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Determination of Organometallic Compounds Using Species Specific Isotope Dilution and GC-ICP-MS

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1. Introduction

Isotope dilution is used in speciation analysis to determine the concentration of specific chemical forms of an element, offering the possibility for correcting eventual loses or alteration of the species that can occur during the analytical process. Gas Chromatography (GC) is known as the strongest separation method used for separation of volatile and thermo stable organic compounds. Among the detection methods that are used, inductively coupled plasma - mass spectrometry (ICP-MS) is marked as the detector of choice for organometallic compounds. The coupling of GC with ICP-MS is extended by making use of the capability of ICP-MS to detect several isotopes simultaneously. Isotope dilution (ID) which was originally used in organic mass spectrometry is now extensively used in speciation analysis of inorganic compounds. This requires that the analyte of interest has more than one stable isotope. Chemical forms of mono-isotopic elements such as Arsenic (m/z 75) can therefore not be determined using isotope dilution. Other elements such as lead (4 isotopes), tin (10 isotopes), mercury (7 isotopes) and chromium (4 isotopes) have all been determined using isotope dilution [1]. According to IUPAC (International Union of Pure and Allied Chemistry) definition speciation of an element is "*the distribution of that element amongst defined chemical species in a system*" and speciation analysis is "*analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample*". Information about the total concentration of an element is usually not enough for a satisfactory characterization of a sample. Inorganic tin is for example harmless compared to organotin compounds (OTCs). The total concentration of tin, determined in a sample contaminated with the more toxic OTCs, does therefore not give meaningful data for risk assessment, making speciation analysis of the organotin compounds very essential.

In ID a known amount of an isotopically enriched element is added to a sample material. A simple question that one may raise is: what does this addition of an isotopically enriched element lead to? To answer this question Mikael Berglund referred to a work done by an enthymologist named C.H.N Jackson back in 1933[2]. Jackson used fly dilution to study the density of tsetse flies in a region in the village Ujiji at Tanganyka lake in Africa [3]. Jackson released a 'synthetic'' Tsetse fly population, which consisted of marked Tsetse flies, into a natural Tsetse fly population. After complete mixing he caught a representative sample of Tsetse flies and counted the marked and unmarked flies. Knowing the total number of

marked and released flies in the sample he could calculate the number of unmarked flies in the region. In a similar fashion we can imagine of counting the number of blue balls (?) in container A in figure 1. To this we add a known amount of identical, red colored, balls from container B. After a thorough mixing we take a portion from the mixture M, and count how many of each ablue nd red balls we have in this portion {P}.

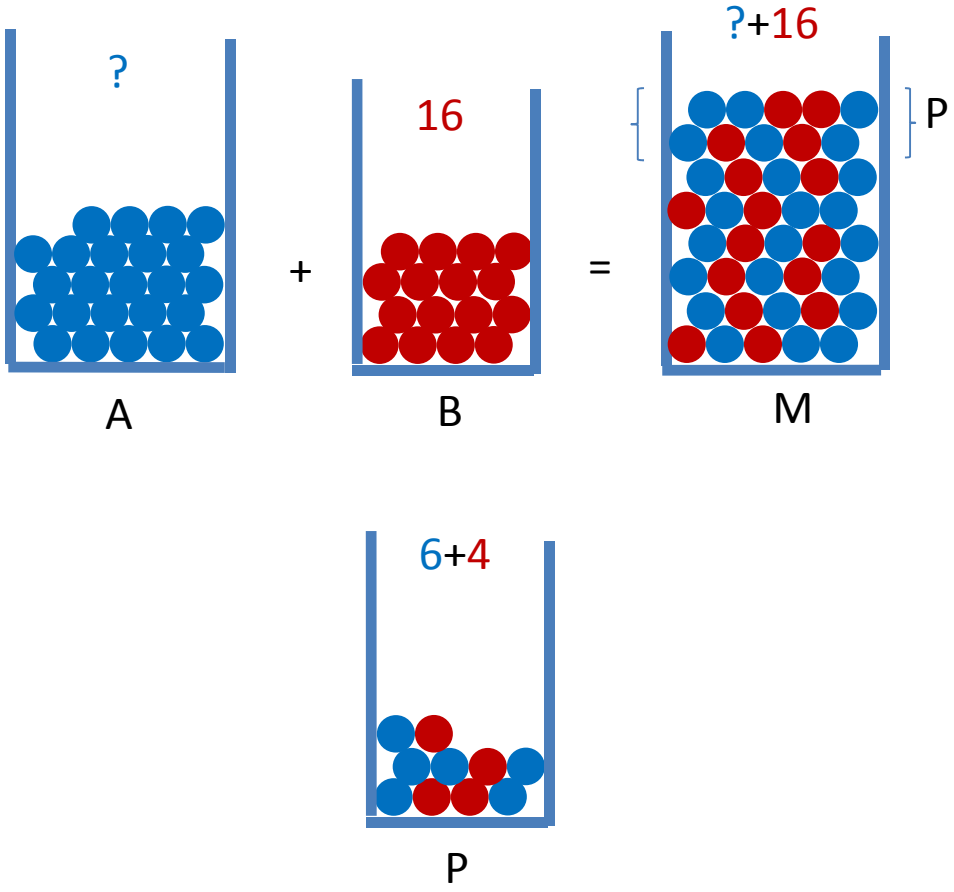

Fig. 1. A: container containing unknown number of blue balls; B: container containing known number of red balls; M: mixture of blue balls in A and red balls from B; P a portion of balls taken from the mixture M.

Using analogous equation to that used by Jackson back in 1933 we can calculate the number of blue bolls in M which also is equal to that we have in container A.

$$\frac{Number\ of\ red\ bolls\ in\ P}{Number\ of\ blue\ bolls\ in\ p} = \frac{Total\ number\ of\ red\ bolls\ in\ C}{Total\ number\ of\ blue\ bolls\ in\ C} \qquad (1)$$

$$\frac{4}{6} = \frac{16}{Total\ number\ of\ blue\ bolls\ in\ C}$$

Total number of blue bolls in M = 24 = number of blue bolls in container A.

This quantification principle, exemplified by the ball addition, is in fact the same as that used in isotope dilution (ID) where isotopically marked analyte (called spike) is added to a sample, containing a natural analyte.

In ID a known amount of enriched isotope (called the "spike") is added to a sample. After equilibration of the spike isotope with the natural isotope in the sample the isotopic ratio is measured. Normally a mass spectrometer is used to measure the altered isotopic ratio(s). The measured isotope ratio of isotope A to isotope B, R_m, can be calculated using equation 1.

$$R_m = \frac{A_x C_x + A_s C_s W_s}{B_x C_x W_x + B_s C_s W_s} \quad (2)$$

where A_x and B_x are the atom fractions of isotopes A and B in the sample; A_s and B_s are the atom fractions of isotopes A and B in the spike; C_x and C_s, are the concentrations of the analyte in the sample and spike, respectively; and W_x and W_s are the weights of the sample and spike, respectively. The concentration of the analyte in the sample can then be calculated using equation 2.

$$C_x = \left(\frac{C_s W_s}{W_x}\right)\left(\frac{A_s - R_m B_s}{R_m B_x - A_x}\right) \quad (3)$$

It should however be noted that depending on the analytical method at hand, the stability of the species under investigation, and the need for addressing the resulting uncertainty in the analytical result the mathematical expression used for calculation of species concentration can look different and get more complicated [2, 4, 5]. In the forthcoming sections speciation analysis of organtin compounds will be discussed. Organotin compounds are one of the most investigated organometallic compounds, using isotope dilution GC-ICPMS, and will therefore fit perfect in this chapter for elaborating the analytical methodology and explain why isotope dilution is needed for speciation analysis.

2. Speciation of organotin compounds (OTCs)

Tin in its inorganic form is generally accepted as being non-toxic, but the toxicological pattern of organotin compounds is very complex. The biological effects of the substances depend on both the nature and the number of the organic groups bound to the Sn cation. Figure 2 shows the ionic form of the most extensively studied types of organotin compounds.

The ecotoxicological effects of organotin compounds (OTC), mainly tributyltin (TBT) and triphenyltin (TPhT) but also their di- and monosubstituted degradation products are well documented. Nowadays, the release of TBT from antifouling paints is recognized worldwide as being one of the main contamination problems for the marine environment, and the use of TBT-based antifouling paints is almost everywhere restricted by law. In order to evaluate the environmental distribution and fate of these compounds, and to control the effectiveness of these legal provisions, many analytical methods have been developed among which gas chromatography coupled to inductively coupled plasma mass spectrometry (GC-ICP-MS) is the most powerful.

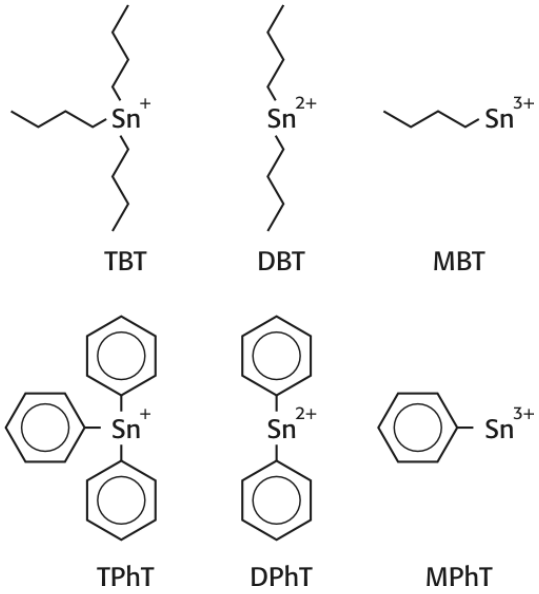

Fig. 2. The ionic form of butyltins and phenyltins of varying substitution.

TBT-based paints are used on vessels hulls to prevent growth of aquatic organisms that create roughness giving rise to increased drag, resulting in reduced vessel speed per unit energy consumption. The antifouling paint consists of a film-forming material with a biocidal ingredient and a pigment. It works by releasing small amounts of the biocide from the painted hull into the water, forming a thin envelope of highly concentrated TBT around the boat. The toxic concentration repels the settling stages of fouling organisms, like barnacles, seaweeds, or tubeworms on the boat's water-immersed surfaces [6].

However, the constant release of OTC from anti-fouling paints has led to toxic effects for nontarget aquatic species in the aquatic environment, where they cause deleterious effects, such as shell anomalies in oysters and imposex in gastropods, even at concentrations as low as nanograms per liter. Despite the restrictions organotin compounds (mainly TBT) are still used in paint formulation for large vessels, and about 69 % of all large ships are reported to use them [7]. Once released into the aquatic environment, organotin compounds may undergo a variety of degradation reactions until they are finally adsorbed onto suspended solids and sediments. Sediments are considered to be the ultimate sinks for organotin compounds [8].

In water, TBT decomposes into less toxic DBT and MBT species. The problem is that this favourable decomposition takes place far more slowly in sediments, creating an ecotoxicological risk long after its release into a given area, making sediments a secondary source of pollution. TBT and its degradation products DBT and MBT have been detected in different environmental compartments, both marine (waters, sediments, and biota) and terrestrial (waters and soils). The occurrences of the less toxic MBT and DBT compounds in the environment have so far been related to the degradation of TBT caused by microbial

activity and/or photochemical reactions, but recently evidence for direct input of MBT and DBT was found. In fact the major application of organotin compounds (about 70 %) is the use of mono and dialkyltin derivatives as heat and light stabilizer additives in PVC processing [6].

3. Speciaition analysis of OTCs

Several techniques, based on species specific analytical methods, have been developed for the determination of butyltins in environmental matrices. Hyphenated systems, based on on-line coupling of gas chromatography (GC), liquid chromatography (LC), or supercritical fluid chromatography (SFC), to mass spectrometry (MS), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), and microwave induced plasma atomic emission spectrometry (MIP-AES) are in current use [7]. Among the different techniques, the coupling of GC to ICP-MS appears to be one of the most popular techniques, due to high sensitivity and multi-elemental and multi-isotopic capabilities [9]. Some of the most frequently used sample pretreatment methods for the determination of OTC, in various samples, are summarized in Table 1.

The coupling of chromatography with ICP-MS and application of calibration methods that are based on ID permits detection of several compounds. ID is a technique based on isotope ratio measurement whereby the natural isotopic abundance ratio of an analyte is altered by spiking with a standard that has a different isotopic abundance ratio. The prerequisite for the technique is that the analyte of interest should have more than one stable isotope [1]. In the case of tin (10 isotopes) the isotope of highest abundance is ^{120}Sn, usually referred as the reference isotope, and the spike isotope is generally one of the less abundant natural isotopes. For the purposes of speciation analysis, where OTCs are to be determined, there is a requirement for the isotopically enriched element-species to be synthesized. If two interference-free isotopes of a given element can be found, isotope dilution ICP-MS (ID-ICP-MS) can be performed, which generally provides superior accuracy and precision over other calibration strategies, including external calibration and the method of standard addition, because a ratio rather than an absolute intensity measurement is used in the quantification of the analyte concentration. Once equilibration is achieved between the analyte in the sample and the added spike, ID-ICP-MS 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-ICP-MS may therefore be considered as a primary method of analysis and can play a crucial role for quality assurance in trace element chemical speciation of environmental and biological samples. ID-ICP-MS coupled with gas chromatography (GC) ID-GC-ICP-MS [9] and to less extent liquid chromatography (LC) ID-LC-ICP-MS [1] are the techniques that are used for speciation of organotin compounds. The derivatisation step is not needed in the case of LC (omitting one source of error), but the coupling with GC is superior when it comes to analysing samples of very low concentrations (pg/g) of OTC.

The presently available techniques for the determination of OTCs involve several analytical steps such as extraction, pre-concentration, cleanup, derivatisation (when gas chromatography is used), separation, and finally detection by element- or molecule specific techniques such as ICP-MS. The multitude of analytical steps is causing errors at various levels, making speciation analysis a difficult task.

4. Derivatization of OTCs for gas chromatographic separation

In order to enable separation by GC, the ionic organotin compounds need to be converted into volatile species such as the hydrides, or their fully alkylated form (derivatisation). For the reduction to hydrides, sodium tetrahydroborate ($NaBH_4$) is commonly used, whereas derivatisation through alkylation can be carried out with Grignard reagents, sodium tetraethylborate ($NaBEt_4$), or sodium tetra (n-propyl) borate ($NaBPr_4$). Derivatisation with $NaBEt_4$ makes the sample preparation faster and easier because it combines an *in situ* derivatisation with extraction of the ethylated organotin compounds into an organic phase [1].

Sample type	Sample pretreatment	Compound studied (amount found (ng))	Separation and detection technique	Ref
Marine sediment from contaminated harbour area in Mar piccolo (Italy)	Add 3 mL HCl and 6 mL methanol to 1 g of sample. Shake and sonicate for 15 min in ultrasonic bath. Add 3 mL of acetate buffer (pH 5.3) and centrifuge the leached sediment at 4000 rpm for 15 min. Pipette 1 mL supernatant into a 15 mL glass vial and add 6 mL of HOAc/NaOAc buffer (pH 5.3). Close the vial with a septum and add 1 mL of $NaBEt_4$ solution with a syringe. Sonicate the reaction mixture and pierce the SPME needle into the septum and expose the fiber into the headspace.	MBT = 8 DBT = 10 TBT = 1	Headspace micro-extraction GC/MS	28
Sediment samples Samples 1 and 2 from the harbour of Ostend, sample 3 from a dry dock in the harbour of Antwerp, and sample 4 from a leisure craft maintenance place located in the province of Limburg, all in Belgium	Add 2 ml of HCl (32 %) and 8 ml H_2O to 1 g sample in a centrifugation vessel. Add 25 ml of hexane-ethyl acetate mixture (1:1) containning 0.05 % tropolone. Sonicate the mixture for 1 h, followed by centrifugation at 3000 rpm for 5 min. Transfer the organic phase into an extraction vessel and evaporate to dryness using rotary evaporation. Add 0.5 zml of hexane containing Pe_3SnEt as an internal standard and derivatize by adding 1 ml of $NaBEt_4$ solution together with 50 ml of acetate buffer solution. Shake the mixture manually for 5 min	**Sample 1** MBT= 0.14 ± 0.02 DBT= 0.44 ± 0.03 TBT= 0.14 ± 0.02 **Sample 2** MBT= 0.36 ± 0.02 DBT= 1.11 ± 0.12 TBT= 2.33 ± 0.14 **Sample 3** MBT= 8.13 ± 0.33 DBT= 10.0 ± 0.6 TBT= 26.4 ± 1.8 **Sample 4**	GC interfaced with AAS and AES	14

Sample type	Sample pretreatment	Compound studied (amount found (ng))	Separation and detection technique	Ref
	and seperate the hexane phase. Introduce the extract into a clean-up column (a pasteur pipette filled with alumina to form a plug of 5 cm). Add an additional volume of 1 ml diethyl ether and evaporate the added diethyl ether using a gentle stream of nitrogen.	MBT= 1.55 ± 0.09 DBT= 1.67 ± 0.20 TBT= 6.60 ± 0.18		
Sediment samples Two samples (1&2) from the harbour of Ostend (Belgium).	Add 4 ml H_2O, 1 ml acetic acid (96 %), 1 ml DDTC in pentane, and 25 ml hexane into a 100 ml Erlenmeyer flask containing 1 g sample. Sonicate the mixture for 30 min and decant the organic phase into a 100 ml beaker. Repeat the extraction with 25 ml of hexane and stir magnetically for 30 min. Centrifuge the mixture for 5 min at 3000 rpm. Dry over Na_2SO_4 and evaporate to dryness on a rotary evaporator. Add 250 µl of n-octane, containing Pr_3SnPe and pentylate with 1 ml of 1 M n-PeMgBr. Destroy excess Grignard reagent by adding 10 ml of 0.5 M H_2SO_4. Introduce the octane layer into a clean-up column (a pasteur pipette filled with alumina to form a plug of 5 cm). Add an additional volume of 1 ml diethyl ether and evaporate the added diethyl ether using a gentle stream of nitrogen.	**Sample 1** DBT= 0.43 ± 0.02 TBT= 0.31 ± 0.03 **Sample 2** DBT= 1.39 ± 0.06 TBT= 2.67 ± 0.08	GC interfaced with AAS and AES	14
Water 1. Sea water from Sahrm el Sheikh harbour in South Sinai (Egypt) 2. Harbour water from Wädenswil, Lake Zurich (Switzerland)	Add 0.5 mL of acetic acid/acetate buffer solution (pH 5) and 1.45 g of sodium chloride to 50 mL water sample. After shaking spike with 100 µl of deuterated standard solution mixture (12.5 ng/L of each species in MeOH). Shake and add 150 µl of 1.5 % (w/v) $NaBEt_4$ aqueous solution. Add 1 ml of hexane and shake in the dark for	**1. Sahrm el Sheikh** MBT = 3.4 ± 7.6 DBT = 2.1 ± 24 TBT = 2.6 ± 17 MPhT= 1.5 ± 6.7 DPhT = 0.5 ± 76 TPhT = 4.8 ± 2.1 **2. Wädenswil** MBT = nd	LLE, Large volume injection GC/MS	29

Sample type	Sample pretreatment	Compound studied (amount found (ng))	Separation and detection technique	Ref
	12 h at 25 ºC. Transfer 180 µl of the hexane extract to a 1 mL auto-sampler vial and spike with 10 µl of 0.2 ng/µl TeBT in hexane. Inject 50 µl in to the GC.	DBT = 3 ± 17 TBT = nd MPhT = 37 ± 3 DPhT = 23 ± 6 TPhT = 353 ± 3		
Sediment Certified reference material PACS-2 (0.98 ± 0.13 TBT ng/L)	Put 0.5 g of PACS-2 in a Prolabo microwave digester and spike with 0.04 mL of ^{117}Sn-enriched TBT solution. Add 5 mL of acetic acid and heat at 60 % power for 3 min. Centrifuge at 2000 rpm for 5 min. Transfer 1 mL volume of the supernatant to a reaction vial and add 1 mL of deionized water. Adjust to pH 5-6 with 1.2 mL ammonium hydroxide. Buffer the content with 0.8 mL of ammonium citrate (2 mol L^{-1}) and dilute to 10 mL with methanol.	TBT = 1.018 ± 0.0315	Microwave extraction, ID-HPLC-ICP-MS	30
Sediment/Sludge 1. Sediment pore water from (1-20 cm) from Stansstaad harbour, Lake Lucerne (Switzerland) 2. Sewage sludge from four wastewater treat-ment plants in the Zurich canton (Switzerland)	Weigh 2.5 g freeze dried sediment or sludge in a beaker. Spike homogeneously with 500 µl of deuterated standard solution mixture (12.5 ng/L of each species in MeOH). Mix with 9 g of quartz sand and transfer the mixture to 11 mL extraction cells. After two hours fill the extraction cells with a mixture of 1 M sodium acetate and 1 M acetic acid in MeOH, using ASE. Extract with three to five static cycles of 5 min. Renew 4 mL of solvent between each static extraction. Rinse the cells with 4 mL of solvent and purge with nitrogen. Transfer the combined extracts to 250 mL volumetric flasks containing 7.3 g of NaCl. Add water and adjust the pH to 5 with 1 M NaOH. Add 1 mL of aqueous solution of 5 % (w/v) $NaBEt_4$ and fill the bottles to 250 mL with water. Add 2	1) Sediment pore water MBT = 11.0± 1.9 DBT = 4.5 ± 4.7 TBT = 9.6 ± 4.4 MPhT = 12.3 ± 4 DPhT = 2.5 ± 14 TPhT = 4.1± 3 2) Sewage sludge MBT = 300 ± 4 DBT = 253 ± 5 TBT = 45 ± 5 MPhT = 7 ± 21 DPhT = nd TPhT = 7 ± 38	ASE, Large volume injection GC/MS	29

Sample type	Sample pretreatment	Compound studied (amount found (ng))	Separation and detection technique	Ref
	mL of hexane and shake for 12 h. Transfer 500 µl of the hexane extract to 2 mL GC vials and spike with 10 µl of TeBT (5 ng/µl). For sewage sludge transfer the hexane extract to 10 mL centrifuge tubes containing 0.9 g of deactivated silica gel and 2 mL water. Shake vigorously and centrifuge.			

Table 1. Some of the most commonly used methods for the determination of OTCs in various samples along with the analysis results obtained.

5. Species Specific Isotope Dilution (SSID)

In the last few years different procedures for the speciation of organotin compounds have been proposed, among which species specific isotope dilution (SSID) is one [10-11]. The use of isotope enriched spike solutions has not only the potential for accurate, precise and simultaneous determination of OTCs but also the evaluation of different extraction and derivatization protocols for the analysis of sediments and biological sample in which species interconversion/decomposition can take place during sample preparation. Organotin compounds synthesized from isotopically enriched tin metals are used for preparing spikes that can be used for the determination of concentrations using species specific isotope dilution (SSID). Different types of calibration strategies based on SSID have been used for the determination of organotin compounds:

i. Single isotope - species specific isotope dilution (SI-SSID): where a mixture containing organotin species, all with the same isotope, is used as a spike. For example: a mixture containing ^{116}Sn-enriched MBT, ^{116}Sn-enriched DBT, and ^{116}Sn-enriched TBT is used for the determination of butyltins.
ii. Multiple isotope - species specific isotope dilution (MI-SSID): where a mixture containing the organotin species, each with different isotope, is used as a spike. For example: a mixture containing ^{119}Sn-enriched MBT, ^{118}Sn-enriched DBT, and ^{117}Sn-enriched TBT is used for the determination of butyltins.

Single isotope species specific isotope dilution (SI-SSID) has been used during the last fifteen years for speciation analysis of bromine [12], chromium [13], iodine [14], selenium [15,16], mercury [17] and tin [18-20]. Recently multi-isotope species specific isotope dilution (MI–SSID) has been used to monitor and correct for the degradation/transformation that takes place during sample preparation [21-23]. The use of multi isotope spike can also be extended to compare different extraction procedures and study the inherent procedural parameters that govern the process of degradation/transformation. If these problems are fully understood and addressed, appropriate strategies can be set to facilitate accurate speciation analysis of the compounds of interest in various types of samples.

One of the drawbacks with SI-SSID is the difficulty to assess the degradation/redistribution of the OTs that can take place during sample workup. Another problem is the inability to match the concentrations of the organotin compounds in the spike with those present in the sample, thus affecting the accuracy and precision of the results [18, 24-26]. The latter problem can however be circumvented by using MI-SSID [5, 23].

Owing to the problems with degradation of phenyltin species during the sample pretreatment steps, poor precisions within and large spreads between laboratory results were obtained when certifying organotin compounds in the BCR CRM-477. As a consequence, only indicative values were given for phenyltin species [27]. Figure 3 shows deconvoluted chromatograms obtained for the multi-isotope standard where no peaks, corresponding to redistribution products of the enriched phenyltin standard, are visible. This indicates that the integrity of the phenyltin species is preserved during the ethylation and the detection procedure.

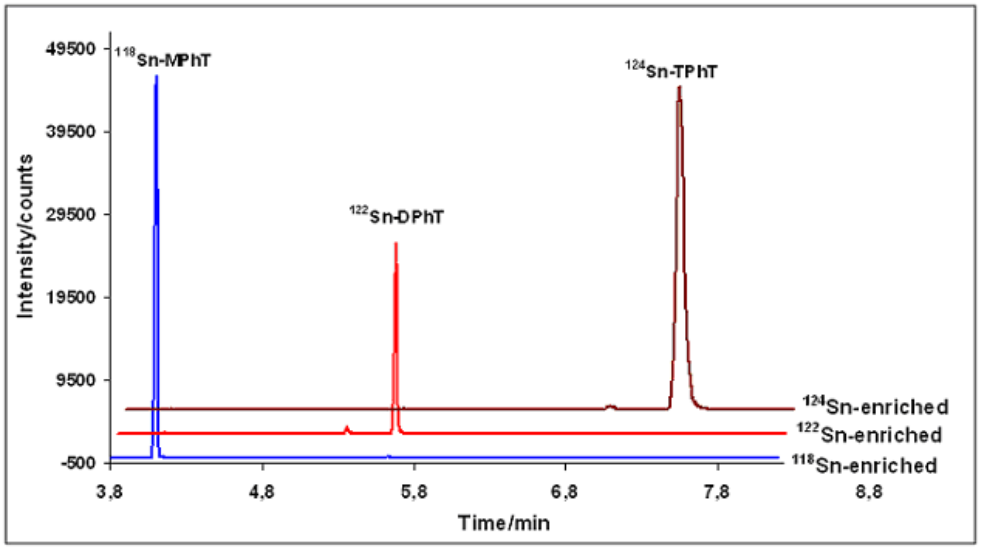

Fig. 3. Deconvoluted GC-ICPMS sub-chromatograms obtained for a mixture of ^{118}Sn-enriched MPhT (106.5 ng g^{-1}), ^{122}Sn-enriched DPhT (57.4 ng g^{-1}) and ^{124}Sn-enriched TPhT (239.0 ng g^{-1}). The chromatograms are shifted from each other by 0.1 minute and 3000 counts for clarity. [*From Van D.N., Muppala S.R.K., Frech W., Tesfalidet S., Preparation, preservation and application of pure isotope-enriched phenyltin species Anal. Bioanal. Chem., 2006, 386, 1505*]

The presently available techniques for the determination of OTCs involve several analytical steps such as extraction, pre-concentration, cleanup, derivatisation (when gas chromatography is used), separation, and finally detection by element- or molecule specific techniques such as ICP-MS. The multitude of analytical steps is causing errors at various levels, making speciation analysis a difficult task.

In the determination of PhTs, species transformation/interconversion is one of the most serious obstacles which affects the reliability of the analytical results. Using MI-SSID, one can assess the degradation/transformation processes, enabling calculation of degradation-corrected concentrations for the species of interest. Possible degradation/transformation pathways of PhTs are depicted in Fig. 4

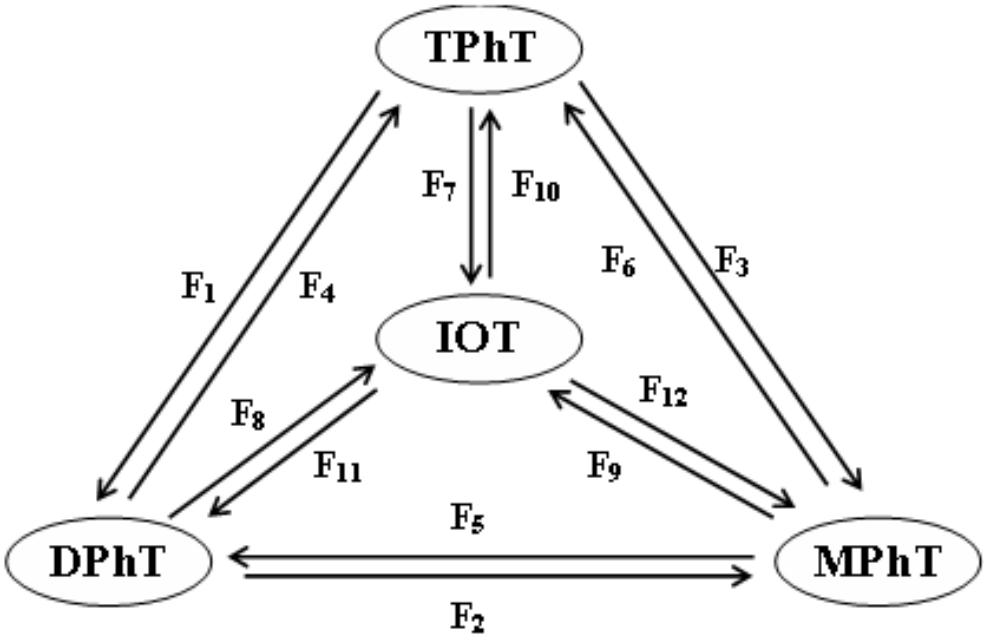

Fig. 4. Possible interconversion pathways of inorganic tin (IOT) and PhTs. F_i is interconversion factor corresponding to the interconversion reaction i. TPhT triphenyltin, DPhT diphenyltin, MPhT monophenyltin. [*From Van D.N., Bui T.T.X., Tesfalidet S. "The transformation of phenyltin species during sample preparation of biological tissues using multi-isotope spike SSID-GC-ICPMS, Anal. Bioanal. Chem 2008, 392, 737*]

6. Instrumentation and operating conditions for the GC and ICP-MS

A Varian 3300 gas chromatograph (Palo Alto, CA, USA) fitted with an on-column injector liner and a methyl silicone capillary column (30 m by 0.53 mm i.d., 1.5 μ film thickness; SPB-1, Supelco, Bellafonte, PA) was used for separation of Sn species and an Agilent 7500 ICP-MS (Foster City, CA) was used for detection. The GC was coupled to the ICP-MS *via* a custom made interface. The operating parameters of the ICP-MS were selected by optimizing the sensitivity for ^{129}Xe, by adding Xe gas at 0.5 ml/min to the Ar carrier gas flow. Oxygen was added to the plasma to prevent carbon deposits on the sampler/skimmer Pt cones. The operating parameters of the GC and ICP-MS are given in Table 2.

GC parameters	
Injection volume	1 μL
Carrier helium gas flow	22 mL min^{-1}
Injector temperature	180 °C
Oven temperature	130 °C ⇧ 40 °C min^{-1} ⇧ 210 °C, hold 0.5 minutes 210 °C ⇧ 7 °C min^{-1} ⇧ 225 °C, hold 0.5 minutes 225 °C ⇧ 40 °C min^{-1} ⇧ 280 °C, hold 2.0 minutes
Transfer line temperature	200 °C
ICP-MS parameters	
ICP RF power	1200 W
Plasma argon gas flow	15 L min^{-1}
Nebulizer argon gas flow	1.0 L min^{-1}
Auxiliary argon gas flow	0.9 L min^{-1}
Auxiliary oxygen gas flow	3 mL min^{-1}
Sampler/Skimmer cones	Platinum
Dwell time	100 ms for $^{116}Sn^+$, $^{117}Sn^+$, $^{118}Sn^+$, $^{119}Sn^+$, $^{120}Sn^+$ and $^{124}Sn^+$

Table 2. Operating conditions for the GC and ICP-MS.

7. Determination of OTCs in biological samples

Various extraction procedures have been used for the analysis of organotin compounds in biological samples. Pellegrino et al. [31] compared twelve selected extraction methods to evaluate the extraction efficiencies obtained for a certified reference material (CRM). The organic solvent, the nature and concentration of the acid used for leaching, and the presence/absence of tropolone used as complexing agent, were all found to influence the grade of transformation that takes place during extraction [32]. Although the performance of the extraction procedures can be validated by recovery tests using certified reference materials (CRMs) or fortified samples, the validity of these procedures on real samples cannot be guaranteed [18,33]. This is because the adsorption/binding forces of the species of interest to the solid are strongly dependent on the matrix [10]. A mild extraction technique could, for example, give rise to incomplete extraction of organotin species from the solid sample while a harsher extraction technique facilitates the degradation/rearrangement of the species of interest [32,34,35]. In both cases, the results of speciation analysis will fail to reflect the real speciation in the solid sample. As a result, it has yet not been able to certify the concentration of phenyltins in the reference material BCR CRM-477 and only indicative values are available [36].

8. Analysis of PhTs in mussel tissue

When analyzing PhTs in certified reference material, mussel tissue, BCR CRM-477, 8.7±0.9% of TPhT was converted to DPhT and 9.0±0.2% of DPhT to MPhT. Other dephenylation/phenylation products were not observed. Peaks indicating the degradation of ^{124}Sn-enriched TPhT and ^{122}Sn-enriched DPhT are shown in Fig. 5 by signals for ^{124}Sn-enriched DPhT and ^{122}Sn-enriched MPhT [5].

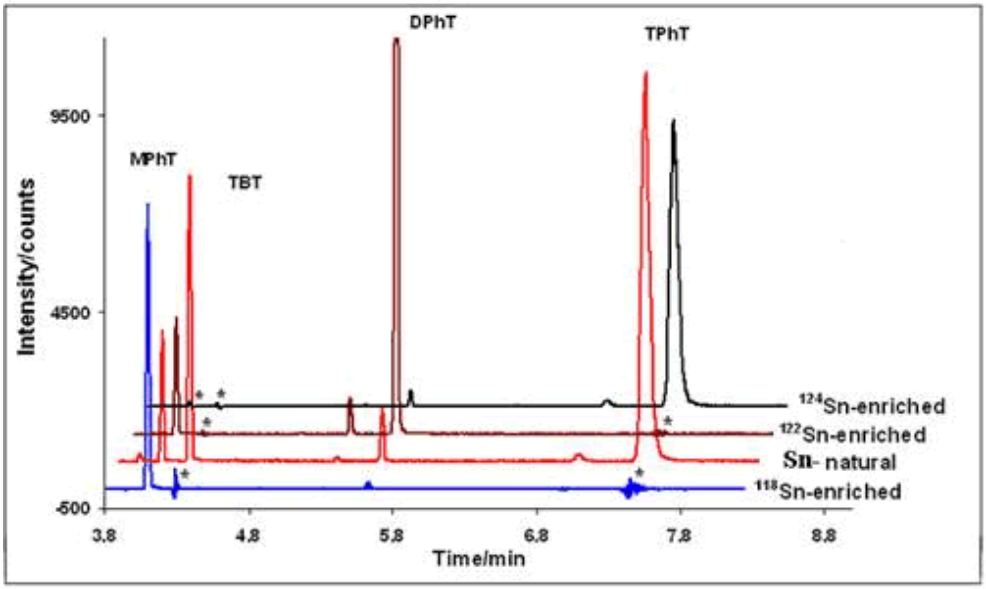

Fig. 5. Deconvoluted GC-ICPMS sub-chromatograms obtained for 0.2g BCR CRM-477 spiked with 0.14g ^{118}Sn-enriched MPhT (90.2 ng g^{-1}), ^{122}Sn-enriched DPhT (367 ng g^{-1}) and ^{124}Sn-enriched TPhT (315 ng g^{-1}). (*) is the fluctuation of the baseline due to deconvolution. The chromatograms are shifted from each other by 0.1 minute and 700 counts for clarity. [*From Van D.N., Muppala S.R.K., Frech W., Tesfalidet S., Preparation, preservation and application of pure isotope-enriched phenyltin species Anal. Bioanal. Chem., 2006, 386, 1505*]

8.1 Equations for calculating the inter-conversion factors and concentrations of PhT species

Various equations have been proposed for the calculation of concentrations in isotope dilution [17, 37-38]. The system of equations used for calculating the inter-conversion factors and concentrations of PhT species presented here are similar to those presented by Rodriguez-Gonzalez et.al. [37] and Nguyen D.N. et.al [5].

A deconvoluted chromatogram of the type shown in figure 5 visualizes how each added isotope-enriched species is distributed between the PhT species. Deconvolution is performed for each species by solving the system of four linear equations (Eq. 4) in an Excel spreadsheet:

$$\begin{aligned}
\sum S_{118} &= R_{118/118}.S_{118} + R_{118/120}.S_{120} + R_{118/122}.S_{122} + R_{118/124}.S_{124} \\
\sum S_{120} &= R_{120/118}.S_{118} + R_{120/120}.S_{120} + R_{120/122}.S_{122} + R_{120/124}.S_{124} \\
\sum S_{122} &= R_{122/118}.S_{118} + R_{122/120}.S_{120} + R_{122/122}.S_{122} + R_{122/124}.S_{124} \\
\sum S_{124} &= R_{124/118}.S_{118} + R_{124/120}.S_{120} + R_{124/122}.S_{122} + R_{124/124}.S_{124}
\end{aligned} \tag{4}$$

To determine the degree of inter-conversion between PhTs, the isotope ratios 120/118, 120/122 and 120/124 for each PhTs were calculated. The following inter-conversion factors were considered: F_1; TPhT degradation to DPhT, F_2; DPhT to MPhT and F_3; TPhT to MPhT F_4; DPhT phenylation to TPhT, F_5; MPhT to DPhT and F_6; MPhT to TPhT. The calculations of two inter-conversion factors related to the formation of TPhT (F_4 and F_6) are exemplified in equations 5&6. Once all the inter-conversion factors are calculated, mass balance equations for each species can be established and used for computing concentrations of each species in the sample. For example, the third row in the matrix (equation 8) is obtained by rearranging Equation 4, the mass balance of TPhT.

The symbols used in the equations represent the following: N_s^{MPhT}, N_s^{DPhT} and N_s^{TPhT} are the number of moles of each species present in the sample; N_{sp}^{X} is the number of moles of the PhTs X (X = MPhT, DPhT or TPhT) spiked into the sample; At_s^i is the abundance of tin isotope $\boldsymbol{i}$ ($\boldsymbol{i}$ = 118, 120, 122 or 124) in the sample; $At_{i,sp}^{MPhT}$, $At_{i,sp}^{DPhT}$ and $At_{i,sp}^{TPhT}$ are the abundance of tin isotope $\boldsymbol{i}$ in the spiked ^{118}Sn-enriched MPhT, ^{122}Sn-enriched DPhT and ^{124}Sn-enriched TPhT, respectively. $N_{124,m}^{X}$, $N_{122,m}^{X}$, $N_{120,m}^{X}$ and $N_{118,m}^{X}$ are the number of moles of the corresponding isotopic PhTs X in the blend; $R_{j/k,m}^{X}$ is the tin isotope ratio of the reference isotope $\boldsymbol{j}$ ($\boldsymbol{j}$ = 120) to the spiked isotope $\boldsymbol{k}$ ($\boldsymbol{k}$ = 118, 122, 124) measured by GC-ICPMS for the phenyltin **X** in the blend after mass bias correction.

$$
\begin{aligned}
&N_{sp}^{TPhT}\left(\frac{At_{120,sp}^{TPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{TPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}-\frac{At_{120,sp}^{TPhT}-R_{120/122,m}^{TPhT}At_{122,sp}^{TPhT}}{R_{120/122,m}^{TPhT}At_{122,s}-At_{120,s}}\right)\\
&+F_4N_{sp}^{DPhT}\left(\frac{At_{120,sp}^{DPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{DPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}-\frac{At_{120,sp}^{DPhT}-R_{120/122,m}^{TPhT}At_{122,sp}^{DPhT}}{R_{120/122,m}^{TPhT}At_{122,s}-At_{120,s}}\right)\\
&+F_6N_{sp}^{MPhT}\left(\frac{At_{120,sp}^{MPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{MPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}-\frac{At_{120,sp}^{MPhT}-R_{120/122,m}^{TPhT}At_{122,sp}^{MPhT}}{R_{120/122,m}^{TPhT}At_{122,s}-At_{120,s}}\right)=0
\end{aligned}
\tag{5}
$$

$$
\begin{aligned}
&N_{sp}^{TPhT}\left(\frac{At_{120,sp}^{TPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{TPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}-\frac{At_{120,sp}^{TPhT}-R_{120/118,m}^{TPhT}At_{118,sp}^{TPhT}}{R_{120/118,m}^{TPhT}At_{118,s}-At_{120,s}}\right)\\
&+F_4N_{sp}^{DPhT}\left(\frac{At_{120,sp}^{DPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{DPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}-\frac{At_{122,sp}^{DPhT}-R_{120/118,m}^{TPhT}At_{118,sp}^{DPhT}}{R_{120/118,m}^{TPhT}At_{118,s}-At_{120,s}}\right)\\
&+F_6N_{sp}^{MPhT}\left(\frac{At_{120,sp}^{MPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{MPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}-\frac{At_{120,sp}^{MPhT}-R_{120/118,m}^{TPhT}At_{118,sp}^{MPhT}}{R_{120/118,m}^{TPhT}At_{118,s}-At_{120,s}}\right)=0
\end{aligned}
\tag{6}
$$

$$
\begin{aligned}
&F_6N_s^{MPhT}+F_4N_s^{DPhT}+N_s^{TPhT}=N_{sp}^{TPhT}\frac{At_{120,sp}^{TPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{TPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}\\
&+F_4N_{sp}^{DPhT}\frac{At_{120,sp}^{DPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{DPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}+F_6N_{sp}^{MPhT}\frac{At_{120,sp}^{MPhT}-R_{120/124,m}^{TPhT}At_{124,sp}^{MPhT}}{R_{120/124,m}^{TPhT}At_{124,s}-At_{120,s}}
\end{aligned}
\tag{7}
$$

$$\begin{pmatrix} 1 & F_2 & F_3 \\ F_5 & 1 & F_1 \\ F_6 & F_4 & 1 \end{pmatrix} \begin{pmatrix} N_s^{MPhT} \\ N_s^{DPhT} \\ N_s^{TPhT} \end{pmatrix} = \begin{pmatrix} X_{MPhT} \\ Y_{DPhT} \\ Z_{TPhT} \end{pmatrix} \quad (8)$$

Solving Matrix 8 by setting the F values (both on the left side and even included in X_{MPhT}, Y_{DPhT}, and Z_{TPhT}) equal to zero will correspond to degradation-uncorrected concentrations of the phenyltins.

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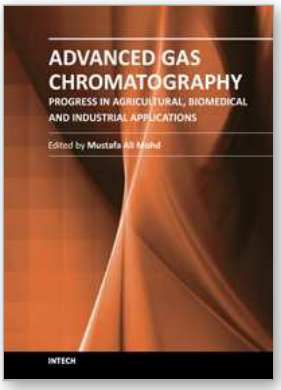

Advanced Gas Chromatography - Progress in Agricultural, Biomedical and Industrial Applications

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Progress in agricultural, biomedical and industrial applications' is a compilation of recent advances and developments in gas chromatography and its applications. The chapters cover various aspects of applications ranging from basic biological, biomedical applications to industrial applications. Book chapters analyze new developments in chromatographic columns, microextraction techniques, derivatisation techniques and pyrolysis techniques. The book also includes several aspects of basic chromatography techniques and is suitable for both young and advanced chromatographers. It includes some new developments in chromatography such as multidimensional chromatography, inverse chromatography and some discussions on two-dimensional chromatography. The topics covered include analysis of volatiles, toxicants, indoor air, petroleum hydrocarbons, organometallic compounds and natural products. The chapters were written by experts from various fields and clearly assisted by simple diagrams and tables. This book is highly recommended for chemists as well as non-chemists working in gas chromatography.

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