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Evaluation of Extraction Techniques for the Determination of Butyltin Compounds in Sediments Using Isotope Dilution-GC/ICPMS with ^{118}Sn and ^{119}Sn-Enriched Species

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Different liquid−solid extraction techniques, including room-temperature leaching with mechanical shaking, ultrasonic, and microwave-assisted extractions, have been evaluated for the quantitative speciation of tin for mono-, di-, and tributyltin (MBT, DBT, and TBT, respectively) in PACS-2 and BCR-646 certified reference materials. A methanol−acetic acid mixture was used as the extractant reagent in all cases. For this purpose, a mixed spike containing ^{119}Sn-enriched MBT (79.7 At%), ^{118}Sn-enriched DBT (86.7 At%), and ^{119}Sn-enriched TBT (83.1 At%), was synthesized, characterized, and used for isotope dilution analysis. The isotopic composition of the mixed spike was determined by gas chromatography/ICPMS after aqueous ethylation using sodium tetraethylborate, and the determination of the concentration of the different species in the spike was performed by means of reverse isotope-dilution analysis using natural MBT, DBT, and TBT standards. In the analysis of the certified sediments, the sample was spiked with the mixed spike, extracted under different conditions, derivatized with sodium tetraethylborate, and extracted into hexane, and the isotope ratios 120/118 and 120/119 were measured as peak area ratios for all butyltin species after GC/ICPMS. Mass bias was corrected using a derivatized natural standard every three sample injections. Sequential degradation reactions during extraction (from TBT to DBT, from DBT to MBT, and from MBT to inorganic tin) were assumed, and mathematical equations were developed that allowed the determination of the correct species concentration and the decomposition factor for each of the transformation reactions. For ultrasonic extraction and mechanical shaking, negligible degradation reactions were observed. However, for microwave assisted extractions, degradation factors up to 7% (TBT to DBT) and 16% (DBT to MBT) were obtained for both reference materials when high-MW energy was applied in the extraction step. For the three extraction techniques tested, the DBT and TBT concentration values obtained for PACS-2 closely matched the certified values. However, for MBT the concentrations found by microwave and ultrasonic extraction were much higher than the certified value. This was not the case for mechanical shaking. The results obtained for BCR-646 using microwave assisted extraction were in good agreement with the certified values for all tin species.

Current measurement procedures based on the coupling of a powerful separation technique and an atomic detection technique, can provide highly selective, sensitive, rapid, and precise measurements for organometallic speciation analysis. However, the initial sample preparation steps are still the "Achilles heel" of the analytical speciation procedures[1,2] that prevent consistently reliable speciation results from being achieved routinely. This statement refers especially to the speciation of biological and environmental solid samples, for which the major difficulty is not the quantification itself, but rather, the quantitative extraction of those compounds from the complex solid sample matrix without degradation or species transformation.[3] This fact is critical for butyltin compounds in sediments that require special extraction procedures, depending on the type of sediment being tested.[4]

Organotin compounds, and tributyltin[5] (TBT) in particular, are highly toxic substances introduced into the environment through their use as antifouling paints on ships and also as wood preservatives, fungicides, biocides, and polymer additives. Although the use of TBT as a biocide has been regulated in many countries,[4] its concentration levels in waters and coastal sediments remain high enough to pose a toxicological risk to marine organisms.[6] Special attention must be given to sediment samples, because they can accumulate butyltin compounds after their release into the environment, creating a toxicity risk long after the anthropogenic sources have been eliminated in a given area.

Different extraction techniques have been developed for the determination of organotin compounds in environmental samples.[7] Traditional techniques involved acid leaching by mechanical

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(1) Quevauviller, P.; Morabito, R. *Trends Anal. Chem.* **2000**, 19, 86−96.
(2) Adams, F. C.; Slaets, S. *Trends Anal. Chem.* **2000**, 19, 80−85.
(3) Quevauviller, P.; Donard, O. F. X.; Maier, E. A.; Griepink, B. *Mikrochim. Acta* **1992**, 109, 169−190.
(4) Quevauviller, P.; Astruc, M.; Morabito, R.; Ariese, F.; Ebdon, L. *Trends Anal. Chem.* **2000**, 19, 180−188.
(5) Maguire, R. J. *Applied Organomet. Chem.* **1987**, 1, 475−498.
(6) Kortlandt, E.; Stronkhorst, J. *TBT in Marine Antifouling Paints*, Direct Dutch BV; National Institute for Coastal and Marine Management: Amsterdam, 1998.
(7) Pellegrino, C.; Massanisso, P.; Morabito, R. *Trends Anal. Chem.* **2000**, 19, 97−106.

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shaking in a polar medium and the use of chelating agents, such as tropolone, to improve the extraction efficiency of MBT.[8,9] Later, ultrasound,[10] microwave-assisted extraction,[11] supercritical fluid extraction,[12] and solid-phase microextraction[13] were also evaluated. Those alternative extraction techniques provided certain advantages as a result of the lower analysis time required and an increased extraction efficiency, particularly for MBT.

Quantitative recoveries, however, of the different organotin compounds from real life solid samples cannot be warranted. Certified reference materials (CRMs) are a very useful tool (much more reliable than spiked or artificial samples). Unfortunately, at present, they are scarce, and some of their certifications have involved a lot of problems as a result of the widespread of the interlaboratory results.[4] Furthermore, although a specific liquid−solid extraction method has proved to be adequate for the determination of organotin compounds in a given certified material, such quantitative extraction may not necessarily be so applied to another material, because the adsorption/binding forces of the species of interest to the solid are strongly dependent on the matrix.

On the other hand, rearrangement reactions between the species and degradation reactions cannot be controlled when more aggressive conditions are used in the extraction step in the search for quantitative recoveries. In such cases, the speciation information might be altered.[11] It is evident that the borderline between quantitative extraction and alteration of the species is probably small and will depend on the matrix itself and on the lability of the sought species.[2] All of these factors make organotin speciation in particular and speciation analysis in general a very difficult task.

An alternative approach to the use of CRMs for speciation could be to resort to highly qualified primary methods, such as isotope dilution (ID)-inductively coupled plasma mass spectrometry.[14] The high accuracy and precision provided by ID-ICPMS in the species-specific spiking mode[15−18] can be used to correct for the majority of systematic errors that occur in speciation analysis (low derivatization yields, nonquantitative separation procedures, signal drift, quantification measurements errors, etc); however, the species-specific spike isotope-dilution mode operated in the typical way (a mixture of species with a single enriched isotope) cannot compensate for nonquantitative extraction from the solid or for species degradation reactions in that initial liquid−solid extraction step.

Evaluating and correcting for these species transformations in this critical first step could be attained if a spike solution containing organotin species isotopically labeled with a different tin isotope were used, in a manner similar to previous studies for the speciation of Cr and Hg.[19−21] The additional degrees of information provided by this spiking mode would allow not only obtaining the correct species concentrations but also the degradation factors from one species to another when decomposition has taken place. Moreover, the possibility of measuring and compensating for degradation reactions would open the way for using harsher liquid−solid extraction conditions while ascertaining whether quantitative extraction of the different species has been obtained.

In this paper, we evaluate and compare three different liquid−solid extraction techniques using a mixture of ^{118}Sn- and ^{119}Sn-enriched species previously synthesized (double spike).[18,22] Mathematical equations are developed that allow computation of both the correct species concentrations by isotope dilution analysis and the possible degradation factors from one tin species to another.

EXPERIMENTAL SECTION

Instrumentation. A Hewlett-Packard (Palo Alto, CA) gas chromatograph, model 6890, fitted with a split/splitless injector and a HP-5 capillary column (cross-linked 5% phenyl methyl siloxane, 30 m × 0.32 mm × 0.25 μm coating), was used for the separation of the organotin species. The gas chromatograph was coupled to a HP-4500 inductively coupled plasma mass spectrometer (Yokogawa Analytical Systems, Tokyo, Japan) using the transfer line described in detail previously.[23]

A Waters high-pressure pump, model 510 (Millipore, MA), a Rheodyne 7125 injection valve (Berkeley, CA) fitted with a 500-μL loop, and a Zorbax 300-SCX cation exchange column (Hewlett-Packard, 25 cm long, 4.6-mm i.d., 5-μm particle size) were used for the preparative separation of the organotin species in the ^{119}Sn-enriched mixed spike. For the optimization of the separation conditions, the outlet of the HPLC column was directly connected to the nebulizer of the ICPMS with a piece of PTFE tubing.

For the extraction of the organotin compounds from the sediments with methanol−acetic acid, a mechanical shaker (Heidolph reax 2, Kelheim, Germany); a microwave oven, model 1200 (Milestone, Socisole, Italy) with an AC-100 open/close module equipped with middle pressure PTFE vessels; and a high-intensity ultrasonic processor (Sonics and Materials Inc., Danbury, CT) were used.

Caution: *Safety guidelines regarding work with microwave fields should be observed.*

Reagents and Materials. Tributyltin chloride (96%), dibutyltin dichloride (97%), and monobutyltin trichloride (95%) were obtained from Aldrich (Steinheim, Germany). Stock solutions were prepared by dissolving the corresponding salt in methanol (Merck, Darmstadt, Germany). All organometallic standard solutions were kept in the dark at 4 °C, and diluted working solutions were

(8) Zhang, S.; Chau, Y. K.; Li, W. C.; Chau, A. S. Y. *Appl. Organomet. Chem.* **1991**, 5, 431−434.
(9) Ceulemans, M.; Adams, F. C. *Anal. Chim. Acta* **1995**, 317, 161−170.
(10) Cai, Y.; Rapsomanikis, S.; Andreae, M. O. *Mikrochim. Acta* **1992**, 109, 67−71.
(11) Donard, O. F. X.; Lalère, B.; Martin, F.; Lobinski, R. *Anal. Chem.* **1995**, 67, 4250−4254.
(12) Chau, Y. K.; Yang, F.; Brown, M. *Anal. Chim. Acta* **1995**, 304, 85−89.
(13) Moens, L.; De Smaele, T.; Dams, R.; Van den Broeck, P.; Sandra, P. *Anal. Chem.* **1997**, *69*, 1604−1611.
(14) Sanz-Medel, A. *Spectrochim. Acta* **1998**, 53B, 197−211.
(15) Heumann, K. J.; Gallus, S. M.; Rädlinger, G.; Vogl, J. *Spectrochim. Acta* **1998**, 53B, 273−287.
(16) Creed, J. T.; Brockhoff, C. A. *Anal. Chem.* **1999**, 71, 722−726.
(17) Snell, J. P.; Stewart, I. I.; Sturgeon, R. E.; Frech, W. *J. Anal. At. Spectrom.* **2000**, 15, 1540−1545.
(18) Ruiz Encinar, J.; Monterde Villar, M. I.; Gotor, V.; García Alonso, J. I.; Sanz-Medel, A. *Anal. Chem.* **2001**, 73, 3174−3180.
(19) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. *Spectrochim. Acta* **1998**, 53B, 299−309.
(20) Hintelmann, H.; Falter, R.; Ilgen, G.; Evans, R. D. *Fresenius J. Anal. Chem.* **1997**, 358, 363−370.
(21) Huo, D.; Kingston, H. M.; Larget, B. Elemental speciation: New Approaches for Trace Element Analysis, 1rst ed.; Elsevier: Amsterdam, **2000**; Chapter 10.
(22) Ruiz Encinar, J.; García Alonso, J. I.; Sanz-Medel, A. *J. Anal. At. Spectrom.* **2000**, 15, 1233−1239.
(23) Montes Bayón, M.; Gutierrez Camblor, M.; García Alonso, J. I.; Sanz-Medel, A. *J. Anal. At. Spectrom.* **1999**, 14, 1317−1322.

Table 1. Isotopic Composition (at %) for All Isotopically Enriched Solutions Used

isotopes	natural tin	^{118}Sn-enriched DBT[22]	^{119}Sn-enriched butyltin mixture[18] (ave of all species)	^{118}Sn-^{119}Sn enriched spike (this work)		
				MBT	DBT	TBT
117	7.68	0.13	0.114	0.150	0.129	0.118
118	24.22	98.44	14.33	17.13	86.69	13.86
119	8.59	0.70	82.40	79.73	12.23	83.08
120	32.58	0.62	3.13	2.988	0.949	2.936

prepared daily before the analysis in methanol. Ethylation of these species was perfomed using sodium tetraethyl borate (Strem Chemicals, Bisheim, France).

Caution: *Butyltin compounds are toxic materials. Sodium tetraethyl borate decomposes rapid in the presence of air and light, and it is also extremely flammable.*

^{119}Sn-enriched and ^{118}Sn-enriched tin metal were purchased from Cambridge Isotope Laboratories (Andover, MA). A ^{118}Sn-enriched DBT, prepared as described previously,[22] was used without further treatment and mixed with ^{119}Sn-enriched MBT and TBT obtained after preparative liquid chromatography of a mixture of all three organotin species. The synthesis of the ^{119}Sn-enriched mixture of MBT, DBT, and TBT has been described in a previous publication.[18] The isotopic composition of both ^{118}Sn- and ^{119}Sn-enriched spikes is given in Table 1. Sediment reference materials tested were PACS-2 and CRM 646, purchased from NRCC (Ottawa, Ontario, Canada), and BCR (Retieseweg, Geel, Belgium), respectively.

The HPLC eluent for the preparative chromatography consisted of 0.1 M diammonium hydrogencitrate (Probus, Barcelona, Spain), 5% (v/v) acetic acid (Merck), and 20% (v/v) methanol in Milli-Q water (Millipore, Molsheim, France), with final degassing using He for 10 min.

Procedures. *Preparative Liquid Chromatography.* Optimum operating conditions for the separation of the three tin species by HPLC[24] were obtained using 0.1M diammonium hydrogencitrate, 5% acetic acid, and 20% methanol in Milli-Q water as eluent at 0.8 mL min^{-1} flow rate. The ^{119}Sn-enriched mixed spike[18] was diluted 10 times with the mobile phase, and 500 μL was injected into the column. The fractions containing MBT and TBT were collected and then mixed appropriately with the ^{118}Sn-enriched DBT obtained previously.[22]

Ethylation, Separation, and Determination by GC/ICPMS of the Tin Compounds. Ethylation of the tin species using sodium tetraethyl borate and typical operating conditions used for the GC/ICPMS detection have been described elsewhere.[18,22] Under optimum conditions, a very small peak broadening was observed, despite the fact that the PFA tube operated as the transfer line was not heated.[23] Daily optimization of the ICPMS conditions was performed after connection of the GC to the ICPMS by using $^{40}Ar_2^+$, because it was demonstrated previously[22,25] that the optimum ICPMS detection conditions for a dry plasma differed greatly from those obtained during nebulization.

Measurement of Isotope Ratios Using GC/ICPMS. Integration of the chromatographic peaks was carried out using the commercial GC/MS Agilent software supplied with the ICPMS instrument. Isotope ratios were always computed as peak area ratios. The integration time per tin isotope selected (118, 119, and 120) was 66 ms. In this way, total integration time (200 ms) was short enough to be able to follow accurately the chromatographic peak profile. Longer and shorter integration times led to considerable spectral skew and an increase in the instrumental noise, respectively.[25]

Precision Attainable Using Speciated Isotope Dilution by GC/ICPMS. Previous work carried out in our laboratory[18,22] demonstrated that the uncertainty of the whole speciated isotope dilution procedure was within 2%, a value very similar to the overall isotope ratio precision attainable using GC/ICPMS.[18,22,25] Given this excellent methodological precision, the analyses carried out through this work were simplified by performing only one isotope dilution experiment per sample and only one injection per experiment.

Extraction Conditions. For all the liquid−solid extraction techniques tested, a sample weight of 0.25 g was selected to ensure sample-to-sample homogeneity. Samples were spiked with an appropriate amount of the "double-enriched isotope spike", and immediately, 1 mL of methanol and 3 mL of acetic acid were added in (a) stoppered glass extraction tubes (mechanical shaking), (b) 10-mL vials (ultrasounds), or (c) high-pressure PTFE vessels (microwaves). Then the slurries were exposed to microwaves (90 and 150 W) and ultrasounds (50 W) for different times (up to 15 min) or shaken mechanically for times ranging from 0.5 to 12 h. After centrifugation, 200 μL of the final extracts was ethylated as described above.

RESULTS AND DISCUSSION

Development of the Isotope Dilution Equations for the Computation of Concentrations and Decomposition Factors. The high accuracy of isotope dilution analysis for trace metal speciation is based on the fact that, once isotope equilibration has taken place, decomposition of the species will not affect the final results; however, if the decomposition product of one species is another compound that also has to be determined, errors in the second species concentration will occur. For butyltin compounds, decomposition reactions follow the progressive removal of the butyl chains from the tin atom.[5,26] According to this model, TBT will degrade to DBT, and this, to MBT, and finally, to inorganic tin. Following this argument, the concentration of TBT found by isotope dilution analysis will not be affected by its degradation, but the concentrations of DBT and MBT will be influenced by any degradation reaction occurring from TBT to DBT and from

(24) García Alonso, J. I.; Sanz-Medel, A.; Ebdon, L. *Anal. Chim. Acta* **1993**, 283, 261−271.

(25) Ruiz Encinar, J.; Leal Granadillo, I.; García Alonso, J. I.; Sanz-Medel, A. *J. Anal. At. Spectrom.* **2001**, 16, 475−480.

(26) Craig, P. J. *Organometallic Compounds in the Environment*, 1st ed.; Longman: Essex, 1986; Chapter 3.

DBT to MBT. To correct for species degradation, enriched spikes containing different enriched isotopes can be used.[19,20] In our case, we used ^{119}Sn-enriched TBT and MBT and ^{118}Sn-enriched DBT as spikes and assumed that TBT can be transformed into DBT by a decomposition factor F_1 (the fraction of the amount of TBT present transformed into DBT after liquid−solid extraction), DBT can be transformed into MBT by a decomposition factor F_2 (the fraction of the total amount of DBT present transformed into MBT after extraction), and MBT can be transformed into inorganic tin by a decomposition factor F_3. Then the original concentrations and decomposition factors can be calculated as follows:

If N_s moles of each species in the sample are mixed with N_{sp} moles of the same species in the spike solution, the total number of moles of each species present in the mixture at the end of the extraction step, N_m, can be calculated taking into account the original amount of the species present, their degradation, and the amount of species formed from the degradation of other species. For a polyisotopic element, this mass balance can be performed for each considered isotope. In our case, isotopes 120, 119, and 118 were measured; 120 was the reference isotope in the sample, and 119 and 118 were the reference isotopes in the spike. For TBT, DBT, and MBT, this can be expressed as follows,

$$N^{TBT}_{120,m} = N^{TBT}_{120,s} + N^{TBT}_{120,sp} - F_1(N^{TBT}_{120,s} + N^{TBT}_{120,sp})$$

where TBT will degrade to DBT to form $F_1(N^{TBT}_s + N^{TBT}_{sp})$ moles of DBT, assuming that no isotopic effects are taking place. For DBT, we have

$$N^{DBT}_{120,m} = N^{DBT}_{120,s} + N^{DBT}_{120,sp} + F_1(N^{TBT}_{120,s} + N^{TBT}_{120,sp}) - F_2(N^{DBT}_{120,s} + N^{DBT}_{120,sp}) - F_1F_2(N^{TBT}_{120,s} + N^{TBT}_{120,sp})$$

where all DBT originally present and that formed from TBT will degrade to MBT with a factor F_2. Finally, for MBT, we will have

$$N^{MBT}_{120,m} = N^{MBT}_{120,s} + N^{MBT}_{120,sp} + F_2(N^{DBT}_{120,s} + N^{DBT}_{120,sp}) + F_1F_2(N^{TBT}_{120,s} + N^{TBT}_{120,sp}) - F_3(N^{MBT}_{120,s} + N^{MBT}_{120,sp}) - F_3F_2(N^{DBT}_{120,s} + N^{DBT}_{120,sp}) - F_3F_2F_1(N^{TBT}_{120,s} + N^{TBT}_{120,sp})$$

Similar equations for the isotopes 118 and 119 can be computed as the three equations for the isotope 120. These nine mass balance equations can be transformed into six isotope ratio equations by dividing the equations obtained for isotope 120 by the equations obtained for isotopes 118 and 119. Taking into account that the number of moles for a certain isotope can be related to the total number of moles of the element by the isotope abundances, AT, we obtain the following equations:

$$R^{120/119}_{TBT,m} = \frac{N^{TBT}_s AT^{120}_s + N^{TBT}_{sp} AT^{120}_{TBT,sp}}{N^{TBT}_s AT^{119}_s + N^{TBT}_{sp} AT^{119}_{TBT,sp}} \quad (1)$$

$$R^{120/118}_{TBT,m} = \frac{N^{TBT}_s AT^{120}_s + N^{TBT}_{sp} AT^{120}_{TBT,sp}}{N^{TBT}_s AT^{118}_s + N^{TBT}_{sp} AT^{118}_{TBT,sp}} \quad (2)$$

$$R^{120/119}_{DBT,m} = \frac{N^{DBT}_s AT^{120}_s + N^{DBT}_{sp} AT^{120}_{DBT,sp} + F_1(N^{TBT}_s AT^{120}_s + N^{TBT}_{sp} AT^{120}_{TBT,sp})}{N^{DBT}_s AT^{119}_s + N^{DBT}_{sp} AT^{119}_{DBT,sp} + F_1(N^{TBT}_s AT^{119}_s + N^{TBT}_{sp} AT^{119}_{TBT,sp})} \quad (3)$$

$$R^{120/118}_{DBT,m} = \frac{N^{DBT}_s AT^{120}_s + N^{DBT}_{sp} AT^{120}_{DBT,sp} + F_1(N^{TBT}_s AT^{120}_s + N^{TBT}_{sp} AT^{120}_{TBT,sp})}{N^{DBT}_s AT^{118}_s + N^{DBT}_{sp} AT^{118}_{DBT,sp} + F_1(N^{TBT}_s AT^{118}_s + N^{TBT}_{sp} AT^{118}_{TBT,sp})} \quad (4)$$

$$R^{120/119}_{MBT,m} = [N^{MBT}_s AT^{120}_s + N^{MBT}_{sp} AT^{120}_{MBT,sp} + F_2(N^{DBT}_s AT^{120}_s + N^{DBT}_{sp} AT^{120}_{DBT,sp}) + F_1F_2(N^{TBT}_s AT^{120}_s + N^{TBT}_{sp} AT^{120}_{TBT,sp})]/[N^{MBT}_s AT^{119}_s + N^{MBT}_{sp} AT^{119}_{MBT,sp} + F_2(N^{DBT}_s AT^{119}_s + N^{DBT}_{sp} AT^{119}_{DBT,sp}) + F_1F_2(N^{TBT}_s AT^{119}_s + N^{TBT}_{sp} AT^{119}_{TBT,sp})] \quad (5)$$

$$R^{120/118}_{MBT,m} = [N^{MBT}_s AT^{120}_s + N^{MBT}_{sp} AT^{120}_{MBT,sp} + F_2(N^{DBT}_s AT^{120}_s + N^{DBT}_{sp} AT^{120}_{DBT,sp}) + F_1F_2(N^{TBT}_s AT^{120}_s + N^{TBT}_{sp} AT^{120}_{TBT,sp})]/[N^{MBT}_s AT^{118}_s + N^{MBT}_{sp} AT^{118}_{MBT,sp} + F_2(N^{DBT}_s AT^{118}_s + N^{DBT}_{sp} AT^{118}_{DBT,sp}) + F_1F_2(N^{TBT}_s AT^{118}_s + N^{TBT}_{sp} AT^{118}_{TBT,sp})] \quad (6)$$

From those six isotope ratio equations, and assuming that the isotope ratios in the mixture are experimentally measured for all species, the equations originating from TBT (eqs 1 and 2) contain only one unknown parameter (N^{TBT}_s), which will allow the determination of the concentration of TBT in the sample regardless of its degradation into DBT. Once the concentration of TBT is known, eqs 3 and 4 contain only two unknowns (N^{DBT}_s and F_1). Those two equations will allow the determination of the concentration of DBT in the sample and the degradation factor from TBT to DBT. Finally, eqs 5 and 6 also contain two unknowns (N^{MBT}_s and F_2), because the other parameters have been determined previously. Those isotope ratio equations were derived to extract the corresponding unknowns, and the derived equations are given in Table 2. Unfortunately, the degradation factor from MBT to inorganic tin, F_3, cannot be computed using the experimental set up given here.

As can be observed in Table 2, eq 7 is the usual isotope dilution equation; the TBT isotope ratios will remain constant regardless of its decomposition into DBT. For DBT and MBT (eqs 8 and 10, respectively), the measurement of both isotope ratios (120/118 and 120/119) is needed to include the decomposition factors. Eqs 9, 10, and 11 were shortened by grouping partial equations as letters A, B, C, and D, whose expressions are also given in the table.

The symbols used in the equations represent the following: N^{TBT}_s, N^{DBT}_s, and N^{MBT}_s are the number of moles of each species present in the sample; N^{TBT}_{sp}, N^{DBT}_{sp}, and N^{MBT}_{sp} are the number of moles of each species added in the spike; AT^{118}_s, AT^{119}_s,and AT^{120}_s are the naturally occurring isotope abundances (valid for MBT, DBT, and TBT in the sample); $AT^{119}_{TBT,sp}$, $AT^{120}_{TBT,sp}$, $AT^{118}_{DBT,sp}$, $AT^{119}_{DBT,sp}$, $AT^{120}_{DBT,sp}$, $AT^{118}_{MBT,sp}$, $AT^{119}_{MBT,sp}$, and $AT^{120}_{MBT,sp}$ are the tin isotope abundances for TBT, DBT, and MBT in the multiple spike added to the sample; and finally, $R^{120/119}_{TBT,m}$, $R^{120/119}_{DBT,m}$, $R^{120/118}_{DBT,m}$, $R^{120/119}_{MBT,m}$, and $R^{120/118}_{MBT,m}$ are the corresponding tin isotope ratios measured by GC/ICPMS for TBT, DBT, and MBT in the mixture

Table 2. Equations Developed to Compute Corrected Species Concentrations and Degradation Factors between Them

$$[7]\quad N_s^{TBT} = N_{sp}^{TBT}\left[\frac{AT_{TBT,sp}^{119}R_{TBT,m}^{120/119} - AT_{TBT,sp}^{120}}{AT_s^{120} - AT_s^{119}R_{TBT,m}^{120/119}}\right]$$

$$[8]\quad N_s^{DBT} = \frac{N_{sp}^{DBT}(AT_{DBT,sp}^{120} - AT_{DBT,sp}^{118}R_{DBT,m}^{120/118}) + F_1\left[N_s^{TBT}\left(AT_s^{120} - R_{DBT,m}^{120/118}AT_s^{118}\right) + N_{sp}^{TBT}\left(AT_{TBT,sp}^{120} - R_{DBT,m}^{120/118}AT_{TBT,sp}^{118}\right)\right]}{R_{DBT,m}^{120/118}AT_s^{118} - AT_s^{120}}$$

$$[9]\quad F_1 = N_{sp}^{DBT}\left[\frac{R_{DBT,m}^{120/118}AT_s^{118}\left(AT_{DBT,sp}^{120} - AT_{DBT,sp}^{119}R_{DBT,m}^{120/119}\right) - R_{DBT,m}^{120/119}AT_s^{119}\left(AT_{DBT,sp}^{120} - AT_{DBT,sp}^{118}R_{DBT,m}^{120/118}\right) + AT_s^{120}\left(AT_{DBT,sp}^{119}R_{DBT,m}^{120/119} - AT_{DBT,sp}^{118}R_{DBT,m}^{120/118}\right)}{AT_s^{119}R_{DBT,m}^{120/119}B - AT_s^{118}R_{DBT,m}^{120/118}A + AT_s^{120}(A - B)}\right]$$

$$A = N_s^{TBT}AT_s^{120} + N_{sp}^{TBT}AT_{TBT,sp}^{120} - R_{DBT,m}^{120/119}\left(N_s^{TBT}AT_s^{119} + N_{sp}^{TBT}AT_{TBT,sp}^{119}\right)$$

$$B = N_s^{TBT}AT_s^{120} + N_{sp}^{TBT}AT_{TBT,sp}^{120} - R_{DBT,m}^{120/118}\left(N_s^{TBT}AT_s^{118} + N_{sp}^{TBT}AT_{TBT,sp}^{118}\right)$$

$$[10]\quad N_s^{MBT} = \frac{N_{sp}^{MBT}\left(AT_{MBT,sp}^{120} - AT_{MBT,sp}^{119}R_{MBT,m}^{120/119}\right) + F_2\ C}{R_{MBT,m}^{120/119}AT_s^{119} - AT_s^{120}}$$

$$[11]\quad F_2 = N_{sp}^{MBT}\left[\frac{R_{MBT,m}^{120/118}AT_s^{118}\left(AT_{MBT,sp}^{120} - AT_{MBT,sp}^{119}R_{MBT,m}^{120/119}\right) - R_{MBT,m}^{120/119}AT_s^{119}\left(AT_{MBT,sp}^{120} - AT_{MBT,sp}^{118}R_{MBT,m}^{120/118}\right) + AT_s^{120}\left(AT_{MBT,sp}^{119}R_{MBT,m}^{120/119} - AT_{MBT,sp}^{118}R_{MBT,m}^{120/118}\right)}{AT_s^{119}R_{MBT,m}^{120/119}D - AT_s^{118}R_{MBT,m}^{120/118}C + AT_s^{120}(C - D)}\right]$$

$$C = N_s^{DBT}\left(AT_s^{120} - AT_s^{119}R_{MBT,m}^{120/119}\right) + N_{sp}^{DBT}\left(AT_{DBT,sp}^{120} - AT_{DBT,sp}^{119}R_{MBT,m}^{120/119}\right) + F_1\left[N_s^{TBT}\left(AT_s^{120} - AT_s^{119}R_{MBT,m}^{120/119}\right) + N_{sp}^{TBT}\left(AT_{TBT,sp}^{120} - AT_{TBT,sp}^{119}R_{MBT,m}^{120/119}\right)\right]$$

$$D = N_s^{DBT}\left(AT_s^{120} - AT_s^{118}R_{MBT,m}^{120/118}\right) + N_{sp}^{DBT}\left(AT_{DBT,sp}^{120} - AT_{DBT,sp}^{118}R_{MBT,m}^{120/118}\right) + F_1\left[N_s^{TBT}\left(AT_s^{120} - AT_s^{118}R_{MBT,m}^{120/118}\right) + N_{sp}^{TBT}\left(AT_{TBT,sp}^{120} - AT_{TBT,sp}^{118}R_{MBT,m}^{120/118}\right)\right]$$

after mass bias correction. As can be observed in Table 2, eqs 7, 8, and 10 will provide the correct species concentration, regardless of the transformation of one species into another. In addition the values of F_1 and F_2 will provide additional information that will allow the comparison of different extraction techniques on the basis of their ability to preserve the original speciation information in the sample. It is worth noting that F_3 (degradation of MBT to inorganic tin) cannot be computed using this approach; however, this factor would only be necessary if inorganic tin was being determined by IDA. The concentration values for MBT do not require correction for F_3.

Accuracy of Tin Isotope Ratios by GC/ICPMS. Because no dead time correction was necessary using the HP-4500,[27] the only factor influencing the accuracy of tin isotope ratios was found to be mass bias. A mixture of ethylated natural MBT, DBT, and TBT standards was injected every three samples in order to correct for this effect. As was observed previously,[18,22,25] the mass bias factor turned out to be ~1% per mass unit (ranging from 0.5 to 1.6%), increasing slightly during the analysis time. This mass bias drift did not affect the accuracy of the obtained isotope ratio, because it was compensated for by using the mean of the mass bias factors computed before and after each sample triplicate.

Isolation of ^{119}Sn-Enriched MBT and TBT by Preparative HPLC/ICPMS. Previous work of our group[18] showed that a solution containing a mixture of ^{119}Sn-enriched MBT, DBT, and TBT could be applied to the simultaneous speciated isotope dilution analysis of the butyltin compounds in certified sediments with satisfactory results; however, no degradation factors could be computed from those experiments. To combine this ^{119}Sn-enriched spike with the previously synthesized ^{118}Sn-enriched DBT,[22] the isolation of the ^{119}Sn-enriched MBT and TBT species was required. Cation-exchange HPLC was selected to attempt this separation (to be later used on a preparative scale). Optimization of the HPLC conditions was performed by coupling the HPLC system to the ICPMS and using a natural tin standard mixture of MBT, DBT, and TBT for the optimization. The final separation conditions selected are described in the procedures, and a typical chromatogram showing the separation of all three butyltin species is given in Figure 1. For the separation of MBT, DBT, and TBT

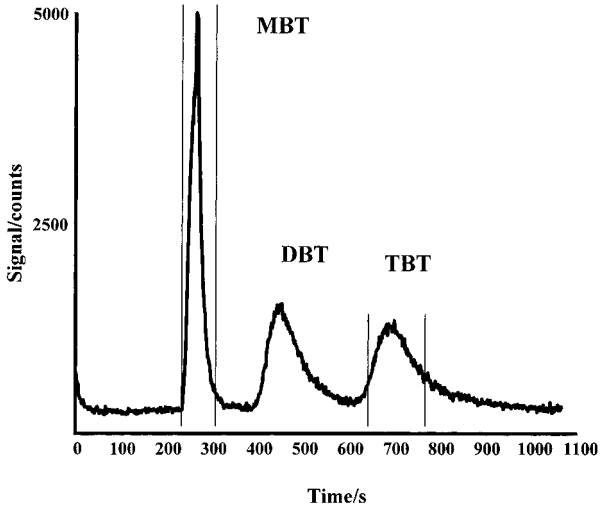


Figure 1. Separation of the butyltin species obtained by cation-exchange liquid chromatography. Lines indicate the MBT and TBT fractions collected.

(27) Ruiz Encinar, J.; García Alonso, J. I.; Sanz-Medel, A.; Main, S.; Turner, P. J. *J. Anal. At. Spectrom.* **2001**, 16, 315−321.

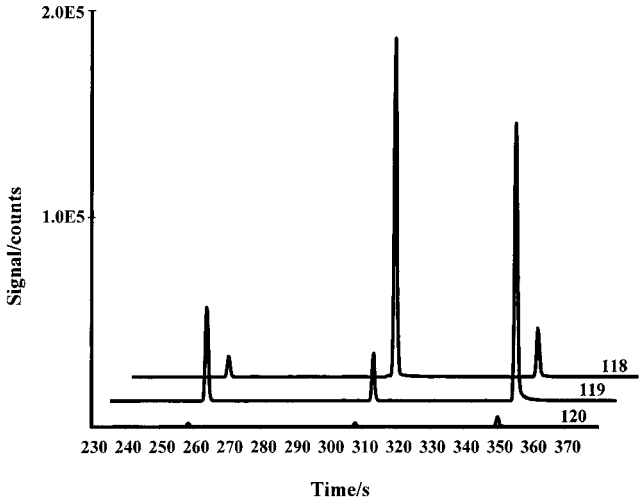


Figure 2. GC/ICPMS chromatogram obtained for the ethylated "double-spike". The different isotopes measured are shifted for clarity.

in the ^{119}Sn-enriched spike solution, it was necessary to dilute the sample 1 to 14 with the mobile phase, because the concentration of methanol and acetic acid in the original spike solution (25 and 75%, respectively) was too high. Finally, the ^{119}Sn-enriched MBT and TBT fractions were collected from 210 to 250 s and from 690 to 790 s. Successive sample injections of 500 μL were performed to collect the required amount of the species of interest.

Preparation and Characterization of the Spike. *Stability with Time.* Once the isolation of the ^{119}Sn-enriched MBT and TBT from the mixed spike solutions was achieved, those species were adequately mixed with the ^{118}Sn-enriched DBT solution to obtain the so-called "double spike". Five GC/ICPMS successive injections of this spike were performed after customary ethylation to compute the isotopic composition of the new spike. Figure 2 shows one of the chromatograms obtained at masses 118, 119, and 120 (shifted for clarity), which shows the preferential enrichment at mass 119 for MBT and TBT and the enrichment at mass 118 for DBT. The isotopic composition of the butyltin species in the spike is also presented in Table 1. Mass bias was corrected using a natural mixed standard injected before and after the injection of the enriched species, as described previously. As can be observed, Table 1 shows the considerable difference between the isotopic composition of tin in DBT and that of TBT and MBT. This fact will allow checking and correction for possible degradation reactions from TBT to DBT and from DBT to MBT, because they will involve an appreciable change in the species isotope ratios. The isotopic composition of natural tin is also included in the table for comparison. As can be observed, mass 120 is the most abundant tin isotope in the natural element, and this is a minor isotope in all organotin species in the spike.

Reverse isotope dilution analysis using natural butyltin standards was carried out in order to determine the concentration of the isotopically labeled MBT, DBT, and TBT in the mixed multiple spike. The stated purity of the commercial standards was assumed for the calculations. Independent spiking experiments ($n = 3$) were carried out for each species to check for possible artifacts during derivatization and detection by GC/ICPMS. The results showed that, in all cases, only the isotopic composition for the spiked species was altered, and the other two species exhibited the original isotopic compositions. That means that no degradation or recombination reactions occurred during derivatization, liquid–liquid extraction, and GC/ICPMS analysis. These experiments were repeated three times (covering 80 days) to check for species stability. The results obtained are represented in Figure 3 for the three organotin species. As can be observed, no degradation with time is evident. The measurement-to-measurement variations were within the 95% confidence interval for the three species, so stability of the species could be safely assumed. For the extraction studies, the average concentrations for the three different determinations performed was used (685, 564, and 317 ng/g as tin for TBT, DBT, and MBT respectively).

Evaluation of Liquid–Solid Extraction Approaches in the Certified Sediments PACS-2 and CRM-646 by the ID-GC/ICPMS Technique. Speciated isotope dilution analysis of the three organotin species was first carried out for the PACS-2-certified sediment under the conditions indicated in the proce-

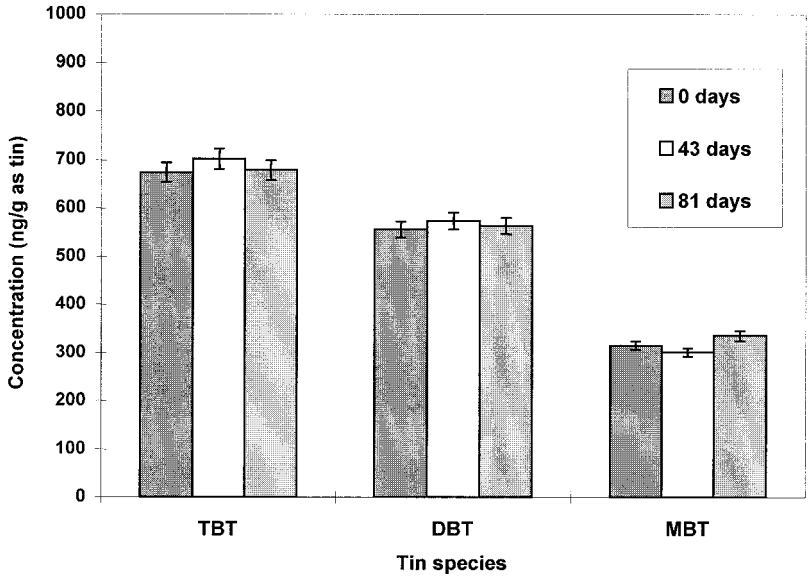
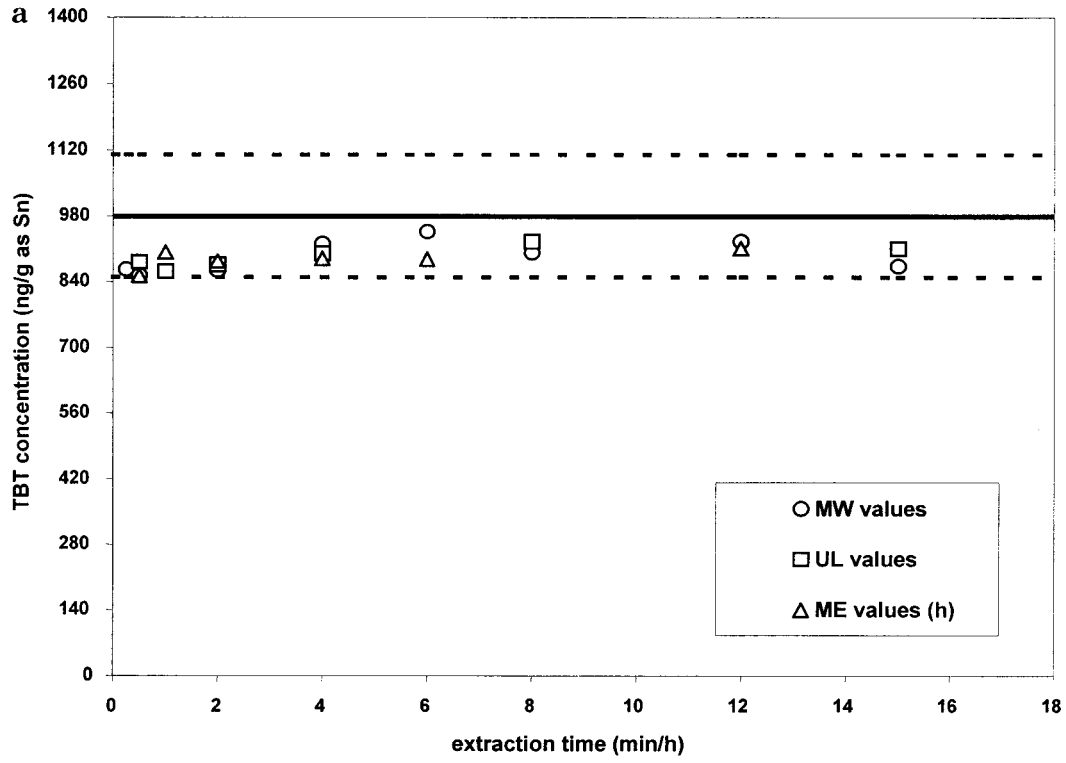
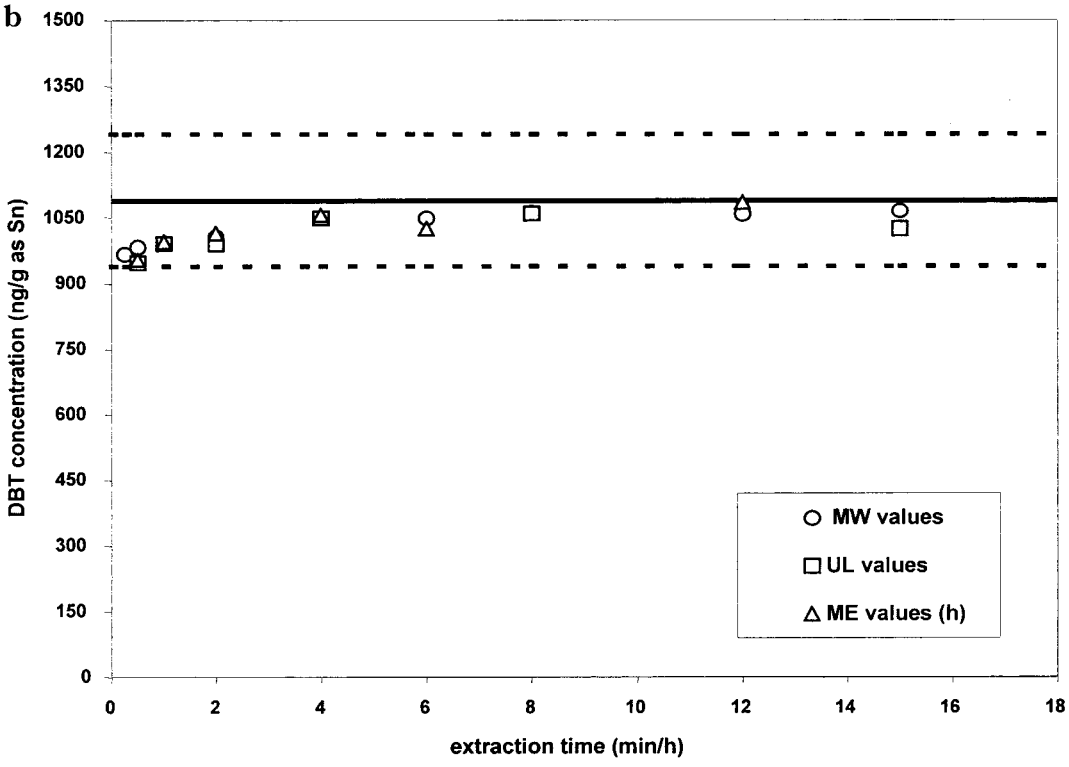


Figure 3. Reverse isotope-dilution analysis of the double spike using natural standards. Three independent spiking experiments were carried out covering 81 days to check for stability.


a
TBT concentration (ng/g as Sn)
1400
1260
1120
980
840
700
560
420
280
140
0
0
2
4
6
8
10
12
14
16
18
extraction time (min/h)
MW values
UL values
ME values (h)


b
DBT concentration (ng/g as Sn)
1500
1350
1200
1050
900
750
600
450
300
150
0
0
2
4
6
8
10
12
14
16
18
extraction time (min/h)
MW values
UL values
ME values (h)

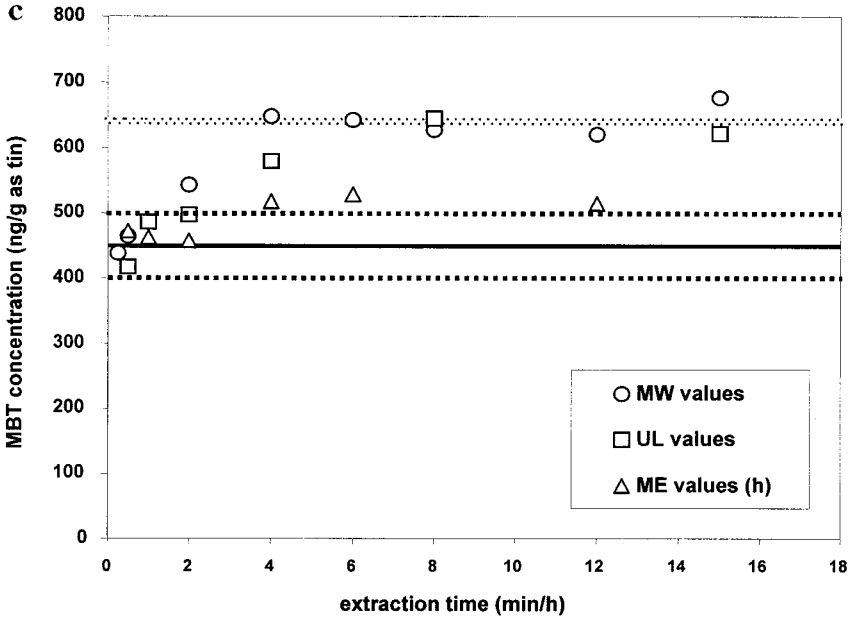


Figure 4. Isotope dilution results for the PACS-2 sediment using MW (circles), UL (squares), and ME (triangles) extraction techniques. Parts a, b, and c correspond to TBT, DBT, and MBT, respectively. Gray line in part c corresponds to the concentration mean of the last points of MW (4, 6, 8, 12, and 15 min) and UL (8 and 15 min).

dures. In all cases, 0.25 g of the dry sediment was spiked with a given amount of the multiple spike, mixed with 4 mL of an acetic acid−methanol mixture (75:25), and subjected to ultrasonic extraction (UL), microwave extraction (MW), or mechanical shaking (ME) for different extraction times. After adequate derivatization and GC/ICPMS measurements, the ratios for 120/118 and 120/119 were evaluated for each species and corrected for mass bias, as indicated, and the tin species concentrations were computed using equations 7, 8, and 10. Results obtained for TBT, DBT and MBT are shown in Figure 4a, b, and c, respectively. For all figures, the horizontal full line corresponds to the certified value, and the two dotted lines, to the 95% confidence interval of the certified value. The extraction time is expressed in minutes for MW and UL and in hours for ME. The power applied using UL was always 50 W, but the power applied using MW was increased from 90 W for the first six points (ranging from 15 s to 6 min) to 150 W for the last three experiments (8, 12, and 15 min). As can be observed, for TBT and DBT in Figure 4a and b respectively, the concentration values obtained were always within the certified range, even when using mild extraction conditions and short extraction times. The results found for these two species are in agreement with those found previously in our laboratory[18] using only a ^{119}Sn-enriched butyltin mixture and 12 h of mechanical shaking. Quantitative extraction for these two species in PACS-2 seemed to be easily achieved in 4 h by ME and in 4 min by both UL and MW.

On the other hand, for MBT in Figure 4c, the concentration values found increased with extraction time up to ca. 640 ng/g when more aggressive extraction conditions were operated (MW and UL, respectively); however, values just above the certified range were obtained by mechanical shaking, in agreement with results found previously with the same extraction system and the ^{119}Sn-enriched spike (510 ng/g).[18] The concentration value found by MW and UL was substantially higher than the certified value (450 ng/g as tin) and in close agreement with the value obtained by Rajendran et al.[28] (620 ng/g) using a tropolone-assisted extraction method that involved three extraction steps (taking 90 min). Thus, it seems that the extraction of MBT from PACS-2 required somewhat harsher conditions than just mechanical shaking. These results indicate also that the MBT species seems to be bound more strongly to the solid matrix than are the other two species.[11]

Microwave-assisted extraction has been previously used for the extraction of butyltin compounds from sediments.[11,29] The results reported in such work for MBT also indicate that higher recoveries are obtained at increasing power and extraction times. Other authors pointed out that to improve the extraction recovery for MBT, the use of chelating agents and strong acidic conditions was required.[9,30] Many of these latter authors suggested that inefficient extraction of MBT could result in low concentration values during certification processes. Unfortunately, the real influence of such a factor was always obscured by the possibility of any degradation reaction (which could not be accounted for).

Figure 5a,b shows the values of F_1 and F_2 found for the three different extraction approaches tested with respect to the extraction time. As can be observed, for ME and UL, the values of F_1 and F_2 are close to 0 under all extraction conditions, indicating that no degradation of the species seems to take place; however, using MW-assisted extraction, the degradation factors increased with extraction time up to ca. 7% for F_1 and 16% for F_2. The fact that similar concentration values above the certified value (Figure 4c) were found for MBT using UL (no degradation) and MW (with degradation) seem to indicate that the certified value for MBT in PACS-2 may be too low.

(28) Rajendran, R. B.; Tao, H.; Nakazato, T.; Miyazaki, A. *Analyst* **2000**, 125, 1757−1763.
(29) Szpunar, J.; Schmitt, V. O.; Lobinski, R.; Monod, J. L. *J. Anal. At. Spectrom.* **1996**, 11, 193−199.
(30) Chau, Y. K.; Yang, F.; Maguire, R. J. *Anal. Chim. Acta* **1996**, 320, 165−169.

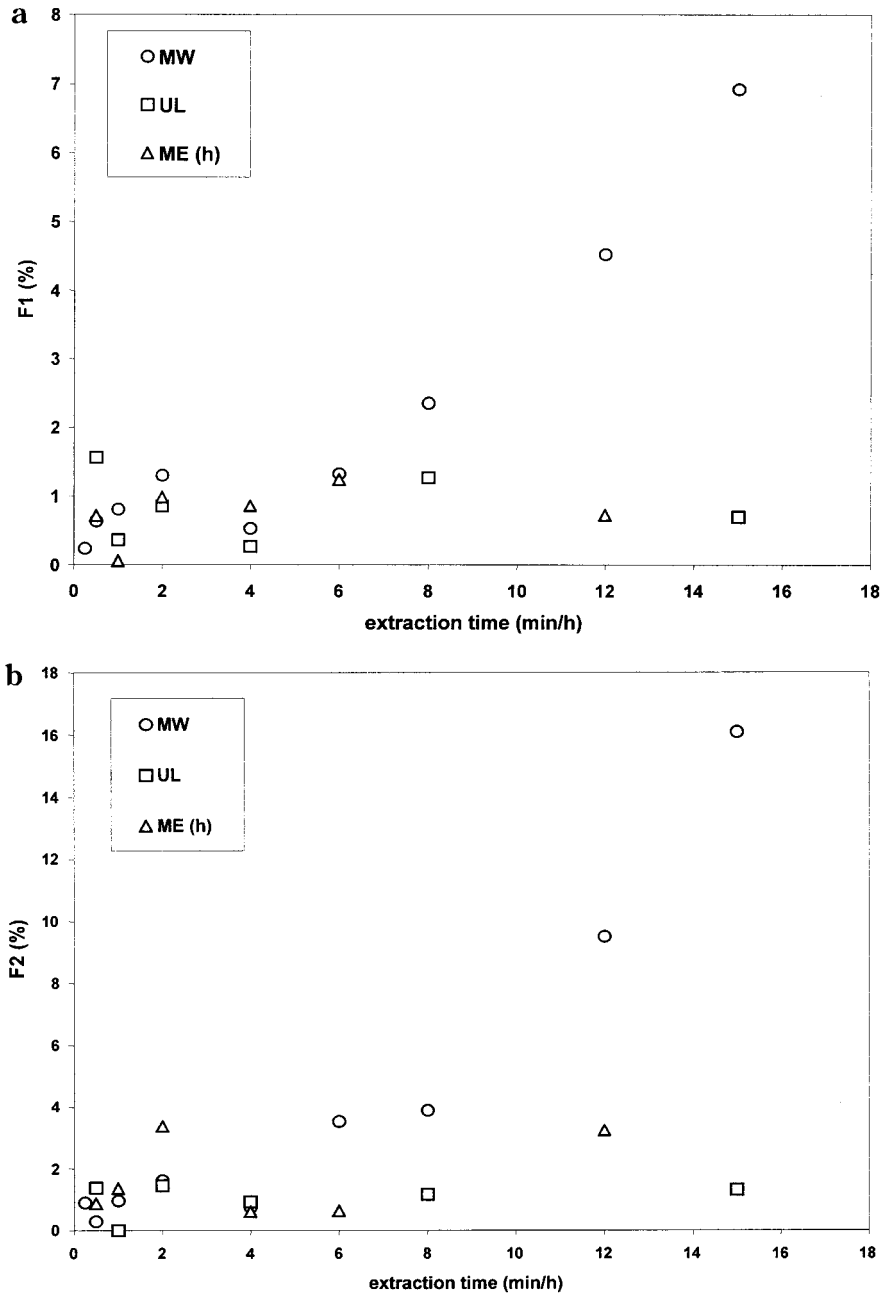
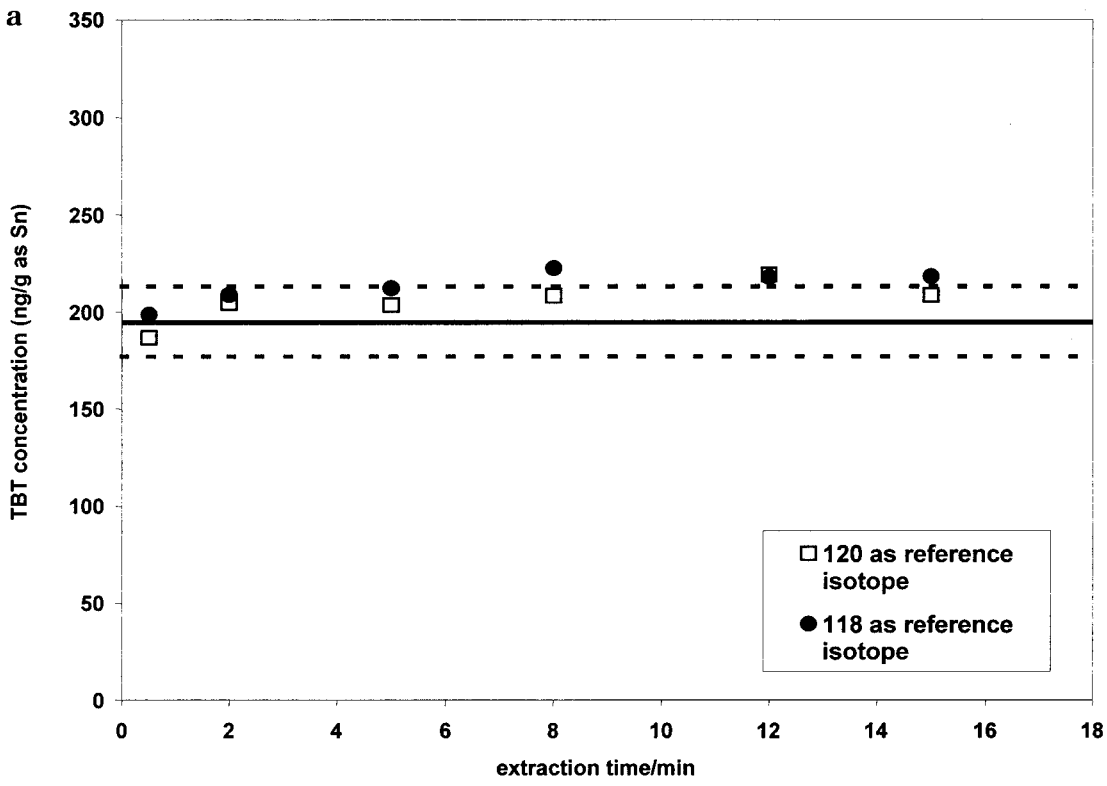
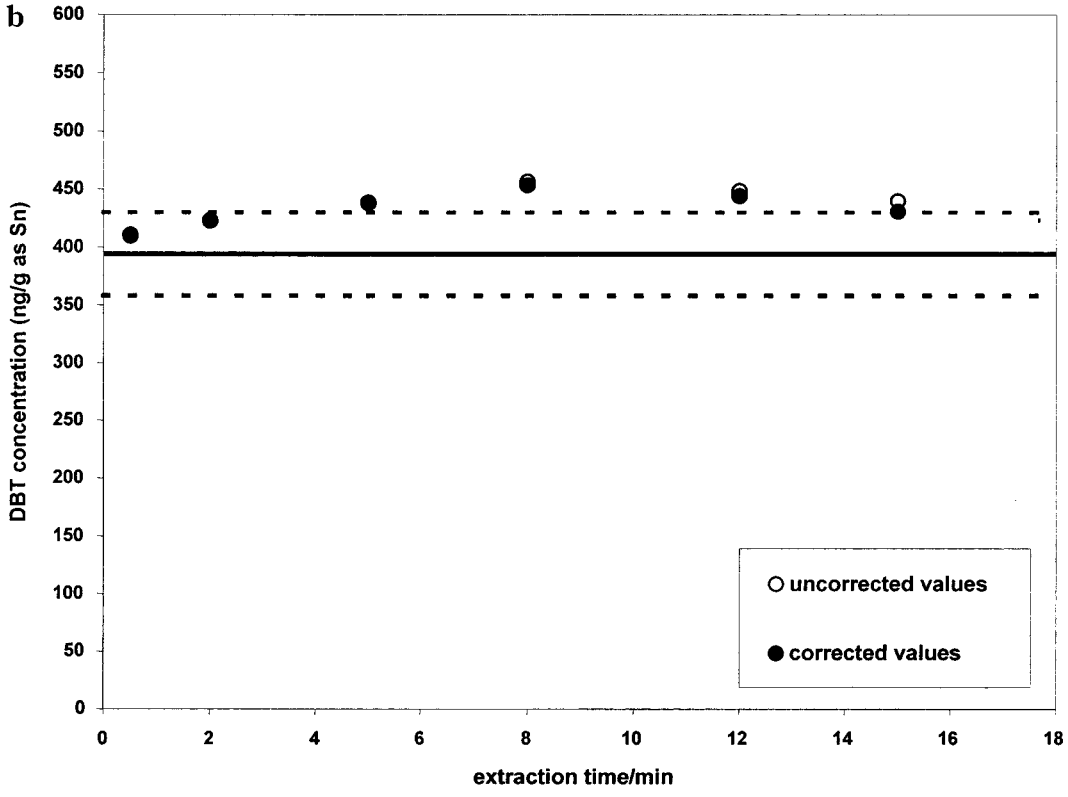


Figure 5. Decomposition factors from TBT to DBT (F1, panel a) and from DBT to MBT (F2, panel b) for the PACS-2 sediment using MW (circles), UL (squares), and ME (triangles).

To study further this peculiar behavior of MBT, another certified sediment/matrix, the CRM 646 from the European BCR, was also speciated for tin. In this case, only the microwave-assisted extraction was used (150 W with exposure times ranging from 30 s to 15 min). Results are presented in Figure 6a, b, and c for TBT, DBT, and MBT, respectively, and in this case, the figure shows somewhat different information from that given in Figure 4 for PACS-2. For example, the results for TBT in Figure 6a were calculated using two reference isotopes for the sample (isotopes 120 and 118), but isotope 119 was used as a reference for the spike. This allowed us to check even for transformations from DBT to TBT during extraction, because the DBT spike was enriched in the ^{118}Sn isotope. As shown in Figure 6a, the results using both reference isotopes were analytically indistinguishable, and so no butylation reactions seem to be taking place. Similar results were obtained for PACS-2 under all extraction conditions tested.

For DBT and MBT, the concentration results obtained are presented in Figure 6b,c using the equations given in Table 2 (black circles) or assuming that no degradation reactions were taking place (open circles). As can be observed, the differences between the degradation corrected and uncorrected concentration values start to be significant only after longer extractions times, during which the degradation factors increase. It is worth noting here that the degradation factors computed for CRM 646 using MW-assisted extraction were very similar to those shown in Figure 5 for PACS-2. That means that the degradation of butyltin compounds during extraction seems to be more dependent on


a
TBT concentration (ng/g as Sn)
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
extraction time/min
120 as reference isotope
118 as reference isotope


b
DBT concentration (ng/g as Sn)
600
550
500
450
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
extraction time/min
uncorrected values
corrected values

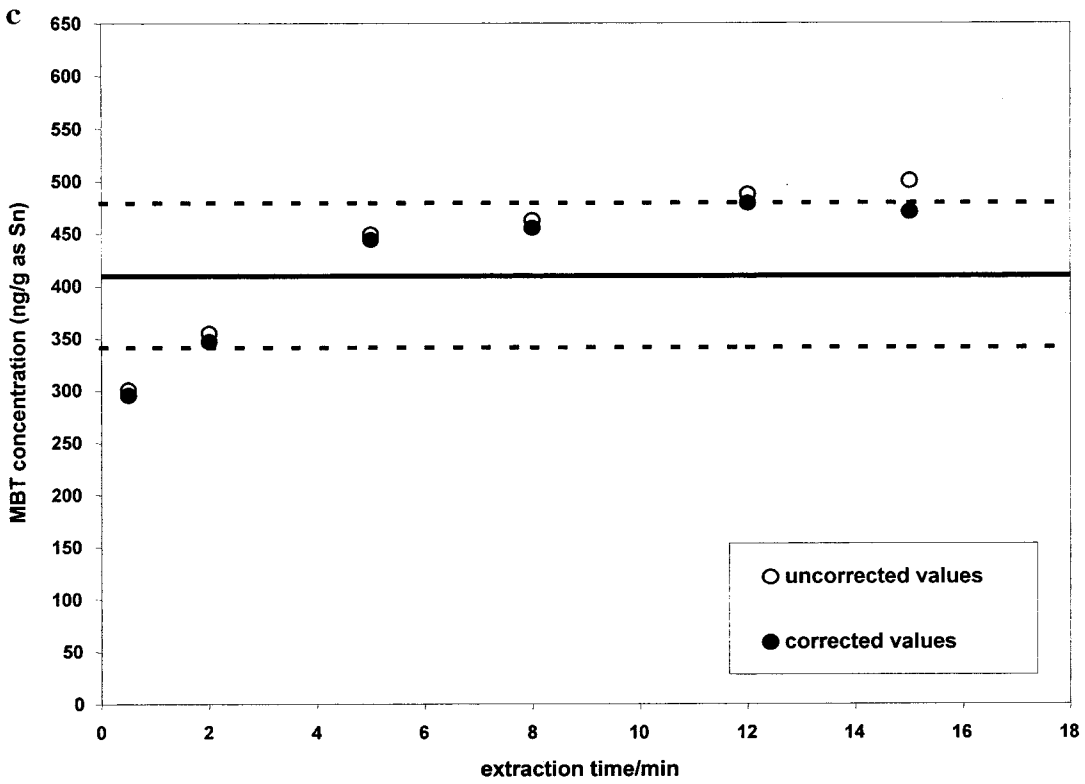


Figure 6. Isotope dilution results for the BCR-646 sediment using MW. Part a corresponds to TBT using as reference the isotope in either sample 120 (squares) or 118 (circles). Parts b and c correspond to DBT and MBT (filled and open circles correspond to the degradation corrected and uncorrected values).

the extraction technique and the extraction time used than on the particular matrix of the sediment.

Finally, with regard to the CRMs values, the concentrations found for DBT and TBT were close to or slightly above the upper certified confidence interval (Figure 6). These new "double spike" results again matched the values obtained previously in our laboratory using only the mixed ^{119}Sn spike.[18] The behavior of MBT in Figure 6c was similar to that obtained for PACS-2 in Figure 4c using MW: the extraction efficiency increased gradually until a plateau was obtained that, in this case, was within the confidence interval for this material (for the degradation corrected data). Unfortunately, by the time that quantitative extraction of MBT is achieved (after ca. 12 min), degradation reactions started to be significant (more than 2% for F_1 and F_2). Thus, a compromise between MBT recovery and species degradation using MW extraction would be necessary for accurate MBT speciation.

It is worth noting that when comparing our current results for MBT in CRM-646 by MW extraction (ca. 470 ng/g) with our previous results using mechanical shaking (388 ng/g),[18] an increase in 21% was observed. Similarly, for PACS-2 in Figure 4c, the difference between MW and ME results was ~25%. In other words, it appears that speciation with MW is able to extract ca. 20% more MBT from both reference materials (although for CRM-646, results using both ME and MW are within the certified range, and this was not the case for PACS-2).

The results given in Figures 4, 5, and 6 for PACS-2 and CRM-646 indicate that ultrasonic extraction might be the method of choice for the extraction of butyltin compounds from sediments by combining high extraction efficiencies for MBT, short extraction times, and no degradation reactions. The absence of degradation reactions by ultrasonic extraction was also observed by Rio-Segade et al.,[31] even for labile species such as MeHg. In any case, summing up all the above results, it is evident that the use of isotopically labeled spikes has proved to be most enlightening to fully understand the extraction behavior of organotin compounds in different biological and environmental samples and could be extended to study other multi-isotopic element speciation analysis issues.

CONCLUSIONS

From the results obtained in the study of the three different extraction approaches, we can conclude that MBT seems to be strongly bound to the solid matrix, and to recover it quantitatively, a harsher extraction technique and longer extraction time are required. On the other hand, the borderline between quantitative extraction of MBT and degradation of TBT and DBT turned out to be very narrow using microwave-assisted extraction. With respect to TBT and DBT, all three extraction techniques tested for PACS-2 were able to recover them quantitatively, even using short extraction times. In addition, the good agreement between previous results obtained for TBT and DBT in CRM-646 using mechanical shaking[18] and the results obtained here using microwave assisted extraction indicate also that those compounds are easily extracted from CRM-646. No evidence of transbutylation reactions was found, on the basis of the results obtained for TBT using 120/119 and 118/119 isotope ratios in both reference materials and under all extraction conditions tested.

When we compare critically the three extraction techniques evaluated here, it can be concluded that ultrasonic extraction

(31) Rio-Segade, S.; Bendicho, C. *J. Anal. At. Spectrom.* **1999**, 14, 263−268.

provided the best results, because no degradation reactions were observed, but recoveries attained for the three tin species were quantitative after 8 min of UL exposure (assuming that the certified value for MBT in PACS-2 needs reevaluation). Microwave-assisted extraction provided adequate data when degradation reactions were taken into account (a difficult task in a routine laboratory). Finally, room-temperature leaching with mechanical shaking provided data in agreement with the certified values for all three species for both PACS-2 (this study) and CRM-646 (ref 18); however, the higher values for MBT found in PACS-2, both by microwave and ultrasonic extraction, raise some doubts about the capability of mechanical shaking to extract MBT from PACS-2 (and, as a result, on the certified value for MBT in this material).

Finally we would like to point out that, despite the disparity between the different extraction techniques and extraction conditions used, the degradation corrected values for all organotin species (in those experiments where quantitative extraction was achieved) exhibited an overall precision around 2−3% RSD. Furthermore, the results for DBT and TBT obtained in this work are in close agreement with previous data obtained using both a ^{118}Sn-enriched DBT spike[22] and a ^{119}Sn-enriched organotin mixed spike,[18] a good indicator of the excellent precision and accuracy that can be obtained using isotope dilution analysis (IDA) for trace metal speciation.

In light of all these results, it seems that multi-isotope-labeled IDA techniques will become a most powerful tool to optimize and validate procedures for trace metal speciation, going from species extraction from the solid to derivatization, separation, and detection of those species for accurate final determinations.

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