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Development of a triple spike methodology for validation of butyltin compounds speciation analysis by isotope dilution mass spectrometry

Part I. Synthesis of the spike, characterisation and development of the mathematical equations†

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A methodology for the determination of butyltin compounds by isotope dilution mass spectrometry based on a triple spike approach is presented. A spike solution containing monobutyltin, dibutyltin and tributyltin, each compound enriched with different tin isotopes, has been synthesised, characterised and applied to the simultaneous determination of the three butyltin compounds by isotope dilution GC-ICP-MS. Mathematical equations correcting for all possible degradation reactions which could occur during sample pre-treatment were developed, allowing the accurate calculation of the three butyltin concentrations sought plus the values of six possible interconversion yields, regardless of the extent of degradation. The triple tin spike solution has shown itself to be stable over more than 7 months, both in isotope composition and elemental concentration, provided that acetic acid is present in the solution. The proposed methodology has been finally evaluated by performing the determination of butyltin compounds in the certified reference material CRM-477 (Mussel Tissue), under different microwave assisted extraction conditions, with satisfactory results.

Introduction

The application of isotope dilution analysis to trace metal speciation has been mainly carried out so far by adding the enriched metallic element after the physical separation of the chemical species of interest (post-column "species-unspecific spiking" mode).[1] However, in the last years an increasing number of stable isotopically-enriched species have become available which allow the development and application of new methodologies based on species-specific IDMS for Hg,[2–8] Cr,[9–12] Se[13,14] and Sn[15–25] speciation. Most of these studies make use of "single isotope" spikes (only one isotope is used to isotopically label one or all species of interest), allowing for correction of some systematic errors which the "species-unspecific" mode is not able to correct (*e.g.*, errors occurring during the sample preparation steps).[26] However, using "single isotope" spikes, possible species rearrangement reactions throughout the speciation procedure cannot be corrected for. In this context, "multiple" spikes (solutions containing different metal species enriched with different isotopes) can be used to investigate and correct for such limitations. Such approaches can be especially important when analysing solid samples (*e.g.*, sediments or biological tissues) as a complete extraction of the species from the solid is required.[27] Using "multiple" spike approaches, more drastic solid–liquid extraction procedures can be evaluated and the results compared with those obtained using more conventional extraction conditions, providing a suitable validation or quality control of the final speciation procedure used.

So far, few applications[2,3,6,10–12,17,22,25] have been published to secure reliable approaches able to correct for or to evaluate the extent of artifact formations or rearrangement reactions by using "multiple" spikes. In the case of the speciation of organotin compounds using "multiple" spikes, previous work in our laboratory[17,22,25] was always based on a double spike approach (118 and 119 enriched Sn), which has been shown to be extremely useful to correct for the stepwise degradation of tributyltin (TBT) during the analysis of sediments. A step forward in this line of using reliable methods for the speciation of organotin compounds is the development of a triple spike IDMS methodology. As shown here, a spike solution containing all three butyltin species (mono-, di- and tributyltin), each of them isotopically labelled with a different isotope, has been synthesised and the mathematical equations required for the application of this powerful tool have been developed. This new approach allows for correction not only of the stepwise degradation of TBT but also for alternative degradation reactions such as the direct debutylation of TBT to monobutyltin (MBT) or possible butylation reactions that could take place during the whole speciation analysis.

Experimental

Instrumentation

A Hewlett-Packard (Palo Alto, CA, USA) gas chromatograph Model 6890, fitted with a split/splitless injector and a HP-5 capillary column (cross linked 5% phenylmethylsiloxane, 30 m × 0.32 mm × 0.25 μm thickness), 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 laboratory made transfer line described in detail previously.[28] A Waters high pressure pump Model 510 (Millipore, Bedford, MA, USA), a Rheodyne 7125 injection valve (Berkeley, CA, USA) fitted with a 100 μl loop and a Zorbax 300-SCX cation exchange column (Hewlett-Packard, 25 cm long, 4.6 mm id, 5 μm particle size) were used for the

† Electronic supplementary information (ESI) available: equations used for the computation of concentrations and degradation factors. See http://www.rsc.org/suppdata/ja/b3/b313437g/

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isolation of MBT from the ^{119}Sn-enriched mixed spike. For the optimisation of the separation conditions the outlet of the column was directly connected to the nebulizer of the ICP-MS with a piece of Teflon tubing. For the extraction of the organotin compounds from CRM-477 (mussel tissue), a microwave oven, Model 1200 (Milestone, Socisole, Italy), equipped with middle pressure PTFE vessels, was used.

Reagents and materials

Tributyltin chloride (96%), dibutyltin dichloride (96%) 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 standards solutions were kept in the dark at 4 °C and diluted working solutions in methanol were prepared daily before the analysis. Acetic acid (Merck) and methanol (Merck) were used for the extraction of the organotin compounds from the solid matrix. Ethylation of these species was performed using sodium tetraethylborate (Galab, Geesthacht, Germany).

Solid ^{119}Sn-enriched and ^{118}Sn-enriched tin metal were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and a solution of ^{117}Sn-enriched tributyltin was supplied by the Laboratory of the Government Chemist (Teddington, UK) during the course of an intercomparison exercise.[29] The synthesis of the ^{118}Sn-enriched dibutyltin (DBT) and the ^{119}Sn-enriched mixture of MBT, DBT and TBT have been described in previous publications.[15,16] The CRM-477 (mussel tissue) was purchased from the Institute for Reference Materials and Measurements (Geel, Belgium). The HPLC eluent for the preparative chromatography consisted of 0.1 M diammonium hydrogencitrate (Probus, Barcelona, Spain), 5% (v/v) acetic acid (Merck) and 16% (v/v) methanol in Milli-Q water (Millipore, Molsheim, France) with final degassing using He for 10 min.

Procedures

Microwave assisted extraction. A sample of 0.1 g of the mussel tissue was directly weighed in middle pressure PTFE vessels, spiked with 0.14 g of the triple spike solution in acetic acid–methanol and, immediately, 4 ml of a mixture of acetic acid and methanol (3 : 1) were added (in all cases the spike to sample ratio was optimised according to the random error propagation theory[30]). The slurries were then exposed to microwaves at 150 W for different extraction times (from 1 to 16 min), left to cool down to room temperature and, finally, 200 μl of the extract were ethylated for further analysis as described below. Due to a technical problem with the pressure and temperature sensor of the microwave oven the extraction temperatures achieved during the microwave procedure have not been reported.

Ethylation, separation and determination by GC-ICP-MS. Ethylation of the tin species was carried out in 7 ml clear glass vials with screw caps (Supelco, Bellefonte, PA, USA). The pH was adjusted to 5.4 with 4 ml of 1 M acetic acid–sodium acetate buffer and ethylation was performed using 0.5 ml of 2% w/v sodium tetraethylborate in 0.1 M NaOH. Finally, 1 ml of hexane was added for liquid–liquid extraction, extracted by manual shaking for 10 min, transferred to a 2 ml chromatographic vial and stored at −18 °C until analysis. This final volume was preconcentrated approximately ten times under a gentle stream of nitrogen just before the GC-ICP-MS measurement.

Blank values were evaluated using the triple spike by spiking 4 ml of the methanol–acetic acid mixture with a 50 times lower amount of the spike than that used for the samples and applying the MW extraction method described. Three independent measurements were performed and the blank values, calculated relative to a typical sample weight of 0.1 g, were 1.33, 0.78 and 1.01 ng g^{-1} Sn for MBT, DBT and TBT, respectively. Due to the low blank values, the concentrations found for the samples were not blank corrected. Detection limits were calculated as three times the standard deviation of the blanks and resulted in being 0.26, 0.10 and 0.19 ng Sn g^{-1} for MBT, DBT and TBT, respectively.

Typical operating conditions and analytical features of these determinations by GC-ICP-MS have been described elsewhere.[15] Daily optimisation of the ion lens of the mass spectrometer was performed after the connection of the GC to the ICP-MS, by using $^{38}Ar^{40}Ar^{+}$ background signal in the ICP-MS. Integration of the chromatographic peaks was carried out by using the commercial GC-MS Agilent software supplied with the ICP-MS instrument. Isotope ratios were always computed as peak area ratios. The integration time per isotope selected was 50 ms and the isotopes measured were 117, 118, 119 and 120. In this way, the total integration time, 200 ms, was short enough to follow the chromatographic peak profiles. Mass bias was corrected by bracketing a natural butyltin standard mixture of MBT, DBT and TBT between each triplicate of samples. The dead time of the detector was automatically corrected by the software of the instrument.

Results and discussion

Synthesis and characterisation of the triple spike

The isolation of MBT from the previously synthesised ^{119}Sn-enriched spike (a mixture of MBT, DBT and TBT)[16] was performed by taking advantage of the different boiling points of the three butyltin species. The removal of TBT (the most volatile) and almost all DBT from a 0.6 g aliquot of the ^{119}Sn-enriched mixture (222 μg g^{-1} of MBT as tin) was achieved by evaporation under a gentle stream of Ar to a few microlitres. This final volume was redissolved in 600 μl of the mobile phase in order to complete the isolation of MBT by semi-preparative HPLC under similar chromatographic conditions to those employed in a previous publication.[17] However, since in this case only the isolation of MBT was required, the percentage of methanol in the mobile phase was decreased from 20% to 16% in order to improve the resolution between MBT and DBT. In this way, baseline separation between these two species was achieved. The 600 μl of the ^{119}Sn enriched MBT solution were injected in five successive 100 μl injections. Fractions were collected during the elution time range of MBT providing a final 5 ml volume of *ca.* 5 μg g^{-1} of ^{119}Sn-enriched MBT. Negligible amounts of DBT or TBT were present, as demonstrated by GC-ICP-MS analysis. Finally, small aliquots of our concentrated stock solutions of the ^{118}Sn-enriched DBT (synthesised in a previous work[15]) and the ^{117}Sn-enriched TBT (supplied by the LGC) were added and the mixture was diluted 1 : 2 with methanol. Final concentrations for MBT, DBT and TBT of 2.6, 4.1 and 5.1 μg g^{-1} (as Sn) were obtained in the final triple spike solution, respectively.

Stability of the triple spike

Instability problems in the triple spike solution were detected after only one month of preparation (July, 2002). Since the three butyltin species were enriched with different tin isotopes its decomposition led to small changes not only in their concentrations but also in their isotopic composition. In previous work the stability of the ^{119}Sn-enriched spike[18] and the double spike solution[22] had been studied with satisfactory results over more than one year. Therefore, since the main difference between these solutions and those obtained here was its acetic acid content, the influence of this reagent on the stability of the spike was checked.

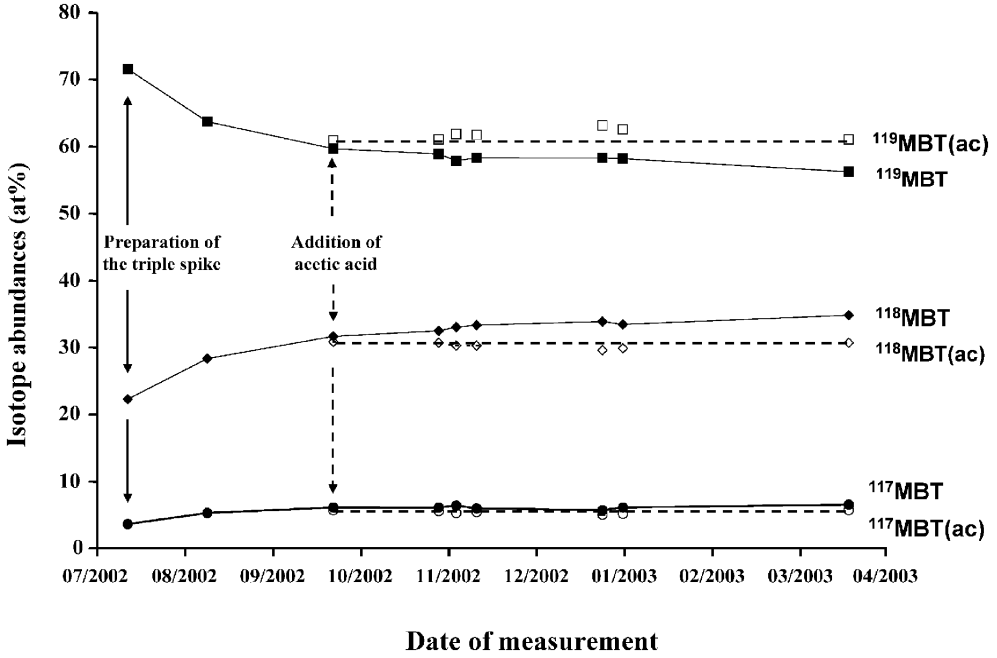

Fig. 1 Variation of the isotopic composition of the ^{119}Sn-enriched MBT in the triple spike solution with time for: 3% (full lines) and 50% (broken lines) acetic acid (v/v).

For this purpose, the triple spike solution was split into 2 aliquots and the first one was diluted (1 + 1) with acetic acid, resulting in a final acid content of around 50%. Fig. 1 shows the changes observed in the abundances of the isotopes 117, 118 and 119 in ^{119}Sn-enriched MBT for both aliquots from July 2002 to April 2003. The horizontal broken and the full lines represent the aliquot with and without acetic acid, respectively. Any decay of TBT to MBT or DBT to MBT will result in an increase in the abundance of the isotopes 117 and 118, respectively, as well as a decrease in the abundance of the isotope 119. As can be observed, from the date of preparation until the addition of acetic acid an important degradation of DBT to MBT is clearly indicated by the increase in isotope 118 abundance (from 22.3% to 31.6%) and by the corresponding decrease of that of 119 isotope (from 71.5% to 59.7%). On the other hand, there was also a slight increase in the abundance of the isotope 117 (from 3.6% to 6.1%), which may be originated by the simultaneous degradation of TBT to DBT or by direct degradation of TBT to MBT. However, after the addition of acetic acid, the isotopic composition of MBT remained constant (as reflected by the horizontal broken lines of Fig. 1, which join the first and last values of the aliquot with acetic acid). As far as the isotopic composition of the ^{118}Sn-enriched DBT is concerned, it is worth stressing that no important changes, even without acetic acid addition, were detected. The abundance for the isotope 118 decreased from 79.7% to 79.0, whereas that for the isotope 117 increased only from 17.8% to 18.5%. No changes in the isotope composition of TBT were detected which ruled out the existence of butylation reactions. In the light of these results, we can conclude that the degradation from DBT to MBT was shown to be the main interconversion reaction in the spike in the absence of acetic acid. This is also in agreement with Rodríguez-Pereiro *et al.*,[31] who reported good stability of those butyltin compounds in acetic acid.

Finally, in the light of such conclusion, the aliquot containing acetic acid was selected for further experiments. The stability of this spike with time was assessed in parallel by measuring the concentration of MBT, DBT and TBT at different time intervals by reverse isotope dilution analysis using commercially available natural butyltin standards (Fig. 2). As can be observed, no degradation of the species was detected from October 2002 to April 2003. Table 1 shows the average isotopic composition of the obtained triple spike and Fig. 3 shows its GC-ICP-MS chromatogram, in which it can be observed that each of the three butyltin species is

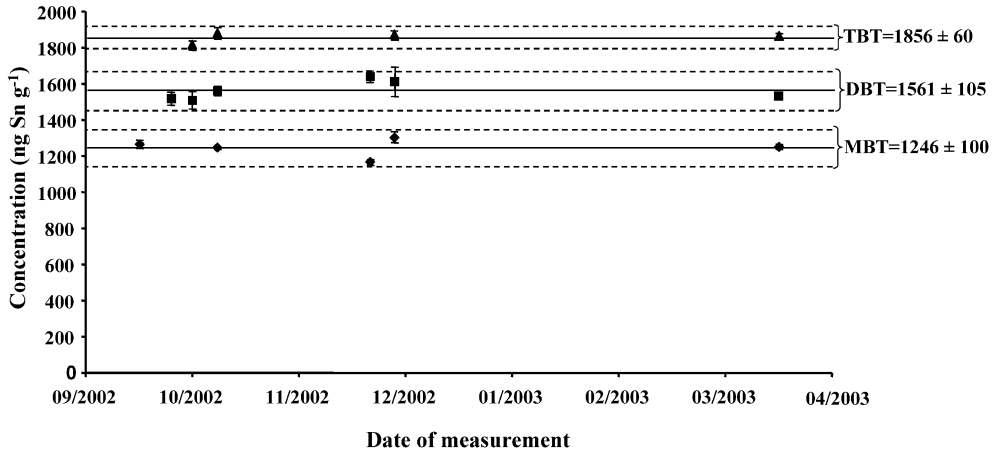

Fig. 2 Six months stability of the butyltin species in the triple spike solution after the addition of acetic acid.

Table 1 Isotopic composition of the triple spike solution (% abundance) for six independent injections[a]

	Isotopic composition (at%)			
Isotopes	Natural tin	MBT	DBT	TBT
116	14.54	0.484 (0.016)	1.421 (0.042)	6.881 (0.156)
117	7.68	5.592 (0.106)	17.451 (0.256)	**92.71 (0.17)**
118	24.22	30.64 (0.18)	**80.12 (0.31)**	0.336 (0.128)
119	8.59	**61.12 (0.34)**	0.516 (0.018)	0.027 (0.002)
120	32.59	2.140 (0.417)	0.454 (0.019)	0.035 (0.005)
122	4.63	0.009 (0.001)	0.020 (0.003)	0.007 (0.001)
124	5.79	0.013 (0.004)	0.018 (0.002)	0.007 (0.002)

[a] Uncertainty corresponds to 95% confidence interval ($n = 6$).

labelled with a different tin isotope. It is worth mentioning that the main natural tin isotope, ^{120}Sn, is now a minor isotope in the spike for the three tin species (Fig. 3).

Development of the isotope dilution equations

The possible interconversion reactions between TBT, DBT and MBT and inorganic tin ($Sn_{(IV)}$) can be illustrated using a tetrahedron in which each compound is a vertex and all possible interconversion reactions are on the edges (Fig. 4). Based on this degradation tetrahedron for butyltin compounds we could theoretically consider up to 12 interconversion reactions: six debutylation reactions (F_1 to F_6) and 6 butylation reactions (F_7 to F_{12}). Of course, some of these chemical reactions are most unlikely. In those particular cases the values of the reaction yields could be assumed close to zero. In the case of the triple spike obtained we can monitor four tin isotopes

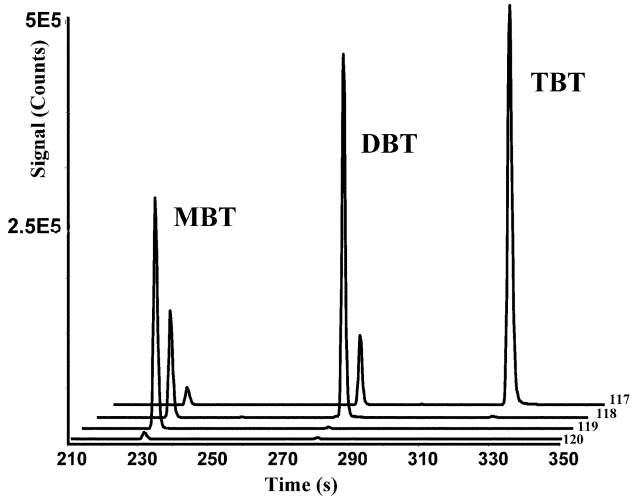

Fig. 3 GC-ICP-MS chromatogram of the triple spike solution with detection at masses 117, 118, 119 and 120.

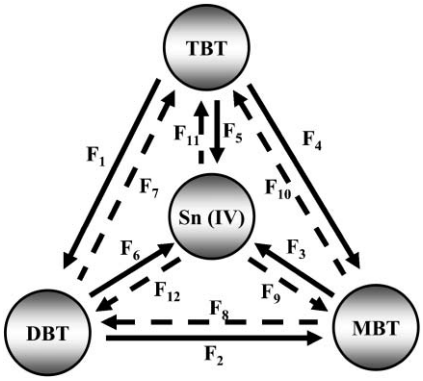

Fig. 4 Possible interconversion pathways of the butyltin compounds.

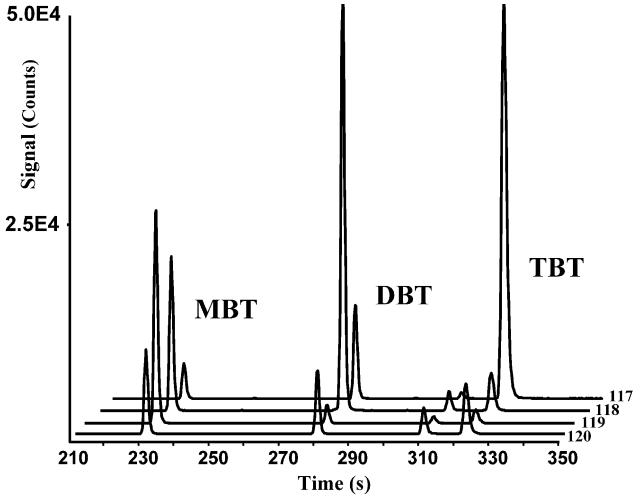

Fig. 5 GC-ICP-MS chromatogram of the spiked CRM 477 reference material.

(117, 118, 119 and 120) for the three butyltin species. Thus, we can measure nine independent isotope ratios, three for each butyltin species separated in the GC-ICP-MS system used. This can be easily seen in Fig. 5, where a typical GC-ICP-MS chromatogram of the spiked CRM 477 is shown.

When we spike the sample (s) with the triple spike (sp) we modify the number of mols for each isotope in each compound in the mixture (m). As the number of mols of spike added is known we can establish 12 mass balance equations (4 for each compound) corresponding to the 4 measured isotopes: 117, 118 and 119 as reference isotopes in the spike, and 120 as reference isotope in the sample. For example, eqns. (1), (2) and (3) show the mass balances for isotopes 117 in TBT, 118 in DBT and 119 in MBT, respectively, in the mixture of sample and spike:

$$\begin{aligned} N^{TBT}_{m,117} = {} & (N^{TBT}_{s,117} + N^{TBT}_{sp,117})\,(1 - F_1 - F_4 - F_5) \\ & + (N^{DBT}_{s,117} + N^{DBT}_{sp,117})\,F_7\,(1 - F_1 - F_4 - F_5) \\ & + (N^{MBT}_{s,117} + N^{MBT}_{sp,117})\,F_{10}\,(1 - F_1 - F_4\,F_5) \\ & + (N^{Sn(IV)}_{s,117} + N^{Sn(IV)}_{sp,117})\,F_{11}\,(1 - F_1 - F_4 - F_5) \end{aligned} \quad (1)$$

$$\begin{aligned} N^{DBT}_{m,118} = {} & (N^{DBT}_{s,118} + N^{DBT}_{sp,118})\,(1 - F_2 - F_6 - F_7) \\ & + (N^{TBT}_{s,118} + N^{TBT}_{sp,118})\,F_1\,(1 - F_2 - F_6 - F_7) \\ & + (N^{MBT}_{s,118} + N^{MBT}_{sp,118})\,F_8\,(1 - F_2 - F_6 - F_7) \\ & + (N^{Sn(IV)}_{s,118} + N^{Sn(IV)}_{sp,118})\,F_{12}\,(1 - F_2 - F_6 - F_7) \end{aligned} \quad (2)$$

$$\begin{aligned} N^{MBT}_{m,119} = {} & (N^{MBT}_{s,119} + N^{MBT}_{sp,119})\,(1 - F_3 - F_8 - F_{10}) \\ & + (N^{DBT}_{s,119} + N^{DBT}_{sp,119})\,F_2\,(1 - F_3 - F_8 - F_{10}) \\ & + (N^{TBT}_{s,119} + N^{TBT}_{sp,119})\,F_4\,(1 - F_3 - F_8 - F_{10}) \\ & + (N^{Sn(IV)}_{s,117} + N^{Sn(IV)}_{sp,117})\,F_9(1 - F_3 - F_8 - F_{10}) \end{aligned} \quad (3)$$

In a similar way we can establish another 9 mass balance equations for the other 3 isotopes for each compound. If we focus on equation (1) we see that we have assumed that the number of mols of ^{117}Sn for TBT in the mixture will consist of:

(i) the sum of the TBT in the sample and the TBT in the spike corrected for degradation (F_1: TBT to DBT, F_4: TBT to MBT and F_5: TBT to $Sn_{(IV)}$);

(ii) the possible small fraction of DBT transformed to TBT through butylation (F_7) corrected for ulterior butyltin degradation as well (F_1, F_4 or F_5);

(iii) the possible small fraction of MBT transformed directly

to TBT through butylation (F_{10}) also corrected for butyltin degradation (F_1, F_4 or F_5);

(iv) the fraction of inorganic tin ($Sn_{(IV)}$) transformed directly to TBT through butylation (F_{11}) also corrected for degradation (F_1, F_4 or F_5).

For the case of DBT and MBT in eqns. (2) and (3), the mass balance equations are very similar to eqn. (1) but introducing the corresponding debutylation and butylation factors indicated in Fig. 4.

Taking a closer look at eqns. (1), (2) and (3), we can observe that the content of inorganic tin in the sample and in the spike appears in all equations. Inorganic tin is not a source of concern in environmental and biological samples so its determination in the sample is not necessary. To simplify data treatment and avoid the need for data on inorganic tin, we have assumed that the butylation yields of inorganic tin to form TBT (F_{11}) or MBT and DBT (F_9 and F_{12}, respectively) are negligible. This seems to be quite a safe assumption as the butylation of inorganic tin requires strong reaction conditions (high temperatures and pressures) and those conditions will not normally be reached during the extraction of butyltin compounds from the samples. We will see later that this assumption proved to be adequate.

Once the 12 mass balance equations are established we can compute nine isotope ratio equations by dividing the mass balance equations for MBT, DBT and TBT at mass 120 for the equations obtained for the other three reference isotopes. For example, the isotope ratio equation for the ratio 120/117 in TBT is shown in eqn. (4):

$$R^{120/117}_{TBT,m}=\frac{N^{TBT}_{m,120}}{N^{TBT}_{m,117}}=\frac{\left(N^{TBT}_{s,120}+N^{TBT}_{sp,120}\right)+F_7\left(N^{DBT}_{s,120}+N^{DBT}_{sp,120}\right)+F_{10}\left(N^{MBT}_{s,120}+N^{MBT}_{sp,120}\right)}{\left(N^{TBT}_{s,117}+N^{TBT}_{sp,117}\right)+F_7\left(N^{DBT}_{s,117}+N^{DBT}_{sp,117}\right)+F_{10}\left(N^{MBT}_{s,117}+N^{MBT}_{sp,117}\right)} \quad (4)$$

As can be seen, only two intercoversion factors remain in this equation: F_7 and F_{10}. The other factors are eliminated in the division. The same occurs for the isotope ratios 120/118 and 120/119 in TBT. For the isotope ratio equations for DBT the factors which remain are F_1 and F_8 and for MBT F_2 and F_4. Factors F_3, F_5 and F_6 (degradation of MBT, DBT and TBT to inorganic tin) are eliminated from the equations and cannot be computed.

Taking into account the isotope composition of tin in the sample and in the different butyltin species in the spike, we can compute the total number of mols of TBT, DBT and MBT both in the sample and in the spike at the different masses. For example, the number of mols of TBT in the sample at mass 120 can be related to the total number of mols of TBT in the sample using the expression: $N^{TBT}_{s,120} = At^{120}_s N^{TBT}_s$, At^{120}_s being the isotope abundance for mass 120 in the sample.

Similar expressions can be used for all tin isotopes and species both in the sample and in the spike. Once this substitution has been made, eqn. (4) can be rewritten for the number of mols of TBT in the sample as follows:

$$N^{TBT}_s = N^{TBT}_{sp}\frac{At^{120}_{sp,TBT}-R^{120/117}_{TBT,m}At^{117}_{sp,TBT}}{R^{120/117}_{TBT,m}At^{117}_s-At^{120}_s}+$$

$$F_7N^{DBT}_{sp}\frac{At^{120}_{sp,DBT}-R^{120/117}_{TBT,m}At^{117}_{sp,DBT}}{R^{120/117}_{TBT,m}At^{117}_s-At^{120}_s}-F_7N^{DBT}_s+ \quad (5)$$

$$F_{10}N^{MBT}_{sp}\frac{At^{120}_{sp,MBT}-R^{120/117}_{TBT,m}At^{117}_{sp,MBT}}{R^{120/117}_{TBT,m}At^{117}_s-At^{120}_s}-F_{10}N^{MBT}_s$$

As can be seen, eqn. (5) is an extension of the usual isotope dilution equation (first term in the equation), but taking into account the formation of TBT from DBT and MBT. Similar equations can be deduced for the other isotope ratios in TBT (120/118 and 120/119) and for the other butyltin compounds (see Electronic Supplementary Information†). This set of nine equations contains only nine unknowns: the three butyltin concentrations and six interconversion yields. The problem of solving these nine equations with nine unknowns was less complex than expected. For example, the three equations obtained for TBT could be simplified to obtain two equations where the only unknowns were F_7 and F_{10} and a similar treatment provided the other four degradation factors sought, using the equations for DBT and MBT, respectively. In that sense, the calculation of the degradation factors was independent of the actual butyltin concentrations in the sample.

Similar to eqn. (5), the following two equations, (6) for DBT and (7) for MBT, respectively, were worked out:

$$N^{DBT}_s = N^{DBT}_{sp}\frac{At^{120}_{sp,DBT}-R^{120/118}_{DBT,m}At^{118}_{sp,DBT}}{R^{120/118}_{DBT,m}At^{118}_s-At^{120}_s}+$$

$$F_8N^{MBT}_{sp}\frac{At^{120}_{sp,MBT}-R^{120/118}_{DBT,m}At^{118}_{sp,MBT}}{R^{120/118}_{DBT,m}At^{118}_s-At^{120}_s}-F_8N^{MBT}_s+ \quad (6)$$

$$F_1N^{TBT}_{sp}\frac{At^{120}_{sp,TBT}-R^{120/118}_{DBT,m}At^{118}_{sp,TBT}}{R^{120/118}_{DBT,m}At^{118}_s-At^{120}_s}-F_1N^{TBT}_s$$

$$N^{MBT}_s = N^{MBT}_{sp}\frac{At^{120}_{sp,MBT}-R^{120/119}_{MBT,m}At^{119}_{sp,MBT}}{R^{120/119}_{MBT,m}At^{119}_s-At^{120}_s}+$$

$$F_4N^{TBT}_{sp}\frac{At^{120}_{sp,TBT}-R^{120/119}_{MBT,m}At^{119}_{sp,TBT}}{R^{120/119}_{MBT,m}At^{119}_s-At^{120}_s}-F_4N^{TBT}_s+ \quad (7)$$

$$F_2N^{DBT}_{sp}\frac{At^{120}_{sp,DBT}-R^{120/119}_{MBT,m}At^{119}_{sp,DBT}}{R^{120/119}_{MBT,m}At^{119}_s-At^{120}_s}-F_2N^{DBT}_s$$

Once the degradation factors were computed the three butyltin concentrations sought were calculated using eqn. (5) for TBT, (6) for DBT and (7) for MBT. Eqns. (5), (6) and (7) can be simplified and expressed in matrix notation as:

$$\begin{pmatrix}1 & F_2 & F_4\\ F_8 & 1 & F_4\\ F_{10} & F_7 & 1\end{pmatrix}\begin{pmatrix}N^{MBT}_s\\ N^{DBT}_s\\ N^{TBT}_s\end{pmatrix}=\begin{pmatrix}Z_{MBT}\\ Y_{DBT}\\ X_{TBT}\end{pmatrix}$$

where Z_{MBT}, Y_{DBT} and X_{TBT} are the sum of all independent terms in eqns. (5), (6) and (7). It is clear that we end up with three equations and three unknowns, which can be easily resolved using Kramer's rule or by inverting the matrix of the factors, computing therefore the concentrations of MBT, DBT and TBT in the sample. For that purpose we have developed a computer spreadsheet that simplifies all the calculations to be performed. In summary, by using the triple spike we can compute six reaction yields plus the three unknown concentrations of MBT, DBT and TBT in the sample. It is clear that the concentrations obtained were those existing in the original sample and that degradation reactions were corrected for in this way. One interesting consequence of the matrix equation given above is that, if all reaction factors are zero, we end up with the standard isotope dilution equations for all three butyltin compounds, as the matrix of the factors contains zero in all its components except for the main diagonal.

Evaluation of the proposed methodology

In order to validate the triple spike methodology developed in this work, the determination of the three butyltin compounds in the certified reference mussel tissue (CRM-477) was carried out under different microwave assisted extraction conditions using an acetic acid–methanol mixture (3 : 1) as extractant. Corrected concentrations and the six factors corresponding to possible rearrangement reactions were calculated under

Table 2 Corrected concentrations (ng Sn g^{-1}) and interconversion factors (%) obtained for CRM 477 Mussel Tissue under different extraction times using microwave assisted extraction at 150 W

Extraction time	MBT	DBT	TBT	F_1	F_2	F_4	F_7	F_8	F_{10}
1 min	1198 ± 50	813 ± 32	899 ± 17	0.8 ± 0.3	3.6 ± 0.7	−0.2	−0.1	−0.3	−0.2
2 min	1185 ± 49	819 ± 32	894 ± 17	0.6 ± 0.3	1.7 ± 0.7	−0.2	0.0	0.0	−0.4
4 min	1223 ± 51	848 ± 33	927 ± 18	0.5 ± 0.3	1.2 ± 0.7	−0.2	−0.1	−0.4	−0.5
8 min	1233 ± 52	843 ± 33	890 ± 17	0.7 ± 0.3	4.6 ± 0.8	−0.3	0.1	−0.3	−0.4
12 min	1227 ± 51	846 ± 33	901 ± 17	3.2 ± 0.4	3.6 ± 0.7	−0.2	0.1	−0.1	−0.3
16 min	1240 ± 52	856 ± 33	907 ± 17	4.3 ± 0.4	34.3 ± 2.2	0.5	0.0	−0.2	−0.3
Mean ± sd	**1218 ± 21**	**838 ± 17**	**903 ± 13**						
RSD (%)	1.7	2.1	1.5						
Certified value	1013 ± 189	785 ± 61	900 ± 78						

increasing heating times from 1 to 16 min at 150 W. The results obtained are shown in Table 2.

As can be observed, slightly higher values than the certified range were obtained for MBT, whereas the concentrations obtained for DBT and TBT were well in agreement with the certified values at all extraction times tested. Concerning the degradation factors, only at 16 min was the decomposition of DBT to MBT (F_2) clearly observed (34%), whereas the interconversion of TBT to DBT (F_1) was only noticeable after 12 min (3%). Direct degradation of TBT to MBT (F_4), as well as all butylation reactions, were found to be negligible under all conditions. This confirms the stepwise degradation mechanism assumed previously[17] for the butyltin compounds.

Uncertainty calculations both on the concentrations and degradation factors F_1 and F_2 were performed using the Kragten spreadsheet approach[32] and are also given in Table 2. The main source of uncertainty was the concentration of MBT, DBT and TBT in the triple spike solution and that explains why the uncertainties in the concentrations are very similar for all experiments. For factors F_1 and F_2 additional sources of uncertainty were found to be the measured isotope ratios in the blend, which had a typical reproducibility of 1%.

The concentrations found for MBT and DBT increased slightly, up to 4 min extraction being within the measurement uncertainty above this extraction time. For TBT, no significant increase was observed from 1 to 16 min. Moreover, it is demonstrated that, even when important degradation reactions are taking place, the proposed methodology allows the calculation of concentrations equivalent to those obtained under extraction conditions that do not promote any degradation. This is demonstrated by the comparatively low relative standard deviation (between 1.5 and 2.1%) that was obtained for the three species by averaging the results obtained under all tested conditions.

Conclusions

The triple spike methodology developed in this work allows the accurate determination of MBT, DBT and TBT concentrations plus six factors corresponding to possible rearrangement reactions (3 butylations and 3 debutylation reactions). Instability problems of the spike solution, reflected mainly in a degradation of DBT to MBT, have been overcome by increasing the acetic acid content up to 50%. The proposed methodology has been evaluated by analysing the three butyltin species in the certified reference material CRM 477 (Mussel Tissue) employing microwave assisted extraction under different conditions. The values obtained for MBT are slightly higher than the certified range while those for DBT and TBT overlap with the certified values under all extraction conditions even when important degradations were taking place (*e.g.*, 16 min of MW exposure). This confirms that the proposed triple spike methodology is able to correct for any interconversion reaction that may occur during the speciation analysis procedure of tin. Butylation reactions and the direct degradation of TBT to MBT were found to be negligible under all conditions, demonstrating the expected stepwise degradation of the butyltin compounds. Moreover, information on the extent of degradation reactions occurring at any experimental conditions used during the solid–liquid extraction of the sought species is easily provided.

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