R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and inorganic Sn standard; C_b is the analyte concentration in the blank (m L^{-1}).

This equation can be simplified (eq 2) for all elements whose isotopic abundance is invariant in nature (hence, $A_x = A_z = A_{xz}$, $B_x = B_z = B_{xz}$, $AW_x = AW_z$, i.e.):

$$C = C_z \frac{m_y}{m_x} \frac{m_z}{m'_y} \frac{A_y - B_y R_n}{B_{xz} R_n - A_{xz}} \frac{B_{xz} R'_n - A_{xz}}{A_y - B_y R'_n} - C_b \qquad (2)$$

Results obtained for total Sn concentration in three butyltin stocks are summarized in Table 3. A value of 5.60 ± 0.35 mg L^{-1} (expanded uncertainty, $k = 2$) found in procedural blanks was used to correct the total Sn concentration in the stock solutions.

Good separation of the hydrides of inorganic tin, MBT, DBT, and TBT was obtained on a slightly nonpolar capillary column for HG-GC/MS, as shown in Figure 2. Only some inorganic Sn and DBT were detected in the natural abundance TBT stock solution based on HG-GC/MS analysis, as shown in Table 3. The sum of all other Sn compounds was less than 0.1% of the TBT concentration and therefore not further considered. Poor precision (6−25% RSD) of the HG-GC/MS results for impurities was obtained, likely a consequence of irreproducible manual gas sampling. However, the final TBT concentration was not significantly affected since the small amount of inorganic Sn and DBT detected in the TBT stock necessitated only a minor correction to the gravimetrically prepared solution concentration. The final natural TBT stock concentration of 1488 ± 51 mg L^{-1} (expanded uncertainty, $k = 2$), obtained by subtracting the inorganic Sn and DBT concentrations found in the TBT stock from the measured total Sn concentration, is close to the gravimetrically expected value of 1483.7 ± 9.2 mg L^{-1} (expanded uncertainty, $k = 2$) calculated from the dissolution of the pure TBT compound in methanol based on the suppliers' 96% purity statement.

Results for TBT in PACS-2 Using HPLC−ICPMS. Chromatographic separation of all three butyltin species was obtained on a strong cation exchange column, as described previously.[7] As shown in Figure 3, good separation of TBT from other butyltins was obtained in a digest of PACS-2, which was spiked with the enriched ^{117}Sn-TBT. As noted earlier, ID ICPMS is capable of compensating for any subsequent loss of analyte during sample manipulation, suppression of ion sensitivities by concomitant elements present in the sample matrix, and for instrument drift, providing equilibration has been achieved between the endogenous anlyte in the sample and the added spike. To achieve accurate and precise results, care must be taken to avoid any contamination during the process, an interference-free isotope pair must be available for ratio measurements, and an optimum measurement procedure must be used to achieve accurate ratio measurements. In this study, contamination control was effected by preparing all samples in a cleanroom environment. In a subsequent experiment, the ^{118}Sn/^{117}Sn ratio from TBT was measured in an unspiked PACS-2 extract using HPLC−ICPMS to investigate possible interferences on the isotopes selected for measurement. A mass bias corrected ratio of 3.148 ± 0.028 (expanded uncertainty, $k = 2$) obtained in unspiked PACS-2 was not significantly different from the expected natural abundance ratio of 3.154. This observation confirmed that no significant spectroscopic interference on either isotope arose from the sample matrix, permitting accurate results to be obtained using the chosen isotope pair.

Equilibration between added spike and the endogenous analyte in the sample is a prerequisite for achieving accurate results using isotope dilution techniques. Results would be biased low if

equilibration between the spike and the sample was not achieved during the ratio measurements (with the spike being recovered with greater efficiency). Although direct proof of equilibration being achieved between the spike and the sample is still lacking at this stage, the effect of sample mass on spike recovery can be used to help elucidate this process. A series of experiments was previously conducted to study the effect of sediment subsample mass on the spike recovery of butyltin species. Sample weights of 0.15, 0.5, 1.0, and 2.0 g of PACS-2 sediment were used and recoveries of the spikes reported.[7] It was noted that quantitative spike recoveries of TBT were obtained for sample weights from 0.15 to 0.5 g. Spike recovery of TBT decreased from 100 to 83% when sample weights increased from 0.5 to 2.0 g. Despite the difference in the spike recoveries obtained, no significant difference in TBT concentrations measured in PACS-2 was observed using standard additions calibration. These results suggest that the added spike mimics the analyte in the sample during the microwave extraction, as equilibration between the added spike and the endogenous TBT in the sample was likely achieved during microwave-assisted extraction. It is thus assumed that 100% extraction efficiency is achieved when quantitative spike recovery is obtained. It is clear at this time that irrefutable evidence of this is lacking, and hence, the strict tracecebility chain is not secured. A spike recovery test (or extraction efficiency test) was again conducted based on microwave extraction of a 0.5 g of PACS-2 sediment with 5 mL of acetic acid. The spike recovery calculation used the same equation reported earlier.[7] A spike recovery of 100 ± 3% (expanded uncertainty, $k = 2$) for TBT was obtained, and this value was subsequently used to estimate the contribution to the standard uncertainty arising from extraction efficiency.

The final analysis of TBT in PACS-2 was performed using HPLC−ICPMS with isotope dilution and reverse spike isotope dilution techniques (steps 9 and 10 in Figure 1). Prior to ratio measurements in samples for TBT determination in PACS-2, the ELAN 6000 was optimized and dead time correction was determined following the procedure recommended by the manufacturer. All sample blanks, spiked samples, and reverse spike isotope dilution samples were analyzed by HPLC−ICPMS immediately after they were prepared. To achieve best accuracy and precision for the ratio measurement, mass bias correction solution was repeatedly introduced between a spiked sample and reverse spike ID sample. The mass bias correction factor was calculated using the expected ratio divided by the ratio measured with the TBT mass bias correction solution. The component of uncertainty associated with this factor was accounted for when the uncertainty calculation in mass bias corrected ratios (R_n or R'_n) was undertaken. The following equation was used for quantitation of TBT in PACS-2:

$$C = C_z \frac{m_y}{w m_x} \frac{m_z}{m'_y} \frac{A_y - B_y R_n}{B_{xz} R_n - A_{xz}} \frac{B_{xz} R'_n - A_{xz}}{A_y - B_y R'_n} E - C_b \qquad (3)$$

where C is the blank corrected total TBT concentration (mg kg^{-1}) based on dry mass; C_z is the concentration of natural abundance TBT standard (mg L^{-1}); m_y is the volume (L) of spike used to prepare the blend solution of sample and spike; m_x is the mass (kg) of sample used; w is the dry mass correction factor; m_z is the volume (L) of natural abundance TBT standard used; m'_y is the volume (L) of spike used to prepare the blend solution of spike and natural abundance TBT standard solution; A_y is the abundance of the reference isotope (^{118}Sn) in the spike; B_y is the abundance of the spike isotope (^{117}Sn) in the spike; A_{xz} is the abundance of the reference isotope in the sample or in the standard; B_{xz} is the abundance of the spike isotope in the sample or in the standard; R_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike; R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance TBT standard; E is the efficiency with which the endogenous TBT is recovered from the subsample (assumed $E = 1.00$); C_b is the analyte concentration of the blank (mg kg^{-1}). The TBT concentration obtained in PACS-2 is shown in Table 4. Excellent agreement between the certified and measured value suggests that equilibration between the added spike (^{117}Sn-enriched TBT) and the endogenous TBT in the sample was achieved during the microwave-assisted extraction.

Table 4. Results for TBT in PACS-2 (mg kg^{-1} as Sn)

	ID method	standard addition method	certified
recommended value	1.018	0.97	0.98 ± 0.13[a]
combined uncertainty, u_c	0.0315	0.0561	
coverage factor, k	2	2	
expanded uncertainty, U	0.063	0.11	
average	1.018	0.97	
std dev ($n = 3$) S_i	0.006	0.02	

[a] Uncertainty expressed as 95% confidence interval.

A method detection limit was derived for the ID HPLC−ICPMS technique based on measurements of three ^{117}Sn-TBT spiked sample blanks. A value of 0.02 mg kg^{-1}, based on three times the standard deviation of measured concentrations of sample blanks normalized to a 0.0005-kg sample, was comparable to the detection limit of 0.03 mg kg^{-1} reported earlier.[7]

Total Combined Standard Uncertainty Calculation. Accounting for all possible sources of uncertainty arising from the measurement process is of fundamental importance for producing an accurate value for a measurement. According to ISO *Guide to the Expression of Uncertainty in Measurement*,[19] the combined standard uncertainty of a measurement result y, designated $u_c(y)$, can be obtained from the following eq 4, where $y = f(x_1, x_2, ..., x_N)$

$$u_c^2(y) = \sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i}\right)^2 u^2(x_i) + 2 \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \left(\frac{\partial f}{\partial x_i}\right) \left(\frac{\partial f}{\partial x_j}\right) u(x_i, x_j) \qquad (4)$$

and is conveniently referred to as the law of propagation of uncertainty. The partial derivatives $\partial f/\partial x_i$ are often referred to as sensitivity coefficients, $u(x_i)$ is the standard uncertainty associated with the input x_i, and $u(x_i, x_j)$ is the estimated covariance associated with x_i and x_j. When parameters in $y = f(x_1, x_2, ..., x_N)$ are independent, the combined standard uncertainty in y can be obtained from eq 5. Details of the means by which standard

$$u_c^2(y) = \sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i}\right)^2 u_{xi}^2 \qquad (5)$$

uncertainties are estimated for each individual parameter are beyond the scope of this study, and calculation procedures for individual parameters were in line with the ISO guide[19] and EURACHEM/ CITAC guide.[23] It is worth noting the following two simplified equations for calculation of the combined standard uncertainty:

$$\left(\frac{u_y}{y}\right)^2 = \left(\frac{u_a}{a}\right)^2 + \left(\frac{u_b}{b}\right)^2 \quad (6)$$

when $y = rab$ or $y = ra/b$, a and b are independent parameters, and r is a constant,

$$(u_y)^2 = (r_a u_a)^2 + (r_b u_b)^2 \quad (7)$$

when $y = r \pm r_a a \pm r_b b$, where r, r_a, and r_b are constants and a and b are independent parameters. Equations 6 and 7 can only be applied when dealing with either an exclusive mix of only additions and subtractions or with a combination of only divisions and multiplications. For example, eq 7 can be used to calculate the combined standard uncertainty of a Sn stock solution obtained by quantitative dissolution of pure Sn metal in HCl. The Sn stock concentration can be expressed as $C_{std} = wp/m$, where w is the mass of Sn metal used, p is the purity of the Sn metal, and m is the final mass of solution. The combined standard uncertainty (u_c) in such case can be calculated using following equation:

$$\left(\frac{u_c}{c}\right)^2 = \left(\frac{u_w}{w}\right)^2 + \left(\frac{u_p}{p}\right)^2 + \left(\frac{u_m}{m}\right)^2 \quad (8)$$

Equation 3 used for calculation of the TBT concentration is much too complicated, and eqs 6 and 7 are no longer adequate to calculate the resulting combined standard uncertainty in the TBT concentration in PACS-2 obtained by isotope dilution and reverse spike isotope dilution techniques. The natural complexity of eq 3 makes combined standard uncertainty calculation a difficult and time-consuming task. Kragten[24] reported a relatively simple spreadsheet method to calculate combined standard uncertainty, and the EURACHEM/ CITAC guide[23] has adopted this approach for application to isotope dilution analysis. This spreadsheet method is based on the following principle: when $y(x_1, x_2, ..., x_N)$ is either linear in x_i or $u(x_i)$ is small compared to x_i, the partial derivatives ($\partial f/\partial x_i$) can be approximated by eq 9; if conditions do not meet these requirements, eq 5 must be used:

$$\frac{\partial f}{\partial x_i} \approx \frac{f(x_i + u(x_i)) - f(x_i)}{u(x_i)} \quad (9)$$

Thus:

$$\frac{\partial f}{\partial x_i} u(x_i) \approx f(x_i + u(x_i)) - f(x_i) \quad (10)$$

By substitution of eq 10 into eq 5, the combined standard uncertainty in C can now be obtained using eq 11:

$$u_C{}^2 \approx \sum (f(x_i + u(x_i)) - f(x_i))^2 \quad (11)$$

Table 5. Components of Uncertainty for Determination of TBT in PACS-2 Sediment

parameter	typical value	type A/B	u_{xi}	$\partial f/\partial x_i$	$(\partial f/\partial x_i)u_{xi}$
C_z (mg L^{-1})	2.0563	B	0.03561209	0.4956813	0.0176522
m_y (L)	0.000400	B	0.00000044	2548.167013	0.0011295
m_x (kg)	0.000500	B	0.00000019	−2038.53361	−0.0003894
w	0.99347	A	0.00017000	−1.0259705	−0.0001744
m_z (L)	0.00025	B	0.00000030	4077.067222	0.0012126
m'_y (L)	0.00015	B	0.00000028	−6795.11203	−0.0018792
A_y	0.0020	B	0.00002887	−0.8494280	−0.0000245
B_y	0.921	B	0.00028868	0.0018446	0.0000005
A_{xz}	0.2422	B	0.00002887	1.8749330	0.0000541
B_{xz}	0.0768	B	0.00002887	−5.9128747	−0.0001707
R_n	0.65392	B	0.00896950	1.9716444	0.0176847
R'_n	1.30630	B	0.00802214	−1.3333160	−0.0106960
E	1.000	A	0.01500000	1.01927	0.0152890
C_b (mg kg^{-1})	0.0011	A	0.00330498	−1.0000000	−0.0033050
combined uncertainty (mg kg^{-1}), u_c					0.03146

In this study, the universal eq 5 was used for calculation of the combined standard uncertainty in order to achieve an accurate estimation of total uncertainty in the TBT concentration measured in PACS-2 using ID HPLC−ICPMS. It is worth noting that, although the extraction efficiency (E) of the method does not affect the final TBT concentration in PACS-2 because equilibration between the added spike and the endogenous analyte was achieved, the total combined uncertainty calculation must still account for the uncertainty contributed by the extraction process. A typical value of 1.00 for E (100%, based on spike recovery data) was used in eq 3 for the calculation of uncertainty. The standard uncertainty of E was estimated from spike recovery data (calculated from the standard deviation of replicate spike recovery results divided by the square root of the number of replicates). The extraction efficiency estimation remains as the weak link in the traceability chain for determined TBT concentration since the true extraction efficiency still cannot be demonstrated at this stage.

Equation 3 was used for the estimation of total combined uncertainty using the ID HPLC−ICPMS method, and individual partial derivatives were derived for all variables in eq 3 and presented in Appendix A. Total combined standard uncertainty of TBT determination in PACS-2 using the method of standard additions was also calculated for comparison purposes using eq 5. Both results are summarized in Table 4. As expected, the combined standard uncertainties for TBT in PACS-2 from both techniques are much larger than the simple standard deviations calculated from their replicate measurements. Much smaller expanded uncertainty characterizes the method of isotope dilution compared to the standard additions technique. The components of uncertainty for determination of TBT in PACS-2 by ID HPLC−ICPMS are summarized in Table 5. It is clear that uncertainty of measurement of the natural abundance TBT concentration, uncertainty associated with the measurement of the isotopic ratio in the spiked sample and in the reverse ID calibration solutions,

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and uncertainty associated with extraction efficiency are the principal sources contributing to the total combined standard uncertainty. This elucidation provides important information for the design of future experiments aimed at reducing the overall uncertainty of the methodology, thereby achieving more precise and accurate results.

CONCLUSION

A general protocol for species-specific ID analysis has been presented and a unique, species-specific ID calibration approach using ICPMS in combination with HG-GC/MS to characterize a natural abundance TBT stock solution is described. This ensured accurate determination of TBT in PACS-2 using ID HPLC−ICPMS. Use of ID techniques for calibration minimizes error introduced in the extraction step. If microwave conditions are not adequately controlled, decomposition of butyltins may occur, as reported by Encinar et al.[25] No alteration of Sn isotopic abundance was observed in this study for DBT and MBT following microwave extraction of ^{117}Sn-TBT spiked sediment. Although ID ICPMS was utilized, the full traceability chain was not secured due to the assumption necessary in estimating the extraction efficiency. This problem plagues all speciation schemes, irrespective of whether ID techniques are used or not. The final TBT concentration measured in PACS-2 sediment is traceable to a gravimetrically prepared high-purity Sn stock. The total combined uncertainty reveals the superiority of the isotope dilution method over the standard addition calibration technique. Future studies aim to improve the precision and accuracy of HG-GC/MS results in order to ensure more accurate and precise final results, such as by employing species-specific ID techniques for determination of impurities in natural abundance stock solutions. Although the resolution of separation of the butyltin species by HPLC is sufficient for the PACS-2 sediment CRM, future studies will be undertaken to improve the resolution of the chromatographic separation.

APPENDIX A

Individual partial derivatives derived from eq 3

$$\frac{\partial C}{\partial C_z} = \frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (12)$$

$$\frac{\partial C}{\partial m_y} = C_z\frac{1}{w m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (13)$$

$$\frac{\partial C}{\partial w} = -C_z\frac{m_y}{w^2 m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (14)$$

$$\frac{\partial C}{\partial m_x} = -C_z\frac{m_y}{w m_x^2}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (15)$$

$$\frac{\partial C}{\partial m_z} = C_z\frac{m_y}{w m_x}\frac{1}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (16)$$

$$\frac{\partial C}{\partial m'_y} = -C_z\frac{m_y}{w m_x}\frac{m_z}{m'^2_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (17)$$

$$\frac{\partial C}{\partial A_y} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{B_{xz}R'_n - A_{xz}}{B_{xz}R_n - A_{xz}}\frac{B_y R_n - B_y R'_n}{(A_y - B_y R'_n)^2}E \quad (18)$$

$$\frac{\partial C}{\partial B_y} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{B_{xz}R'_n - A_{xz}}{B_{xz}R_n - A_{xz}}\frac{A_y R'_n - A_y R_n}{(A_y - B_y R'_n)^2}E \quad (19)$$

$$\frac{\partial C}{\partial A_{xz}} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{A_y - B_y R'_n}\frac{B_{xz}R'_n - B_{xz}R_n}{(B_{xz}R_n - A_{xz})^2}E \quad (20)$$

$$\frac{\partial C}{\partial B_{xz}} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{A_y - B_y R'_n}\frac{A_{xz}R_n - A_{xz}R'_n}{(B_{xz}R_n - A_{xz})^2}E \quad (21)$$

$$\frac{\partial C}{\partial R_n} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{A_{xz}B_y - A_y B_{xz}}{(B_{xz}R_n - A_{xz})^2}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n}E \quad (22)$$

$$\frac{\partial C}{\partial R'_n} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}A_y - B_y A_{xz}}{(A_y - B_y R'_n)^2}E \quad (23)$$

$$\frac{\partial C}{\partial E} = C_z\frac{m_y}{w m_x}\frac{m_z}{m'_y}\frac{A_y - B_y R_n}{B_{xz}R_n - A_{xz}}\frac{B_{xz}R'_n - A_{xz}}{A_y - B_y R'_n} \quad (24)$$

$$\partial C/\partial C_b = -1 \quad (25)$$

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