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**DEVELOPMENT OF ACCURATE AND PRECISE METHODS FOR
THE DETERMINATION OF BUTYLTIN SPECIES IN SEDIMENTS
USING HPLC AND GC SEPARATION IN COMBINATION WITH
MASS SPECTROMETRIC DETECTION**

By

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Doctor of Philosophy

The Department of Chemistry

Carleton University

Ottawa, ON, Canada

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ABSTRACT

Several analytical approaches for the determination of butyltin (BT) species in sediments have been developed. An initial method, based on HPLC separation, utilized a strong cation-exchange column and an ammonium citrate buffer in a 60:40 methanol-water mixture as mobile phase in combination with ICP-MS detection. Separation of monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT) could be achieved in 8 minutes with estimated detection limits [3 times the standard deviation (SD) of sample blanks] of 0.08, 0.05 and 0.03 $\mu\text{g g}^{-1}$ for MBT, DBT and TBT, respectively, normalized to a 0.5 g sub-sample. This approach was used to investigate microwave assisted extraction procedures for determination of BT in PACS-2 sediment certified reference material (CRM), as well as an initial stability study of butyltins in this sediment.

A GC-ICP-MS method was subsequently developed with which chromatographic separation of MBT, DBT and TBT could be achieved in 10 minutes with method detection limits of 0.006, 0.001 and 0.002 $\mu\text{g g}^{-1}$ for MBT, DBT and TBT, respectively, based on a 0.5 g sub-sample. In addition to the slightly better precisions obtained in measured BT concentrations, resolution and sensitivity achieved for all BT peaks by GC-ICP-MS were far better than could be obtained using HPLC-ICP-MS. The GC-ICP-MS method was used to study the effects of γ -sterilization on homogeneity of BTs and their content in candidate sediment CRMs, as well as their stability in PACS-2 sediment.

Since the accuracy of the natural abundance BT standards is critical for obtaining reliable results, a unique approach, using hydride generation (HG) GC-MS, was developed to quantify any inorganic Sn, MBT, DBT and TBT impurities in these BT calibration solutions.

The true natural abundance BT concentrations were obtained following correction for these impurities.

To achieve accurate and precise results, isotope dilution (ID) calibration was applied to the determination of BTs in PACS-2. Significant improvements (close to 4-fold) in precision of measurement of TBT using a ^{117}Sn -enriched TBT spike were obtained with both HPLC- and GC-ICP-MS methods, clearly demonstrating the superiority of ID as compared to the method of standard additions. A performance comparison was also made between a sector field and quadrupole ICP-MS instrument used for these measurements.

In an effort to simplify sample preparation for GC separation, solid phase microextraction (SPME) was investigated for determination of TBT in PACS-2. To overcome poor precision (typically 10 % RSD) associated with SPME, species specific ID was investigated. An eighteen-fold improvement in the precision of TBT concentration measured using ID with SPME GC ICP-MS, in comparison to standard additions calibration, was obtained. A detection limit (3 SD) of 0.09 ng g^{-1} was estimated for TBT in PACS-2 sediment, superior to that obtained by either HPLC- or GC-ICP-MS methods.

As a result of long-term stability study of BTs in PACS-2, its recertification is currently underway. An alternative ID GC-MS method was also developed for quantitation of BTs in PACS-2. Results obtained with this approach are in good agreement with those obtained by ID GC-ICP-MS.

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List of Abbreviations

Atomic Absorption Spectrometry	AAS
Atomic Emission Spectrometry	AES
Butyltin	BT
Certified Reference Material	CRM
Counts Per Second	cps
De-ionized Water	DIW
Dibutyltin	DBT
Electrostatic Analyzer	ESA
Flame Photometric Detection	FPD
Full Width of Half Maximum	FWHM
Gas Chromatography	GC
Gas Chromatography Mass Spectrometry	GC-MS
Graphite Furnace Atomic Absorption Spectrometry	GFAAS
High Performance Liquid Chromatography	HPLC
Isotope Dilution	ID
Marine Environmental Protection Committee	MEPC
Mass Spectrometry	MS
Monobutyltin	MBT
Inductively Coupled Plasma Mass Spectrometry	ICP-MS
International Maritime Board	IMO
Isotope Dilution Mass Spectrometry	ID-MS

Parts per Quadrillion	ppq
Parts per Million	ppm
Polyvinyl Chloride	PVC
Reference Material	RM
Secondary Electron Multiplier	SEM
Standard Deviation	SD
Solid Phase Microextraction	SPME
Supercritical Fluid Chromatography	SFC
Tributyltin	TBT
Tripropyltin	TPrT

List of Publications

1. L. Yang, Z. Mester, and R. E. Sturgeon, Comparison of Sector Field- and Quadrupole-ICP-MS For the Determination of DBT and TBT in Sediment Following GC Separation, *J. Anal. At. Spectrom.*, 2003, 18, 1365-1370.
2. L. Yang, Z. Mester, and R. E. Sturgeon, Determination of Butyltin Species in a Sediment Certified Reference Material by HPLC- and GC-ICP-MS, *Can. Anal. Sci. Spectro.*, 2003, 48, 223-230.
3. L. Yang, C. Bancon-Montigny, Z. Mester, R. E. Sturgeon, S. N. Willie and V. J. Boyko, Irradiation Effects On Butyltins in The Production of Sediment CRMs and Their Stability Study Using GC-ICP-MS, *Anal. Bioanal. Chem.*, 2003, 376, 85-91.
4. L. Yang, Z. Mester, and R. E. Sturgeon, Improvement in Measurement Precision with SPME by Use of Isotope Dilution Mass Spectrometry and its Application to the Determination of Tributyltin in Sediment Using SPME GC-ICP-MS, *J. Anal. At. Spectrom.*, 2002, 17, 944-949.
5. L. Yang, Z. Mester, and R. E. Sturgeon, Species-Specific Isotope Dilution-Based Calibration for Trace Element Speciation and Its Combined Uncertainty Evaluation: Determination of Tributyltin in Sediment by HPLC-ICP-MS, *Anal. Chem.*, 2002, 74, 2968-2976.
6. C. Bancon-Montigny, P. Maxwell, L. Yang, Z. Mester and R. E. Sturgeon, Improvement of measurement precision of SPME determination of TBT using ID-GC-MS, *Anal. Chem.*, 2002, 74, 5606-5613.

CHAPTER 1. INTRODUCTION

1.1. Definition of Speciation

The rapid increase in the levels of environmental pollution in recent decades has resulted in increasing concern for global ecosystems. It is currently recognized that the determination of the total trace metal burden in most biological and environmental samples provides insufficient information on bioavailability and toxicity since such effects depend to a great extent on its chemical form and concentration. Therefore, the determination of the total content of a selected element in the sample is certainly not sufficient for the estimation of toxicity impacts. Speciation of trace elements provides more specific information about the real status and impact of a given element in an environmental or biological system. According to the International Union of Pure and Applied Chemistry (IUPAC) "speciation" is defined as: "the specific form of a chemical element defined according to its molecular, complex, electronic or nuclear structure". For example, tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) are different molecular species of Sn; Cr (III) and Cr (VI) are different oxidation state species of Cr and D-Selenomethionine and L-Selenomethionine are different structural species of Se. Hence, speciation analysis can be considered to be the identification and quantification of the different chemical and structure forms of an element present in a sample.

Recently, much effort has focused on the speciation of metals because of their functions in biologically important enzymes and co-factors as well as toxic effects in the environment. Over the past decade, new and more sensitive speciation techniques have been developed for the investigation of various pollution and poisoning incidents, focusing on, for

example speciation of tin. Organotin compounds exhibit significant differences in their toxicity, depending on the number and nature of attached organic groups. It is recognized that tributyltin (TBT) is the most toxic. The decimation of oyster and sea snail populations observed in the early 1980s was traced to the use of TBT as an antifouling agent¹⁻² in paints for boats and ships.

1.2. Butyltins (BT) in the Aquatic Environment

Three butyltin (BT) compounds persist in the environment, TBT, DBT and MBT. BTs are those compounds which consist of at least one butyl (C_4H_9) group covalently bonded to a tin (Sn) atom, for example, TBT has three butyl (C_4H_9) groups and an associated anion (X^-), as shown in Figure 1.1.

Figure 1.1. TBT Salt

BTs have been introduced into the environment through various industrial and agricultural uses and applications¹⁻². Today, the largest use of organotin compounds is probably as DBT for stabilizing polyvinyl chloride (PVC) polymers². PVC is used extensively in the construction industry (flooring, fencing and piping) and in the food

packaging industry (bottles and films) to allow customers visual inspection of one side of food products such as poultry, pork or beef. Exposure of PVC to heat or ultraviolet light for prolonged periods causes diminished optical clarity and undesirable coloration. To maintain these desirable qualities, DBT is added to the polymers. DBT has also been used as a wood preservative against wood-reducing fungi, as fungicides for bathrooms and on decks and exterior siding and fences; in the textile industry to control odour-producing fungi in socks, undergarments and children's pyjamas; and as biocides for agricultural applications and in antifouling paints.

Since the first day of sail, mariners have been battling "fouling" - the growth of barnacles, seaweeds, tubeworms and other organisms on boat bottoms. This fouling produces roughness that increases turbulent flow, acoustic noise and drag and can increase fuel consumption. For large vessels, fuel costs can reach as much as 50 % of the total operating costs. Thus, for the shipping industry, efficient control of fouling is very important. Organotin compounds are one of the most effective classes of biocides utilized against fouling. In particular, TBT has been used as the major biocide ingredient in most antifouling paints for ships and boats since the 1970s¹⁻³. In 1990, the estimated annual worldwide fuel savings for global shipping industries was $\$2.5 \times 10^9$ (US), achieved through use of TBT antifouling agent¹⁻². Due to its effectiveness, TBT based paints have been used worldwide.

Once TBT enters the aquatic environment, degradation to less toxic DBT and MBT occurs by progressive removal of the butyl groups in the water column and its loss to sediments generally keeps TBT concentrations less than 10 ppt in open water. In aquatic systems, the distribution of TBT species is dependent on pH and salinity. Speciation

products in seawater are composed of a mixture of the hydrated TBT cation, tributyltin chloride, tributyltin carbonate and tributyltin hydroxide². It is widely accepted that toxicity of tributyltin is ascribed to the cation and not to the associated anion. Studies have shown that TBT is not highly persistent in aquatic ecosystems, having a half-life in the water column of a few days due to losses from degradation, adsorption on sediments and possibly volatilization². Microbial degradation in water was found to be the most important process limiting the persistence of TBT in aquatic environments; photolysis and chemical degradation were not considered significant. Degradation of TBT in sediments appears to consist of two independent processes: a rapid abiotic debutylation forming primarily MBT (2-4 days half-life) followed by slower biological degradation having a half-life of up to several months. Therefore, sediment can act as a temporary sink for that portion of TBT that is not rapidly degraded and may release TBT and its degradation products slowly back into the water column, dependent on solubility and equilibrium conditions.

1.3. Toxicity of BTs

TBT is one of the most toxic of BT compounds. In general, the level of toxicity for an aquatic organism to BTs is as follows¹⁻²:

In 1974, oyster growers first reported the occurrence of abnormal shell growth in *Crassostrea gigas*, the Pacific oyster. However, it was not until the early 1980s that researchers in France and the United Kingdom suggested that the use of TBT in antifouling paints was adversely impacting a number of marine species, including the economically important species, *Crassostrea gigas*, which is farmed in coastal waters of England and France⁴⁻⁷. Since then, toxic effects of TBT have been noted on a wide range of other marine organisms, particularly

gastropod molluscs (snails), in which TBT acts as an endocrine disruptor⁸⁻⁹, even at low ng l⁻¹ concentration levels in seawater. The organism most sensitive to the substance is the female dogwhelk, *Nucella lapillus*, a small marine snail. At concentrations as low as half a nanogram (ng) per litre, female snails grow a penis which eventually blocks the oviduct, preventing reproduction and leading to population declines and local extinction. This phenomenon is called “imposex”, the imposition of male sexual characteristics on females of the species.

As a result, a number of countries have adopted policies restricting the use of TBT antifouling paints. In 1982, France was the first to restrict the use of TBT in paints on vessels less than 25 meters in length. Many other countries, including Canada (in 1989), adopted the same regulations; the release rate is restricted to less than 4 micrograms of TBT per square centimeter of hull surface per day from vessels of greater than 25 m length. TBT concentrations in water, sediment and biota have generally declined for the past decade and Oyster culture has recovered in France following national regulations³. But ‘national’ regulations for TBT have only worked in most regulated countries, as the problem has now shifted to the unregulated countries. In many locations, TBT concentrations in sediments were high enough to cause acute and chronic toxicity to aquatic and benthic (living on the water bottom) organisms. In some areas, TBT from contaminated sediment may transfer back into the water column. It also appeared that large harbors handling ships legally painted with TBT antifouling paints continued to experience significant contamination. In 1999, the Marine Environmental Protection Committee (MEPC) of the International Maritime Board (IMO) recommended a complete ban on TBT paints from the year 2003, to protect the sea and its wildlife and help the ecosystem recover.

1.4. Analytical Techniques for Determination of Butyltin Species

The growing concerns over the toxicity of TBT and its DBT and MBT degradation products entering the environment have led to a dramatic increase in interest in the development of accurate and rapid analytical methods for their monitoring in various environmental samples, such as waters, sediments, biological tissue and sewage sludge. Determination of BTs generally involves several analytical steps, including extraction, derivatization (when GC analysis is involved), separation and detection, each of which can contribute to the difficulty of analysis, degrading the accuracy and precision of the results¹⁰⁻¹². Therefore, efficient extraction procedures free of degradation of BT species for sample preparation as well as sensitive and accurate analytical methods and certified reference materials with accurate values of BT for the validation of methodologies are needed for butyltin speciation.

Commonly used separation techniques, such as gas chromatography (GC), high performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC), have been popular. In the last decade, advances in the methods of extraction and separation techniques have been developed in conjunction with highly sensitive and element specific detectors such as atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), flame photometric detection (FPD) and mass spectrometry (MS)¹³⁻⁴⁰. In particular, inductively coupled plasma mass spectrometry (ICP-MS) has gained popularity as a sensitive and selective detector when coupled to HPLC³⁶⁻⁴² and GC^{33-34, 43-49} for determination of BTs.

ICP-MS possesses high sensitivity, a large dynamic range and multi-element capability. Furthermore, if two interference free isotopes of a given element can be found, isotope dilution (ID) ICP-MS can be considered, which generally provides superior accuracy

and precision over other calibration strategies, including external calibration and the method of standard additions. This arises because a ratio, rather than an absolute intensity measurement, is used for quantitation of the analyte concentration⁵⁰. The ID-MS technique is considered to be a primary method of analysis because of its capability for high accuracy and precision⁵¹. Although ID ICP-MS has been widely used for trace element analysis in a variety of sample matrices, its application to species specific determinations has been limited by the commercial non-availability of species specific enriched spikes⁵². If these are available and equilibration between the added spikes and endogenous analyte has been achieved before ratio measurements are undertaken, a number of advantages accrue, including: enhanced precision and accuracy of results as the species specific spike serves as an ideal internal standard; matrix effects are accounted for since quantitation is done by ratio measurements; subsequent analyte loss during sample manipulation and non-quantitative analyte recovery do not impact on the final results, and species alteration during sample work-up can be assessed. Only recently has this approach been applied to the determination of butyltin^{41-42, 45-47} and other species, such as organolead^{41-42, 53} and methylmercury⁵⁴⁻⁵³, using synthetic species specific spikes.

1.5. Solid Phase Microextraction (SPME) Sample Preparation for Determination of BTs

The popularity of GC separation techniques stems from their high resolution. However, sample preparation for GC analysis is usually time-consuming and organic solvents used in the liquid-liquid extraction are generally toxic. Disposal of organic solvent is not only costly but may have adverse impact on the environment. In an effort to simplify sample preparation and minimize solvent usage, while retaining the use of GC, solid phase

microextraction (SPME) was introduced by Pawliszyn and co-workers⁵⁴⁻⁵⁵ in the early 1990s. Since 1993, following the commercialization of SPME, this sampling technique has enjoyed widespread acceptance as a consequence of its simplicity, relatively low cost and ease with which analytes can be transferred to the GC column. The drawback noted with this technique is the degraded precision (typically 10 % RSD). As the volume of the extraction phase provided by the fiber is extremely small, any irregularity/inhomogeneity in the polymer phase/surface may result in a significant effect on its extraction characteristics⁵⁶⁻⁵⁷. In addition, some level of degradation of the fiber generally occurs during repeated usage, with the result that accuracy and precision achieved with the SPME technique can be compromised. The imprecision of the results obtained with different fibers often makes it necessary to employ an internal standard or method of standard additions⁵⁷. More recently, SPME has been applied to the determination of organotin compounds and has evolved as an elegant, solvent-free sample extraction technique^{32-33, 35, 43, 58-63}. The precision of results obtained using this technique is typically in the range of 5 to 15 % RSD for organotin compounds^{32-33, 35, 43, 58-63}.

1.6. Certified Reference Materials (CRMs) for Speciation Analysis

Due to increasing worldwide trade, a large number of measurements are conducted by laboratories in order to assess the quality of various products. Economic losses due to incorrect analyses are therefore not acceptable and measurements must be accurate. Therefore, a new method may be used in routine analysis only after an appropriate validation, which is usually achieved through use of certified reference materials (CRMs) and participation in interlaboratory comparisons.

A reference material (RM), by definition, is a material or substance, one or more of

whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials⁶⁴⁻⁶⁵. A CRM is a RM accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. Chemical measurement traceability is the property of the result of a measurement, either physical or chemical, or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons⁶⁵. The use of CRMs is desirable for all analytical laboratories undertaking methods development to ensure the validity of a proposed new approach.

As mentioned earlier, speciation analysis is a difficult analytical task due to the numerous analytical steps involved in the measurements, including extraction, separation and detection. Uncertainties contributed from these sources often degrade the accuracy and precision associated with the results. Thus, the use of species specific CRMs for the validation of a developed method is critical and necessary for assurance of accuracy. Species specific CRMs are currently available from only a few reference material producers; the suite of analytes is quite limited and includes Sn, Hg, As, Cr and Se⁶⁶. In the case of tin, TBT, DBT and MBT are certified in only a few matrices, including National Research Council Canada (NRC) PACS-2 and HIPA-1 marine sediments, Institute for Reference Materials and Measurements (IRMM) BCR 462 coastal sediment, BCR 477 mussel tissue and BCR 646 freshwater sediment, National Institute of Standards and Technology (NIST) SRM 1941b marine sediment and National Metrology Institute of Japan (NMIJ) NMIJ CRM 7301 marine

sediment. Additional species specific CRMs are needed for method validation in the study of environmental pollutants.

1.7. Objectives of This Research

Efficient extraction procedures free of analyte degradation, sensitive and accurate analytical methods and CRMs certified for concentrations of BTs for the validation of methodologies are needed for the speciation of BTs in environmental samples. The National Research Council Canada has been involved in the production of CRMs for speciation since the 1980s. Certification of an analyte concentration requires good agreement between at least two independent analytical methods. Development of independent methodologies possessing the highest possible accuracy and precision is thus required.

The objectives of this study were four fold. First, was to take advantage of the high sensitivity, selectivity and capability for isotope dilution offered by ICP-MS in combination with HPLC or GC separation for the development of reliable, accurate and precise independent methods for the determination of TBT, DBT and MBT in sediments. A second objective was to develop a robust extraction method for speciation of BTs in sediments. A third objective was to investigate the application of species specific ID techniques with ICP-MS and GC-MS detection for the accurate and precise determination of BTs in sediments, as well as using ID to overcome the generally poor precision associated with SPME sample preparation. The fourth objective was to apply these developed methods to a study of the homogeneity of TBT and DBT in a new NRC sediment CRM (SOPH-1) as well as to a short- and long-term stability study of BTs in PACS-2 sediment CRM subjected to various storage conditions.

CHAPTER 2. EXPERIMENTAL SECTION

2.1. Instrumentation

2.1.1. High Performance Liquid Chromatography (HPLC)

The HPLC used was a Dionex instrument equipped with a model AGP-1 advanced gradient pump (Dionex, Sunnyvale, CA). Samples were injected onto the column through the microinjection valve of a Dionex Model LCM liquid chromatography module, equipped with a nominal 100 μ l volume injection loop. The chromatography column used was a 10 μ m strong cation exchange column (Whatman SCX, 4.6 mm x 250 mm) for separation of BTs. A 0.16 M ammonium citrate solution at pH 4.8 in a 60:40 methanol/water mixture was used as the mobile phase at a flow rate of 1.00 ml min⁻¹. Coupling of the HPLC to the ICP-MS was accomplished by directing the elute from the column to the nebulizer through a 0.3 m length of PEEK tubing (0.254 mm i.d., 1.59 mm o.d.).

2.1.2. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The ICP-MS used in this work was a Perkin-Elmer SCIEX ELAN6000 (Concord, Ontario, Canada) equipped with a GemTip cross-flow nebulizer. The linear dynamic range spans 8 orders of magnitude with a sensitivity of 3×10^7 cps/ppm ¹¹⁵In. A custom-made quartz sample injector tube (0.9 mm i.d.) was used. A double pass Ryton® spray chamber, inert to most mineral acids and organic solvents, was mounted outside the torch box and maintained at room temperature. The main components and their arrangement on the ELAN6000 are shown in Figure 2.1.

Figure 2.1. Schematic diagram of ELAN6000: P_{cps}: pulse counting mode; A_{cps}: analog mode.

ICP-MS utilizes an Ar plasma as an ion source and typically a quadrupole mass analyzer to separate ions according to their mass-to-charge ratio with detection based on an electron multiplier. ICP-MS can handle solid as well as liquid samples. Solid samples are introduced into the ICP as an aerosol by way of laser ablation or a slurry and also based on direct sample insertion. Aqueous samples are introduced by way of a nebulizer which aspirates the sample with high velocity argon, forming a fine mist. The aerosol then passes into a spray chamber where larger droplets are removed via a drain and, typically, only 1-2% of the original mist enters the plasma.

Optimization of the ELAN6000 for nebulizer gas flow, lens voltage, RF power and dead time correction was performed as recommended by the manufacturer. Detailed information on optimization of HPLC or GC ICP-MS systems is presented in subsequent chapters.

2.1.3. Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS)

A SF-ICP-MS instrument was also used in this research to provide a comparison of performance with the quadrupole ICP-MS for determination of TBT and DBT in sediment using species specific ID. The SF-ICP-MS instrument used was a ThermoFinnigan Element2 (Bremen, Germany) equipped with a Scott-type double pass glass spray chamber and a PFA self aspirating nebulizer (Elemental Scientific, Omaha, NE, USA). A plug-in quartz torch with a sapphire injector and a Ag guard electrode (GE) were used. The GE decreases the ion energy spread of the analyte ions and leads to increased ion transmission. The Element2 can be operated at low ($R=300$), medium ($R=4000$) and high ($R=10000$) resolution by automatic switching of the position of the entrance and exit slits (in less than one second) and is characterized by high sensitivity (1.5×10^6 cps/ppb ^{115}In) in low resolution mode with low background (< 0.2 cps dark noise) and a flat response curve. Rather than having the ion beam directly strike the detector to initiate an electron cascade, the secondary electron multiplier implemented in the Element2 uses a conversion dynode at -8 kv, producing a more uniform response across the mass range and linearity over nine decades, from ppq to ppm concentrations. The primary components of the Element2 are shown in Figure 2.3.

The main difference between the Element2 (sector field) and ELAN6000 (quadrupole) is the mass analyzer used. The Element2 uses a magnetic sector mass analyzer followed by an electrostatic analyzer (often termed a double focusing mass spectrometer), in reverse Nier Johnson geometry to focus an entrance slit, the object of an ion optical lens, onto the collector (exit slit, detector). In a magnetic sector, ions are accelerated by an electrical potential (V), are collimated by an entrance slit and pass through a curved flight tube positioned between a high field electromagnet. The flight tube is positioned so that the

path of the ion beam is perpendicular to the magnetic field lines of force:

$$r = \frac{(2Vm/z)^{1/2}}{H} \quad (2.4)$$

where r is the radius of curvature of the ion path, V is the accelerating voltage, m is the mass of the ion, Z is the charge of the ion and H is the strength of the magnetic field. From this equation, it is evident that the radius of the ion path is proportional to the square root of the m/z for a constant accelerating voltage and at a constant magnetic field. By continuously varying either the accelerating voltage, V , or the magnitude of the magnetic field, H , as a function of time, the separate m/z ion beams can be scanned past the exit slit.

Figure 2.3. Schematic diagram of Element2

When ions are passed through an electrostatic analyzer (ESA), a curved flight tube through an electrostatic field, they travel in a circular path of radius r such that the electrostatic force balances the centrifugal force:

$$\frac{mv^2}{r} = Ez \quad (2.5)$$

where E is the electrostatic field strength and z is ion charge. The radius of curvature is dependent on the ion's energy and not its mass. A broad energy distribution is reduced to a very narrow energy distribution, permitting higher resolution to be attained.

From the above description, the magnetic sector is used to separate ions according to their mass and energy (momentum) while the electrostatic analyzer is used to filter the ions according to their kinetic energy, irrespective of their m/z . Both systems provide angular focusing. When the energy dispersion of the magnet and ESA are equal in magnitude but of opposite direction, the magnet and ESA focus both ion angles (first focusing) and ion energies (double focusing) while being dispersive for m/z . The Element2 can be operated in two scan modes: a magnetic scan, which varies the magnetic field at a constant acceleration voltage, resulting in an unlimited mass range wherein the settling time is dependent on the mass jump, producing a relatively slow scan, and an electric scan, which keeps the magnetic field constant while decreasing the acceleration voltage. This results in a limited mass range (30% of the magnet mass) with 1ms settling time independent of mass and is a fast mode. This fast mode is usually used for ratio measurements as well as for acquiring transient signals from introduction sources such as laser ablation, HPLC and GC.

2.1.4. Gas Chromatography (GC)

A Varian 3400 gas chromatograph (Varian Canada Inc. Georgetown, Ontario,

Canada) equipped with either a DB-1 column (1% phenyl, 99% polydimethylsiloxane, 15 m x 0.32 mm x 1.5 μ m) or DB-5 (5% phenyl, 95% polydimethylsiloxane, 30 m x 0.28 mm x 0.5 μ m) was used for separation of the BTs. A 1 μ l volume of iso-octane extract (or SPME sampling) was sampled, in the splitless mode, onto the column at 50°C. Helium carrier gas was set at 32 psi (1.2 ml min⁻¹). The GC was coupled to the ICP-MS using a home-made interface and transfer line similar to that reported by Bayón *et al*⁶⁷. Detailed description and optimization of GC-ICP-MS system is reported in Chapter 6.

2.1.5. Microwave Digesters

Two microwave extraction devices (a closed-vessel system and an open-focused system) were used in this study. The closed-vessel system is a Perkin-Elmer *Multiwave* Microwave Sample Preparation System (Perkin-Elmer, Inc., Ontario, Canada) equipped with integrated pressure and continuous temperature monitoring for all six individual vessels on a single carousel. The unit is designed to deliver unpulsed, continuous microwave energy over the entire range up to a maximum of 1 kW.

A Microdigest model 401 (2.45 GHz, maximum power 300 W) microwave digester (Prolabo, Paris, France), equipped with a TX32 programmer, was used for open vessel microwave assisted extraction of butyltins from sediment samples.

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

2.1.6. Solid Phase Microextraction (SPME)

A manual SPME device equipped with a fused silica fiber coated with a 100 μ m film of polydimethylsiloxane (Supelco, Bellefonte, USA), was used in this research. SPME

sampling of the ethylated butyltins from the headspace above aqueous solutions was used in this research to protect the fiber coating from any possible damage by high molecular weight species and other non-volatile concomitants which may have been present in the sample matrix.

2.1.7. Syringe

A 10 μ l volume liquid sampling syringe (Hamilton Company, Nevada, USA) was used for the injection of iso-octane extract for the determination of BTs using GC-ICP-MS.

2.1.8. Gas Chromatography Mass Spectrometry (GC-MS)

A Hewlett Packard HP 6890 GC-MS (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada) fitted with a DB-5MS column (Iso-Mass Scientific Inc., Calgary Alberta, Canada) was used for the separation of the butyltin hydrides for the determination of impurities in stock solutions of the BTs as well as for the determination of BTs in sediments. Detection was achieved with an HP model 5973 mass selective detector (MS).

2.2. Reagents and Solutions

2.2.1. Acids

Acetic, nitric and hydrochloric acids were purified in-house by sub-boiling distillation of reagent grade feedstocks in a quartz still prior to use.

2.2.2. Ammonium Hydroxide

Environmental grade ammonium hydroxide was purchased from Anachemia Science (Montreal, Quebec, Canada) and used to neutralize and adjust the pH of samples.

2.2.3. Ammonium Dibasic Citrate

The certified A.C.S. grade ammonium dibasic citrate was purchased from Fisher

Scientific (Nepean, Canada) and was used to prepare eluting solution for HPLC.

2.2.4. Methanol

OmniSolv® methanol (glass-distilled) was purchased from EM Science (Gibbstown, NJ, USA).

2.2.5. High Purity De-ionized Water

High purity de-ionized water (DIW), $18.2 \text{ M}\Omega \text{ cm}^{-1}$, was obtained from a NanoPure mixed bed ion exchange system fed with reverse osmosis domestic feed water (Barnstead/Thermolyne Corp, Iowa, USA).

2.2.6. Sodium Tetraethylborate Solution

Sodium tetraethylborate solution, 1 % (m/v), was prepared daily by dissolving NaBEt_4 (Strem, Bischeim, France) in DIW.

2.2.7. Sodium Acetate Buffer Solution

A 1 M sodium acetate buffer was prepared by dissolving an appropriate amount of sodium acetate (Fisher Scientific, Nepean, Ontario, Canada) in water and adjusting the pH to 5 with acetic acid.

2.2.8. Iso-octane

Reagent grade iso-octane was purchased from Caledon Laboratories LTD (Georgetown, Ontario, Canada).

2.2.9. MBT, DBT and TBT Standards

Tributyltin chloride (98.3 %), dibutyltin dichloride (97.9 %) and monobutyltin trichloride (95 %) were purchased from Alfa Products (Danvers, MA, USA). Individual stock solutions of $1000 - 1500 \mu\text{g ml}^{-1}$ as tin were prepared in methanol and kept refrigerated until used. Individual TBT, DBT and MBT working standard solutions were prepared by

diluting the stock solutions with methanol.

2.2.10. Tripropyltin (TPrT) Internal Standard

Tripropyltin chloride (95 %) was purchased from Alfa Products (Danvers, MA, USA). A stock solution of 1000 $\mu\text{g ml}^{-1}$ as tin was prepared in methanol and kept refrigerated until used. A 1.00 $\mu\text{g ml}^{-1}$ TPrT solution was prepared by diluting the stock solution with methanol for use as an internal standard for GC-ICP-MS applications.

2.2.11. ^{117}Sn Enriched TBT and DBT Stock Solutions

Individual ^{117}Sn enriched TBT chloride and DBT dichloride stock solutions (97 % purity), at a nominal concentration of 100 $\mu\text{g g}^{-1}$ in methanol, were obtained from the Laboratory of the Government Chemist (LGC, Teddington, UK). The isotopic composition was provided as follows: ^{112}Sn (0.03 %), ^{114}Sn (0.01 %), ^{115}Sn (0.05 %), ^{116}Sn (7.6 %), ^{117}Sn (92.1 %), ^{118}Sn (0.20 %), ^{119}Sn (0.10 %), ^{120}Sn (0.04 %), ^{122}Sn (0.01 %), and ^{124}Sn (0.01 %). Spike solutions at concentrations of 0.40 and 0.90 $\mu\text{g ml}^{-1}$ as tin for TBT and DBT were separately prepared by volumetric dilution of the stocks with methanol. These spike solutions were used for ID-MS analysis of TBT and DBT in sediments. The TBT and DBT concentrations in the spike solutions were quantified by reverse spike isotope dilution against natural abundance TBT and DBT standards.

2.2.12. ^{116}Sn Enriched MBT Spike Solution

A small amount of ^{116}Sn enriched MBT spike solution was donated by Dr. Wolfgang Frech (Department of Chemistry, Umeå University, Sweden). The isotopic composition was provided as follows: ^{112}Sn (0.053 %), ^{114}Sn (0.053 %), ^{115}Sn (0.053 %), ^{116}Sn (95.47 %), ^{117}Sn (1.02 %), ^{118}Sn (1.51 %), ^{119}Sn (0.36 %), ^{120}Sn (1.11 %), ^{122}Sn (0.17 %), and ^{124}Sn (0.20 %). Spike solution, at a concentration of 0.65 $\mu\text{g ml}^{-1}$ as tin for MBT, was prepared by

volumetric dilution of the stock with methanol and used for determination of MBT in PACS-2 by ID-MS. The MBT concentration in the spike solution was quantified by reverse spike isotope dilution against natural abundance MBT standards.

2.2.13. Inorganic Tin Stock Solutions

Two independent 1000 $\mu\text{g ml}^{-1}$ stock solutions of inorganic Sn were prepared by dissolution of the high purity metal (99.9999%, Johnson, Matthey & Co. Limited, London, UK) in HCl. These solutions were used to prepare working standard for the reverse ID quantitation of the concentration of Sn in a ^{117}Sn enriched spike solution.

2.2.14. Enriched ^{117}Sn Inorganic Tin Spike Solution

Enriched ^{117}Sn isotope was purchased from Isotec Inc. (Miamisburg, OH, USA) as metal and a stock solution of approximately 177 mg l^{-1} was prepared by its dissolution in HCl containing 1 % HF. The concentration of ^{117}Sn spike was verified by reverse spike isotope dilution against the natural abundance high purity metal solutions. This spike solution was used to determine total Sn in BT stock solutions. The isotopic composition was provided as follows: ^{112}Sn (0.08 %), ^{114}Sn (0.04 %), ^{115}Sn (0.06 %), ^{116}Sn (2.34 ± 0.05 %), ^{117}Sn (89.2 ± 0.1 %), ^{118}Sn (4.50 ± 0.05 %), ^{119}Sn (1.12 ± 0.05 %), ^{120}Sn (2.16 ± 0.05 %), ^{122}Sn (0.26 %), and ^{124}Sn (0.28 %).

2.2.15. Certified Reference Material (CRM)

The marine sediment CRM PACS-2 (NRC, Ottawa, Canada) was used as a test sample for method development. This material is certified to contain 0.98 ± 0.13 , 1.09 ± 0.15 and 0.45 ± 0.05 $\mu\text{g g}^{-1}$ TBT, DBT and MBT (as tin), respectively.

2.2.16. Sediment Samples

A CRM candidate sediment, SOPH-1, and sediment CRM HISS-1 were used as test

samples for speciation of the BTs.

2.3. Sample Preparation and Analysis Procedure

Sample preparation was conducted in a class-10 or class-100 clean room environment to minimize possible contamination. Two different microwave assisted heating systems were used for the extraction of organotin species from sub-samples of PACS-2 sediment. Detailed sample extraction and analysis procedures are presented in subsequent related chapters.

CHAPTER 3. DEVELOPMENT OF AN HPLC-ICP-MS METHOD FOR THE DETERMINATION OF BUTYLTIN SPECIES IN PACS-2 SEDIMENT

3.1. Introduction

The National Research Council Canada has been involved in the production of CRMs since the 1980s. In the early certification work of PACS-1³⁶, a HPLC-ICP-MS method was developed for the determination of DBT and TBT using a Whatman SCX cation exchange column. The MBT concentration was not reported due to two major difficulties encountered at the time. Poor extraction efficiency of the species from PACS-1 occurred when using an ultrasonic extraction procedure based on use of 8 ml of HCl and 4 ml methanol for a 4 g sub-sample which was extracted into a 4 ml mixture of toluene/isobutyl acetate (80:20, v:v) containing 1.5 % (w:v) tropolone. Additionally, a high background from inorganic tin co-eluted with MBT during the HPLC gradient elution step when the carrier pH was changed from 6 to 3. NRC certification campaigns require good analytical agreement between at least two independent methods. Development of independent methodologies possessing the highest possible accuracy and precision is thus required. An HPLC-ICP-MS method was first developed as an independent method for the determination of all three BTs in PACS-2 to complement a more established GC procedure.

3.2. Sample Preparation

The sample preparation procedure followed the method outlined by Szpunar *et al.*¹⁶ with some minor modifications. Three sample blanks, five replicate sub-samples of unspiked PACS-2 and five replicate sub-samples of PACS-2 which were spiked were prepared and analyzed immediately for best results. Sub-samples of 0.5 g PACS-2 (0.100 ml

methanol was added to the unspiked sample and 0.100 ml of 5.00 ppm of TBT and DBT and 2.5 ppm MBT mixed standard solution were added for the spiked sample) along with 5 ml acetic acid were heated in the Prolabo microwave digester at 60 % power for 3 minutes. Following cooling, the contents were centrifuged at 2000 rpm for 5 minutes. A 1.00 ml volume of the supernatant was transferred to a 20 ml glass vial along with 1 ml DIW and the mixture was adjusted to pH 5-6 with 1.2 ml of ammonium hydroxide. The contents were then buffered with 0.8 ml of ammonium citrate (2 mol l^{-1}) and diluted to 10 ml with methanol.

In the study of the retention/separation characteristics of butyltin compounds on the SCX cation exchange column, mixed standard solutions containing 20 ng ml^{-1} each of MBT, DBT and TBT were prepared in a similar way as for samples, with the exception that no microwave digestion was applied.

3.3. Optimization of ICP-MS

Prior to coupling to the HPLC, the ELAN6000 ICP-MS was optimized daily for nebulizer gas flow and lens voltage in order to achieve the best signal-to-background ratio for tin at m/z 120. The inorganic tin standard used (10 ng ml^{-1}) was made-up in 0.16 M ammonium citrate solution in a 60:40 methanol/water mixture. It was found that nebulizer gas flow had to be decreased to less than 0.4 l min^{-1} (optimum at 0.5 l min^{-1}) in order to maintain the vacuum during prolonged aspiration (7 hours) of carrier containing high concentrations of methanol. With reduced nebulizer gas flow, no plasma instability was encountered, even at concentrations up to 70% of methanol in water when the argon plasma was operated at a forward power of 1.6 kW. A minor accumulation of carbon was noted on the cones at the end of the day, necessitating that they be cleaned daily. The ICP-MS

operating conditions used in this study are summarized in Table 3.1.

Table 3.1. HPLC-ICP-MS operating conditions

	ICP-MS
Rf power	1600 W
Plasma Ar gas flow rate	15.0 l min ⁻¹
Auxiliary Ar gas flow rate	1.0 l min ⁻¹
Nebulizer Ar gas flow rate	0.35 l min ⁻¹
Sampler cone (nickel)	1.00 mm
Skimmer cone (nickel)	0.88 mm
Lens voltage	7.0 V
Scanning mode	peak hopping
Points per peak	1
Dwell time	50 ms
Sweeps per reading	1
Readings per replicate	6500
Number of replicates	1
Dead Time	55 ns
	HPLC
Injection volume	100 µl
Column	10 µm strong cation exchange column (Whatman SCX, 4.6 mm x 250 mm)
Carrier	0.16 M ammonium citrate solution in a 60:40 methanol/water mixture
Elution mode	Isocratic
Flow rate	1 ml min ⁻¹

Following optimization of ELAN6000, the HPLC was brought on line by directing the elute from the column to the ICP-MS nebulizer through a 0.3 m length of narrowbore

PEEK tubing. The PEEK tubing was connected to the nebulizer with the plasma on and nebulizer gas flow reduced to zero l min^{-1} , otherwise the plasma would be extinguished. After successful connection of the HPLC, the nebulizer gas flow was reset to 0.35 l min^{-1} . Data acquisition for the Elan6000 was manually triggered after an injection of the sample to the HPLC ($100 \mu\text{l}$). The data were acquired by monitoring several Sn isotopes. In order to achieve the best signal-to-noise ratio, the most abundant isotope, ^{120}Sn , was used throughout the study. At the end of the chromatographic run, the acquired data were transferred to an off-line computer for further processing using in-house software that yields both peak height and peak area information. In this work, only data for peak area were used for calculations. Standard additions calibration techniques were used for the quantification of all butyltin compounds.

3.4. Optimization of Chromatographic Separation of Butyltin Species Using HPLC

As noted earlier, MBT was not reported in the initial certification of PACS-1 when a cation exchange column HPLC-ICP-MS method was used because of interference from inorganic tin that coeluted when the pH of the carrier was changed from 6 to 3 (a condition wherein MBT would be eluted). This problem can be minimized by development of chromatographic conditions that separate all three butyltin species using the same cation exchange column without resorting to gradient elution. This approach not only lowers the limit of quantification for MBT, but also significantly reduces the time needed for each analysis. The degree of retention of the butyltin species in a cation exchange column is dependent on the ionic strength of the mobile phase^{29, 36}. Factors having direct impact on separation include the eluent concentration, pH, the methanol-to-water ratio in the carrier and its flow rate. A systematic study of all of these parameters was conducted to optimize the

separation of butyltin species on this cation exchange column.

3.4.1. Effect of Eluent Concentration on Separation of BTs

The effect of the concentration of ammonium citrate on the retention of butyltin species was first examined. In this investigation, ammonium citrate concentrations ranging from 0.04 M to 0.24 M at its natural pH of 6 in 60:40 methanol/water mixture were studied. It was noted that retention of TBT was not affected in any way over the range of ammonium citrate concentrations tested. However, DBT was not detected until 0.12 M was reached and only a small improvement in the elution time could be attained when concentrations higher than 0.16 M were used. In all cases, MBT was not eluted; this species was retained on the column even at an ammonium citrate concentration as high as 0.50 M. Despite such high concentrations, no clogging of the ICP-MS cones was observed. In order to minimize both plasma loading and the blank contribution from the eluent, the ammonium citrate concentration was fixed at 0.16 M for all subsequent work.

3.4.2. Optimization of Methanol/Water Mixtures for the Elution of Butyltin Species

Since the presence of methanol influences the degree of dissociation of ammonium citrate and thus the ionic strength of the carrier, the effect of methanol concentrations on the efficiency of the separation of tin species was studied. The concentration range of methanol used was 0% to 70% (v/v) in 0.16 M ammonium citrate buffer at pH 6. Figure 3.1 illustrates that methanol is needed in order to elute butyltin species from the column. It is clear that DBT is not eluted (10 minutes after an injection) if the concentration of methanol in the carrier is less than 50%. No significant decrease in the retention time of DBT was observed when the methanol content was increased beyond 70%. Under all tested concentrations, MBT was not eluted. This may be due to the more polar nature of MBT, which binds more

Figure 3.1. Effect of methanol concentration on the elution of butyltin compounds: 2 ng each of MBT, DBT and TBT from a 100 μ l injection. Carrier composition: 0.16 M ammonium citrate at pH 6 and a methanol/water ratio a) 0:100, b) 30:70, c) 40:60, d) 50:50, e) 60:40 and f) 70:30.

strongly to the cation exchange column. A more polar eluent is thus needed, since MBT was not detected in any of the methanol concentrations used in this study. The methanol content was therefore limited to 60% in all subsequent studies in an effort to minimize any plasma instability and plasma loading over the course of a full day, as well as to obtain good separation between TBT and DBT in the shortest time possible.

3.4.3. Optimization of Eluent pH for the Elution of Butyltin Species

As MBT is the most polar compound of the three BT species, it is expected that the stationary phase in the cation exchange column will have a stronger affinity for this species. A mobile phase having higher '*polarity*' is thus expected to be more efficient in eluting this species. McLaren and co-workers³⁶ demonstrated that all three butyltin species could be gradient eluted (from pH 6 to 3) from the same strong cation exchange column. Unfortunately, inorganic tin ion was co-eluted with MBT under these conditions, thus rendering this method unsuitable for measurement of low concentrations of MBT. To avoid this problem, isocratic elution conditions were tested. The pH of the carrier was studied in the range of 4 to 6. Figure 3.2 shows the elution characteristics of the three BT standards (20 ng ml⁻¹ each) at various pHs. It is evident that the retention times of MBT and DBT are very much dependent on the pH of the carrier. In fact, MBT was not eluted if the carrier pH was greater than 5, whereas at pH 4.2 (Figure 3.2f) the MBT and DBT were poorly resolved. Therefore, a pH of 4.8 was chosen (Figure 3.2d) for subsequent chromatography work as it provides a good compromise resolution for all three species in the shortest run time. The entire chromatographic process can be accomplished in 8 minutes.

Figure 3.2. Effect of carrier pH on the elution of butyltin compounds: 2 ng each of MBT, DBT and TBT from a 100 μ l injection. Carrier composition: 0.16 M ammonium citrate in a 60:40 methanol/water mix at pH a) 6.0, b) 5.3, c) 5.0, d) 4.8, e) 4.5 and f) 4.2

3.4.4. The Effect of Carrier Flow Rate on the Elution of Butyltin Species

Flow rates from 0.6 to 1.4 ml min⁻¹ for a carrier consisting of 0.16 M ammonium citrate in 60:40 methanol/water at pH 4.8 were tested. Significant peak broadening (for DBT and MBT) and increased retention times for DBT and MBT were observed as flow rates decreased below 0.8 ml min⁻¹. Poor separation of DBT from MBT was observed when the flow rate increased above 1.2 ml min⁻¹. It was noted that the operating pressure of the ELAN6000 also increased as carrier flow rate increased. A flow rate of 1.0 ml min⁻¹ was selected to achieve good separation of the BTs as well as shortest retention time.

To confirm that inorganic tin was not co-eluting with MBT, a subsequent set of experiments was conducted to investigate the characteristics of the various tin species under the chromatographic conditions specified above. Individual standard solutions of inorganic Sn, MBT, DBT and TBT as well as a sample blank were injected. Inorganic tin was not eluted from this strong cation exchange column under the selected operating conditions. However, an intense, broad peak was recorded when 0.5 M ammonium citrate in 60:40 methanol/water at a pH of 3 was pumped through the column at the end of the day. These results, shown in Figure 3.3, confirmed that inorganic Sn does not pose any problem for the determination of any of the butyltin species under the chosen experimental conditions. Thus, HPLC separation of all three butyltins can be achieved using this strong cation exchange column and a simple isocratic elution with a carrier consisting of 0.16 M ammonium citrate in 60:40 methanol/water at a pH of 4.8 at a flow rate of 1.0 ml min⁻¹.

Figure 3.3. Chromatograms of tin species obtained under “optimum” elution conditions using 0.16 M ammonium citrate in a methanol/water (60:40) mixture at pH 4.8 as carrier, 100 μl injection. a) procedural blank, b) 60 $\mu\text{g l}^{-1}$ of inorganic tin, c) 20 $\mu\text{g l}^{-1}$ of MBT, d) 20 $\mu\text{g l}^{-1}$ of DBT, e) 20 $\mu\text{g l}^{-1}$ of TBT and f) mixed standard, 20 $\mu\text{g l}^{-1}$ of MBT, DBT and TBT

3.5. Quantitation of MBT, DBT and TBT in PACS-2 Sediment

As shown in Figure 3.4, PACS-2 contains three BTs and good separation of these species is obtained using a cation exchange column. Results from a preliminary study confirmed the low signal intensity for MBT in an extract of PACS-2 even under optimized conditions. For maximum sensitivity, the most abundant ^{120}Sn isotope is preferred. To ensure that this isotope is free of polyatomic or other isotopic interferences, an initial matrix matched standard additions quantitation using the two most abundant isotopes, ^{118}Sn and ^{120}Sn , was performed for all three butyltin compounds in PACS-2. In particular, for ^{120}Sn , the data were collected without interference correction from ^{120}Te . The calculated mean concentrations and their respective variances based on both isotopes were compared against a t-test criterion. At $P = 0.05$, there is no statistical difference in the calculated concentrations derived from either isotope, suggesting that both are interference free and the isobaric contribution from ^{120}Te on ^{120}Sn intensity is negligible.

In an attempt to achieve the best signal-to-background ratio, only the ^{120}Sn isotope was monitored for final measurements, despite the multi-ion detection capability of the Elan6000. The linearity of the standard additions calibration curve was verified and BT standards containing amounts equivalent to 1-, 2-, 4- and 6-fold the concentrations of the individual butyltin species present in a 0.5 g sub-sample of PACS-2 sediment were sequentially added. Linear responses having correlation coefficients of 0.9959, 0.9995 and 0.9999 for MBT, DBT and TBT, respectively, were obtained.

Figure 3.4. Chromatogram obtained from an extract of PACS-2 using HPLC-ICP-MS

Only one addition was made for the final determination of TBT, DBT and MBT in PACS-2 to minimize analysis time and increase sample throughput. Samples were injected as unspiked sample1, spiked sample1 and then unspiked sample1 to counter any instrument drift and maintain accurate results. Concentrations of 0.96 ± 0.03 , 1.08 ± 0.07 and $0.46 \pm 0.03 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=5$) as tin with precisions of 3.1, 6.5 and 6.5 % were obtained for TBT, DBT and MBT, respectively, based on standard additions calibration. These values are in agreement with the respective certified concentrations of 0.98 ± 0.13 , 1.09 ± 0.15 and $0.45 \pm 0.05 \mu\text{g g}^{-1}$ (as 95 % confidence interval).

3.6. Method Detection Limit

Method detection limits based on three times the standard deviation estimated from 10 repetitive measurements of sample blanks were 0.08 , 0.05 and $0.03 \mu\text{g g}^{-1}$, normalized to a 0.5 g of sub-sample, for MBT, DBT and TBT, respectively.

3.7. Conclusions

An accurate and rapid HPLC-ICP-MS method was developed for separation of BT species and their quantitation in PACS-2 sediment. All three butyltin compounds can be separated within 8 minutes in a single HPLC chromatographic run under isocratic elution conditions using a carrier consisting of 0.16 M ammonium citrate in $60:40$ methanol/water at a pH of 4.8 and a flow rate of 1.0 ml min^{-1} . Concentrations of MBT, DBT and TBT measured in PACS-2 agree with their certified values. Method detection limits (3 SD based on a 0.5 g sub-sample) of 0.08 , 0.05 and $0.03 \mu\text{g g}^{-1}$ for MBT, DBT and TBT, respectively, are well suited for the determination of typical concentrations of BTs in sediments.

CHAPTER 4. DEVELOPMENT OF A FAST AND EFFICIENT MICROWAVE ASSISTED EXTRACTION PROCEDURE FOR SPECIATION OF BUTYLTIN IN SEDIMENTS

4.1. Introduction

Efficient extraction procedures free of degradation are needed for accurate determination of BT species in environmental samples. Earlier published methods are not only labor intensive, but also inefficient in terms of extraction recovery⁶⁸. As highlighted by Zhang *et al.*⁶⁹, only three of ten sample extraction methods for the analysis of sediments published in 1991 were able to recover more than 90% of TBT present and none of them was able to quantitatively recover MBT. Pannier *et al.*⁷⁰ investigated three different sample pre-treatment methods and two analytical procedures. They reported good general agreement amongst the methods. More recently, Morabito and co-workers⁷¹ systematically compared twelve selected extraction methods using the same derivatisation and clean-up step for the determination of butyl- and phenyltin compounds in a mussel tissue (CRM477). Agreement amongst the different extraction methods was poor. Similarly, MBT was not certified in PACS-1, in part, due to the inefficient extraction method used at the time³⁶. Clearly, the uncertainty in the extraction procedure hampers the success of speciation in real samples. In the mid 1990s, promising progress was reported on the extraction of tin species by means of microwave assisted heating in an open-vessel¹⁴⁻²⁵. This approach is appealing, as a microwave system offers a potentially fast and efficient means of extraction.

An effort was thus made to develop microwave assisted extraction procedures based on both a closed and open-focused geometry for the determination of BTs in sediment CRM

PACS-2. An HPLC-ICP-MS procedure, described in Chapter 3, was used for quantitation.

4.2. Sample Preparation

4.2.1. Open-Vessel Microwave Assisted Extraction

Sample preparation using an open-vessel microwave assisted extraction was undertaken for the purpose of providing a comparison study to the closed-vessel microwave approach and followed the procedure outlined by Szpunar *et al.*¹⁶ described in detail in Chapter 3 (section 3.1). A 1 g sub-sample of PACS-2 (0.200 ml methanol was added to the unspiked sample and 0.200 ml of 5.00 ppm of TBT and DBT and 2.5 ppm MBT mixed standard solution was added for the spiked sample) and 10 ml of either glacial or 1+1 acetic acid were heated in the Prolabo microwave digester at 60 % power for 3 minutes. The contents were then centrifuged at 2000 rpm for 5 minutes. Depending on the initial acetic acid concentration, an aliquot of the supernatant (1.00 or 2.00 ml) was adjusted to pH 5-6 with 1.2 ml of ammonium hydroxide. The mixture was further buffered with 0.8 ml of 2 M ammonium citrate and diluted to 10 ml with methanol and water so as to result in a final 60:40 methanol/water ratio.

4.2.2. Closed-Vessel Microwave Assisted Extraction

The Perkin-Elmer *Multiwave* system has the capacity to handle a maximum of six reaction vessels at a time. In one typical heating cycle, four vessels were used for sample treatment while the remaining two vessels were used as a means to evaluate the procedural blank. For samples, 0.5 g of PACS-2 sediment was weighed into each of the four pre-cleaned quartz high pressure / high temperature vessels. Into two of the sample vessels, 0.100 ml of each of the 10 $\mu\text{g ml}^{-1}$ butyltin standard was added (spiked samples) while 0.300 ml of 60:40 methanol/water was added into the two remaining sample containers. For the

procedural blank, a set of spiked and unspiked sample blanks was also prepared, as above. Into each vessel, either 5 ml of acetic acid (glacial) or 5 ml of (1+1) acetic acid were added. The vessels were sealed and heated at 450 W power for 6 minutes to a temperature of 100°C. After cooling, samples were transferred to glass vials and centrifuged at 2000 rpm for 5 minutes. Depending on the initial acetic acid concentration, an aliquot of the supernatant (1.00 or 2.00 ml) was transferred and adjusted to pH 5-6 with 1.2 ml of ammonium hydroxide. The mixture was further buffered with 0.8 ml of 2 M ammonium citrate and diluted to 10 ml with a suitable mix of methanol and water so as to result in a 60:40 methanol/water ratio.

For best results, regardless of the type of extraction method used, samples were analyzed on the same day as they were prepared.

4.3. Optimization of Parameters for Closed-Vessel Microwave Assisted Extraction of BTs From PACS-2

Extraction of butyltin compounds for speciation analysis requires several considerations in the design of the experiments which are crucial to achieving optimal extraction efficiency as well as ensuring that the integrity of the extracts is not compromised by the process. In the case of microwave leaching, factors such as temperature, duration of extraction and the behavior of the spike are important features in recovery of species. Szpunar *et al.*¹⁶ reported on the rapid extraction of butyltin compounds from sediments using an open-vessel focused microwave digester (Prolabo). One obvious disadvantage of such a method is the potential loss of volatile components during the heating cycle, resulting in erratic recoveries of the species without the use of a condenser. In addition, this digester is capable of processing only one sample at a time, making the procedure not only more time

consuming, but also making the precision of the extraction process potentially dependent on maintaining an identical interaction of the samples with the microwave energy for every extraction run. The latter can be particularly problematic if the recovery is not quantitative. As the Perkin-Elmer *Multiwave* Sample Digester has the capability of accommodating six vessels in the same heating cycle, throughput as well as the accuracy of quantitation when a standard additions calibration protocol is implemented are improved. In addition, the ability to continuously monitor the individual temperatures and pressures in each vessel offers further insight into the stability and recovery of species in the extract.

For the following studies, a 1 g sub-sample of PACS-2 sediment was used for extraction and the volume of liquid phase was similar to that employed by Szpunar *et al.*¹⁶, *i.e.* 10 ml of (1+1) acetic acid.

4.3.1. Extraction Temperature

Temperature is important in sample extraction as it may not only have a direct impact on the integrity of the extracted species, but will also affect the speed and efficiency of analyte recovery. In general, a study of the recovery of a spike from a sample will provide information about the efficiency of the extraction step as long as extraction characteristics of such an added spike are the same as those for the endogenous analyte of interest^{16, 71}. The recoveries of the analyte as well as the spike can be further quantified by a second matrix matched external calibration measurement (addition of a separate spike after the microwave extraction process). In total, two separate standard additions steps are needed in order to quantify the extraction efficiencies of both the spiked standard and analyte in the sample. For the spiked samples, a 0.200 ml volume of each of the three 10 $\mu\text{g ml}^{-1}$ butyltin standards was added to the sediment. For the unspiked samples, in order to match the matrix, a volume

containing 0.600 ml of 60:40 methanol/water was also added. The BT species were extracted in a closed vessel microwave assisted system over a temperature range 75°C to 125°C for a fixed extraction time of 6 minutes. After the extraction, a second set of standard additions measurements was performed by adding 0.040 ml each of the 10 $\mu\text{g ml}^{-1}$ of MBT, DBT and TBT standards to 2.00 ml of the extracted supernatant. The spike recoveries were then calculated using the following equation:

$$R = \frac{I_{sp} - I_{un}}{I_{std} - I_{un}} \cdot 100\% \quad (4.1)$$

where R is the percent recovery; I_{un} is the signal intensity obtained from the original sample aliquot (unspiked) after microwave extraction; I_{sp} is the signal intensity from the sample aliquot containing the spike standard added prior to microwave extraction and I_{std} is the signal intensity measured for the solution containing the standard spike added after the extraction process.

The results, summarized in Table 4.1A, show that 90% or more of the DBT and TBT, but less than 50% of the MBT can be recovered from the PACS-2 sediment. Moreover, the recoveries of all three butyltin species are similar for extraction temperatures ranging from 95°C to 115°C. Below this range, the recovery of MBT is low. At temperatures higher than 115°C, it appears that the recoveries of all three species are somewhat degraded. It is conceivable that the butyltin compounds become less stable in this elevated temperature environment. Further research to substantiate this claim is required. An extraction temperature of 100°C was chosen to ensure a gentle extraction medium without compromising the recovery of the three butyltin species.

Table 4.1. Recovery of butyltin species under different microwave extraction conditions using the *Multiwave*

A) Recovery of butyltins as a function of extraction temperature (heating time 6 minutes)			
Recovery (%)*			
Temperature	MBT	DBT	TBT
75°C	26±4	85±9	92±10
85°C	37±6	91±15	88±8
95°C	42±3	98±3	90±2
100°C	45±2	93±4	93±2
105°C	45±2	92±8	91±6
115°C	48±3	98±5	94±3
125°C	36±2	85±2	85±3
B) Recovery of butyltins as a function of extraction time (extraction temperature 100°C)			
Recovery (%)*			
Time (minutes)	MBT	DBT	TBT
2.5	39±3	85±5	81±4
5.0	43±4	94±3	93±5
6.0	45±2	93±4	93±2
7.5	46±3	94±4	91±2
10	51±3	83±3	87±5
15	45±2	86±5	89±3

* Results are reported as mean and one standard deviation, n=3.

4.3.2. Heating Time

To efficiently extract a species from a given medium as well as avoid degradation of the species, the interaction time must be kept to a minimum. With sample preparation and measurement conditions as described earlier, the effect of heating time on the results using an

extraction temperature of 100°C was studied. The data, summarized in Table 4.1B, show no discernible difference in the recoveries of all three butyltin species for heating times ranging from 5 to 7.5 minutes. Lower recoveries were evident for heating time less than 5 minutes. When the heating process was 10 minutes or longer, a slight decrease in the recoveries for both DBT and TBT was noted while that for MBT remained the same. It appears that the DBT and TBT are degraded under a 'prolonged' heating environment (even for a temperature as low as 100°C). As a consequence, a compromise heating time of 6 minutes was used in all subsequent studies.

4.3.3. Recovery of MBT From Sediment

Using an optimized closed-vessel microwave extraction procedure, the recovery of MBT remains less than 50% when using 1 g sub-samples of sediment, significantly lower than its quantitative recovery reported by Szpunar *et al.*¹⁶, who used an open-focused microwave extraction system. It is possible that either the recovery, and thus the interaction between MBT and the sediment, is dependent on the absolute amount of sediment used (0.1-0.2 g used in reference 16) for a given extraction phase volume, or the recovery of the analyte is related to its ultimate concentration in the extraction phase. The latter concern can be addressed by studying the recoveries of spikes of the three butyltin standards over a range of concentrations for a fixed mass of sediment sample. The microwave extraction conditions, sample preparation and measurement protocols were similar to those described in the previous section. Synthetic standards containing amounts equivalent to 1-, 2-, 4- and 6-fold the concentrations of the individual butyltin species were sequentially added to 1 g sub-samples of PACS-2 sediment. The recovery data for the extractions indicated linear responses with correlation coefficients for MBT, DBT and TBT of 0.9959, 0.9995 and

0.9999, respectively. As the spike concentrations were equivalent to a range of 0 to $7 \mu\text{g g}^{-1}$ for DBT and TBT and roughly half for MBT, these findings suggest that consistent extraction efficiencies for all three butyltin compounds have been achieved. Therefore, the method of standard additions is valid within the context of this work.

The issue of non-quantitative recovery of MBT remains. Since Szpunar *et al.*¹⁶ used only 0.1 - 0.2 g sub-samples of sediment for extractions, a further series of experiments was conducted to study the effect of amount of sediment on the recovery of butyltin species. Sample masses of 0.15, 0.5, 1.0 and 2.0 g of PACS-2 sediment were used. Recoveries of the spikes are summarized in Table 4.2.

Table 4.2. Recovery of butyltin species in PACS-2 sediment using the *Multiwave*

Sample mass (g)	Recovery (%) ^a			Acetic acid
	MBT	DBT	TBT	
0.150	72±2	97±2	97±3	(1+1)
0.500	54±3	102±2	100±2	(1+1)
1.000	42±2	88±2	87±2	(1+1)
2.000	35±2	84±2	83±2	(1+1)
0.500*	102±2	100±3	98±3	glacial

* Results are reported as mean and one standard deviation, n=3.

It is interesting to note that the recovery of all three species decreases with increasing sample mass and it is especially pronounced with MBT, for which more than 70% can be recovered when 0.15 g of sediment was used. As MBT is the most polar of the three butyltin species, these results can be rationalized by considering the partition coefficients of the

butyltin species between the solid phase (sediment matrix) and the liquid phase (1+1 acetic acid). In this situation, the MBT has a greater tendency to bind with or be adsorbed onto the numerous active sites of the sediment²⁹. Thus, for a fixed volume of liquid phase, as the mass of sediment (numbers of active sites) increases, the percentage of MBT partitioned into the liquid phase decreases. In contrast, as DBT and TBT are both less polar, their interactions with the exchange sites on the sediment are expected to be less prominent. Thus, the effect of sediment mass on the recoveries of these species will be less evident. These findings suggest that the recovery of all three butyltin compounds, in particularly MBT, can be improved by decreasing the mass of sediment used or by increasing the polarity of the extraction medium (but not to the point where possible species degradation may occur).

As dilute acetic acid (1+1) was previously used, this was changed to glacial acetic acid for the following studies. A spike recovery test based on a 0.5 g sub-sample of PACS-2 sediment was undertaken. Quantitative recoveries of all three species were obtained, as shown in Table 4.2. A subsequent comparable spike recovery test was also performed using the open-vessel Prolabo digester, again based on a 0.5 g sub-sample and 10 ml of acetic acid. Recoveries of 99 ± 3 , 101 ± 3 and 100 ± 3 % for MBT, DBT and TBT, respectively, were obtained.

4.4. Quantitation of MBT, DBT and TBT in PACS-2 Sediment

The original intent of this study was to compare a closed-vessel microwave assisted extraction method to the more established open-vessel extraction technique pioneered by the Łobiński group¹⁶⁻³². The performance and robustness of the closed-vessel extraction system was evaluated by comparing the measured concentrations of the three butyltin species in PACS-2 using the two extraction systems. HPLC-ICP-MS was consistently used for

quantitation. Results, summarized in Table 4.3, show that there is no significant difference between the two extraction techniques and that the measured concentrations of the three species are in excellent agreement with their certified values. It is also interesting to note that, despite the large difference in the recovery of MBT using the closed-vessel method (recovery of 50% or less using (1+1) acetic acid as the extraction medium compared to quantitative recovery obtained with glacial acetic acid), identical measured concentrations have been obtained. This further confirms that equilibrium has been achieved between the added spiked standards and the analyte species within the system during the microwave heating process.

Table 4.3. Determination of TBT, DBT and MBT in PACS-2

Type of Extraction	Measured Concentration ($\mu\text{g g}^{-1}$ as Sn)		
	MBT	DBT	TBT
Prolabo Open-vessel [@]	0.42 $\pm$ 0.03	1.07 $\pm$ 0.05	0.97 $\pm$ 0.02
<i>Multiwave</i> , (1+1) acetic acid ⁺	0.44 $\pm$ 0.02	1.08 $\pm$ 0.03	0.95 $\pm$ 0.03
<i>Multiwave</i> , glacial acetic acid [*]	0.45 $\pm$ 0.02	1.11 $\pm$ 0.03	0.96 $\pm$ 0.02
Certified value	0.45 $\pm$ 0.05	1.09 $\pm$ 0.15	0.98 $\pm$ 0.13

Results are reported as mean and one standard deviation, for [@] n=9, ⁺ n= 8 and ^{*} n=3.

4.5. Conclusions

A relatively fast, efficient closed-vessel microwave extraction method has been developed for the extraction of BT species from sediments. Results are comparable to the more established open-vessel microwave extraction technique. It is interesting to note that, despite the difference in the recoveries of spikes obtained for BTs based on varying masses of sub-sample or different concentrations of acetic acid used (1+1 acetic acid vs. glacial), no

significant difference in BT concentrations measured in PACS-2 was observed using standard additions calibration. These observations confirm that equilibrium between the added synthetic spikes and the analyte species in the sample is achieved during the microwave heating cycle. Therefore, quantification by means of standard additions calibration is valid even when the extraction recovery is less than 50%.

CHAPTER 5. CHARACTERIZATION OF BUTYLTIN STOCK SOLUTIONS

5.1. Introduction

Since the purity of commercial BT compounds is usually less than 99 % and such data are usually not available for these compounds, the accuracy of the concentrations of BT in solutions prepared from these compounds is questionable. The accuracy of the natural abundance BT standards is critical for obtaining reliable results and their concentrations must therefore be verified before they can be used for establishing calibration curves or for use in reverse spike isotope dilution analysis to accurately quantify the concentration of the analyte in an enriched species specific spike. Therefore, a unique approach, using hydride generation (HG) GC-MS, was developed to quantify the inorganic Sn, MBT, DBT or TBT impurities in the natural abundance BT standards. The true concentration of the natural abundance BT standard was obtained following correction for these impurities.

5.2. Sample Preparation

5.2.1. Sample Preparation for Determination of Total Sn in Butyltin Stock Solutions

Isotope dilution calibration was used for the determination of total Sn in BT stock solutions. Triplicate 0.10 ml aliquots of each 1000 - 1500 $\mu\text{g ml}^{-1}$ TBT, DBT and MBT stock solution were accurately pipetted into individual pre-cleaned Teflon digestion vessels. A suitable amount of the enriched inorganic ^{117}Sn was then added to each vessel. Three process blanks (spiked with 10 % of the amount of enriched isotope solution used for the samples) were processed along with the samples. After 4 ml nitric acid, 2 ml HCl and 0.2 ml H_2O_2 were added, the vessels were capped and digested in a CEM MDS-2100 microwave oven. The heating conditions were: 10 min. at a pressure of 20 psi and 40 % power, 10 min.

at 40 psi and 50 % power, 10 min. at 80 psi and 50 % power, 15 min. at 100 psi and 60 % power, 25 min. at 120 psi and 70 % power. After cooling, 0.1 ml volumes of the digested solutions were transferred to pre-cleaned polyethylene screw-capped bottles and diluted to 25 ml with 2 % HCl.

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

The digested spiked samples and the six reverse spike ID calibration samples were analyzed by ICP-MS on the same day, following a measurement sequence approach similar to that reported by Watters *et al.*⁷² wherein a spiked sample and reverse spike isotope dilution sample were bracketed between mass bias drift correction solutions for best accuracy and precision. Optimization of the ELAN6000 was performed as recommended by the manufacturer and typical operating conditions are similar to those summarized in Table 3.1 with a Rf power of 1100 W, nebulizer Ar gas flow rate of 0.75 l min^{-1} , sweeps per reading of 100, readings per replicate of 3, number of replicates of 5 and dead time of 50 ns.

5.2.2. Sample Preparation and Procedure for Purity Assessment of Butyltin Stock Solutions Using HG GC-MS

Aliquots of the 1000 - 1500 $\mu\text{g ml}^{-1}$ stocks of natural abundance TBT, DBT, MBT and Sn were diluted 10-fold in 50:50 (v/v) methanol/water. Triplicate samples of each working solution were then prepared and individually analysed. A 0.5 ml aliquot of each 100 - 150 $\mu\text{g ml}^{-1}$ Sn (or MBT, DBT and TBT) working solution was accurately pipetted into a 15 ml vial. After 1 ml of 0.6 M HCl was added, the vial was capped with a septum. A glass gas sampling syringe of nominal volume 10 μl was inserted into the vial through the septum. A 1 ml volume of a 1 % NaBH_4 in 0.02 M NaOH was injected into the vial through the septum and the hydrides so formed accumulated in the head-space from which they were sampled by the gas syringe. The sampled hydrides were immediately injected onto the GC column for separation and detection. The GC-MS program used is summarized in Table 5.1.

5.3. Results and Discussion

Preliminary studies revealed that up to 30 % loss of TBT could occur from a 100 $\mu\text{g ml}^{-1}$ standard solution kept in an open vial at 50 °C for one hour. No detectable loss was found for DBT and MBT standard solutions under these same conditions. Loss of TBT even at room temperature from acidified solutions (HCl and acetate) was also reported by Mester *et al.*⁷³⁻⁷⁴. These observations confirm that TBT is a volatile compound and care must be taken during preparation and storage of its solutions and samples to minimize possible loss and consequent errors. Experiments were therefore designed to obtain the true concentration

Table 5.1. GC-MS operating conditions

Column	HT8 (50 m x 0.22 mm x 0.28 μ l)
Split/splitless injector	Splitless mode
Injector temperature	250 °C
Oven temperature program	50°C to 270 °C at 20°C/min (temperature hold 5 min)
Transfer line temperature	290 °C
MS	HP model 5973 mass selective detector
SIM parameters	Dwell times: 50 ms for each m/z
MS quad temperature	150 °C
MS source temperature	230 °C
Transfer line temperature	250 °C

of natural abundance TBT, DBT and MBT stock solutions, which were used for the quantitation of these species in sediments or for the reverse spike isotope dilution determination of the concentration of a ^{117}Sn enriched TBT or DBT spike. The entire process is outlined in the flow chart illustrated in Figure 5.1.

Step1 employed reverse spike isotope dilution ICP-MS to determine the concentration of the enriched ^{117}Sn spike using a natural abundance Sn stock solution prepared from the high purity metal. In step 2, total inorganic Sn concentrations in the digested natural abundance TBT, DBT and MBT stock solutions were quantified by isotope dilution ICP-MS using the

Figure 5.1. Flow chart for the determination of TBT, DBT and MBT concentrations in stock solutions.

enriched ¹¹⁷Sn spike. Step 3 was used to quantify the inorganic Sn impurity in the natural abundance MBT stock solution by HG-GC-MS. In step 4, the true concentration of MBT in the natural abundance MBT stock solution was calculated. In step 5, the MBT impurity in the natural abundance DBT stock solution was determined by HG-GC-MS. Step 6 was used to obtain the true concentration of DBT in the natural abundance DBT stock solution. Inorganic Sn and DBT impurities determined in the natural abundance TBT stock solution by HG-GC-MS were quantified in step 7 and in step 8 the true concentration of TBT in the

natural abundance TBT stock solution was calculated. Once the characterizations of natural abundance BT stock solutions (steps 1 to 7) were established, these data (shown later in Table 5.2) could be used in future studies without having to repeat these determination steps. Once the true concentrations of MBT, DBT and TBT in the stock solutions were obtained, they can be used for the quantitation of BTs in sediments or for reverse spike ID determination of the enriched BT spike concentrations, as shown in step 9.

5.3.1. Concentrations of Total Sn in BT Stock Solutions

The following equation was used to quantify the concentration of total Sn (step 1 and 2 in the flow chart) in the TBT, DBT and MBT stock solutions using isotope dilution and reverse spike isotope dilution techniques:

$$C = C_z \cdot \frac{v_y}{v_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R'_n - A_z}{A_y - B_y \cdot R'_n} \cdot \frac{AW_x}{AW_z} - C_b \quad (5.1)$$

where C is the blank corrected concentration ($\mu\text{g ml}^{-1}$) of total Sn in the stock solution; C_z is the concentration of primary assay Sn standard ($\mu\text{g ml}^{-1}$); v_y is the volume (ml) of spike used to prepare the blend solution of sample and spike; v_x is the volume (ml) of butyltin stock solution used; v_z is the volume (ml) of primary assay Sn standard; v'_y is the volume (ml) of spike used to prepare the blend solution of spike and primary assay Sn standard solution; A_y is the abundance of the reference isotope (^{118}Sn) in the spike; B_y is the abundance of the spike isotope (^{117}Sn) in the spike; A_x is the abundance of the reference isotope in the sample; B_x is the abundance of the spike isotope in the sample; A_z is the abundance of the reference isotope in the primary assay standard; B_z is the abundance of the spike isotope in the primary assay standard; AW_x is the atomic weight of analyte in the sample; AW_z is the atomic weight of Sn in the standard; R_n is the measured reference/spike isotope ratio (mass bias corrected)

in the blend solution of sample and spike; R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and inorganic Sn standard; C_b is the analyte concentration in the blank ($\mu\text{g ml}^{-1}$). This equation can be simplified (equation 5.2) for all elements whose isotopic abundance is invariant in nature (hence $A_x = A_z = A_{xz}$, $B_x = B_z = B_{xz}$, $AW_x = AW_z$, i. e.) to yield:

$$C = C_z \cdot \frac{v_y}{v_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} - C_b \quad (5.2)$$

Results obtained for the concentration of total Sn in the three butyltin stock solutions are summarized in Table 5.2. A concentration of $5.60 \pm 0.21 \mu\text{g ml}^{-1}$ (mean and one standard deviation) found in procedural blanks was used to correct the results.

Table 5.2. Characterization of natural abundance butyltin stock solutions

	$(\mu\text{g ml}^{-1} \text{ as Sn})$		
	TBT stock	DBT stock	MBT stock
Total measured Sn	1523 $\pm$ 7	1259 $\pm$ 10	1087 $\pm$ 7
Inorganic Sn determined	0.95 $\pm$ 0.08	< 0.5	13.8 $\pm$ 0.8
MBT determined	< 0.5	2.9 $\pm$ 0.7	
DBT determined	34 $\pm$ 8		< 0.5
TBT determined		< 0.5	< 0.5
Corrected butyltin concentration	1488 $\pm$ 11	1256 $\pm$ 10	1073 $\pm$ 7

Results are reported as mean and one standard deviation, n=3.

5.3.2. Measured Impurities in BT Stock Solutions

Good separation of the hydrides of inorganic tin (Sn), MBT, DBT and TBT was

obtained using a slightly non-polar capillary column for HG-GC-MS, as shown in Figure 5.2.

Quantitation of Sn MBT, DBT and TBT impurities was achieved using one point external calibration prepared from their corresponding standard solutions. Results are summarized in Table 5.2. Some inorganic Sn and DBT was detected in the natural abundance TBT stock solution while only some MBT and inorganic Sn was detected in the DBT and MBT stock solutions, separately, based on analysis by HG-GC-MS. The sum of all other Sn compounds was less than 0.1 % of the BT concentrations and therefore not further considered. Poor precision (6 to 25 % RSD) of the HG-GC-MS results for impurities was obtained, likely a consequence of irreproducible manual gas sampling. However, the final TBT, DBT and MBT concentrations which were corrected for detected impurities, were not significantly affected, since impurities found in the BT stock solutions were minor.

The final natural abundance TBT, DBT and MBT concentrations of 1488 ± 11 , 1256 ± 10 and $1073 \pm 7 \mu\text{g ml}^{-1}$ (mean and calculated standard uncertainty based on error propagation) in the stock solutions, obtained by subtracting the concentrations of impurities found from the measured concentration of total Sn, are close to the volumetrically expected values of 1484 1180 and $1006 \mu\text{g ml}^{-1}$, respectively, calculated from the dissolution of the pure TBT, DBT and MBT compounds in methanol and based on the suppliers' statement of 96, 96.5 and 95 % purity, respectively.

Figure 5.2. Chromatogram of inorganic Sn, MBT, DBT and TBT hydrides obtained by injection of a mixed standard solution (0.5 ml each of $100 \mu\text{g ml}^{-1}$) using HG-GC-MS; $10 \mu\text{l}$ gas injection.

5.4. Conclusions

A unique approach, using hydride generation GC-MS, was developed to quantify the inorganic Sn, MBT, DBT or TBT impurities in solutions of natural abundance BT standards. The true concentrations of the natural abundance BT standards were obtained following correction for these impurities. The concentrations of total Sn in the natural abundance BT standards were determined by ID analysis using an enriched inorganic ^{117}Sn following closed vessel mixed acid digestion. Calibration of the enriched inorganic ^{117}Sn standard was achieved by reverse ID analysis against a natural abundance inorganic tin standard prepared from the high purity metal. Concentrations of 1488 ± 11 , 1256 ± 10 and $1073 \pm 7 \mu\text{g ml}^{-1}$ measured for TBT, DBT and MBT, respectively, in the stock solutions using the present method are close to the volumetrically expected values of 1484, 1180 and $1006 \mu\text{g ml}^{-1}$, respectively.

CHAPTER 6. DEVELOPMENT OF A GC-ICP-MS METHOD FOR THE DETERMINATION OF BUTYLTIN SPECIES IN SEDIMENT

6.1. Introduction

Although the separation of the butyltin species by HPLC is sufficient for their quantitation in PACS-2 sediment CRM, the peak shape for MBT is quite broad using this cation exchange column and thus might affect its accurate determination at low concentrations. Enhanced separation of butyltins with higher sensitivity may be required for samples containing other organotin species or much lower concentrations of MBT. As mentioned earlier, GC is one of the most popular techniques used for speciation analysis due to its high resolution for separation and high sensitivity. A GC-ICP-MS method was therefore developed for determination of BTs in sediments.

6.2. Sample Preparation

Three sample blanks, five replicate unspiked sub-samples of PACS-2 and five replicate spiked sub-samples of PACS-2 were prepared for analysis by GC-ICP-MS using the method of standard additions calibration. Each 0.5 g mass of PACS-2 (0.100 ml methanol was added to the unspiked sample and 0.100 ml of $5.00 \mu\text{g ml}^{-1}$ of TBT, DBT and $2.5 \mu\text{g ml}^{-1}$ MBT mixed standard was added for the spiked sample) with 0.100 ml of $1.00 \mu\text{g ml}^{-1}$ TPrT and 10 ml acetic acid were heated in the Prolabo microwave digester at 60 % power for 3 minutes. The contents were centrifuged at 2000 rpm for 5 minutes and a 5 ml volume of the supernatant was transferred to a 50 ml glass vial. After 10 ml of 1 M NaAc buffer solution, 5 ml ammonium hydroxide, 1 ml of 2 % NaBeT_4 and 2 ml iso-octane were added, the vial was capped and shaken manually for five minutes. The iso-octane layer was then transferred to a

2 ml glass vial for analysis by GC-ICP-MS after phase separation facilitated by centrifuging at 2000 rpm for 5 minutes.

6.3. Coupling GC to ICP-MS

The GC was coupled to the ICP-MS using a home-made interface and transfer line similar to that reported by Bayón *et al*⁶⁷, essentially consisting of a 1/4 in od. stainless steel Swagelok® “T” mounted on the top of the GC chassis where a conventional detector would normally be located. The lower arm of the “T” was connected to a concentric assembly of a 90 mm length of 15 mm od. brass rod, the latter housed inside the heated GC oven and maintained at 300 °C with the use of the detector temperature controller (no additional heating elements required). This concentric rod, threaded at each end, served as a relatively massive heat sink through which the DB-1 column was passed, as illustrated in Figure 6.1. The other arm of the “T” was connected, *via* a stainless steel reducing union, to a 110 cm length of PTFE tubing (0.02 in id. x 1/16 in od.) which served to conduct the GC effluent to the ICP-MS injector. The side arm of the “T” was used to admit a stream of unheated Ar carrier gas at a flow rate of 0.30 l min⁻¹ to aid in the rapid transfer of eluent to the ICP. The PTFE transfer line was subsequently inserted into the quartz torch injector (terminating 15 mm from the injector tip) and secured with an air-tight Swagelok (1/16 in to 1/4 in) fitting to an adapter mounted on the torch box.

Figure 6.1. Schematic diagram of the GC-ICP-MS interface

6.4. Optimization of the GC-ICP-MS System

Optimization of the ELAN6000 was first performed as recommended by the manufacturer using a standard liquid sample introduction system. A 10 ng ml^{-1} inorganic Sn standard solution was nebulized and the Ar gas flow, lens voltage and RF power were adjusted for maximum response. The plasma was then extinguished and the spray chamber and nebulizer assembly replaced with the transfer line and its adapter. The final optimization of lens voltage, RF power and Ar carrier gas flow for dry plasma conditions was performed by either monitoring the ^{120}Sn intensity over the last portion of the chromatographic run during the column cleaning cycle (holding temperature at 270°C) following injection of $1 \mu\text{l}$

of a 1000 ng ml^{-1} solution of ethylated butyltin standard in iso-octane for a quick optimization, or by the injection of $1 \mu\text{l}$ of a 25 ng ml^{-1} ethylated butyltin standard in iso-octane for more precise optimization. It is interesting to note that no significant difference in parameters for lens voltages, torch position and RF power was evident between wet and dry plasma conditions.

Various GC heating programs were initially tested to achieve good separation of the BTs as well as the shortest retention time possible. The GC-ICP-MS operating conditions used are summarized in Table 6.1.

6.4.1. The Effect of Ar Carrier Gas Flow Rate

The optimum Ar carrier gas flow rate is dependent on that of the He effluent from the GC column as the Ar was introduced through the side arm of the interface. The effect of He pressure (a surrogate for flow rate) on response was investigated by injection of $1 \mu\text{l}$ of a 25 ng ml^{-1} standard of ethylated butyltin in iso-octane. A significant effect of He pressure on butyltin retention time was observed, the latter decreasing as He pressure increased with separation slightly deteriorating. A peak for TBT was not obtained when the He pressure was reduced below 20 psi and a He pressure of 32 psi was thus selected so as to produce the shortest retention time for butyltin while maintaining good separation. An Ar carrier gas flow rate in the range of 0.2 to 1.2 l min^{-1} was investigated using a He pressure of 32 psi. Highest sensitivities were found using an Ar carrier gas flow rate in the range of 0.280 to 0.32 l min^{-1} ; sensitivities decreased significantly at either lower or higher flows. Thus, an optimum Ar carrier gas flow rate of 0.30 l min^{-1} was selected for the dry plasma conditions, resulting in a He flow rate of 1.2 ml min^{-1} .

Table 6.1. GC-ICP-MS operating conditions

	ICP-MS
Rf power	1200 W
Plasma Ar gas flow rate	15.0 l min ⁻¹
Auxiliary Ar gas flow rate	1.0 l min ⁻¹
Nebulizer Ar gas flow rate	0.30 l min ⁻¹
Sampler cone (nickel)	1.00 mm
Skimmer cone (nickel)	0.88 mm
Lens voltage	8.0 V
Scanning mode	peak hopping
Points per peak	1
Dwell time	50 ms
Sweeps per reading	1
Readings per replicate	5000
Number of replicates	1
Dead Time	50 ns
	GC
Injection mode	Splitless
Injection volume	1 μ l
Injector temperature	250 °C
Column	MXT-5 (20 m x 0.28 mm x 0.5 μ m)
Carrier gas	He at 32 psi, 1.3 ml min ⁻¹
Oven program	60 °C (1 min) to 200 °C at 20 °C min ⁻¹ to 270 °C at 30 °C min ⁻¹ (2 min)
Interface temperature	300 °C

The optimum Ar carrier gas flow rate obtained is significantly different from the optimum flow rate of 0.50 l min⁻¹ used with wet plasma conditions. A further study was conducted to determine whether a make-up gas, introduced through a T connection at the end

of the transfer line to the torch (to avoid any pressure build up at the end of GC column at the interface), would improve sensitivities. No significant improvement was observed as the make up gas flow rate was increased from 0.1 to 0.9 l min⁻¹, producing a total sample Ar flow rate of 0.4 to 1.1 l min⁻¹.

As a result of the above studies, optimization of the GC-ICP-MS system was simplified for ease of operation in the final study. The Elan6000 liquid sample introduction system was brought on line once a week to optimize the instrument. It was then removed to permit GC sample introduction. The Ar carrier gas flow rate was then optimized by injection of a 25 ng ml⁻¹ butyltin standard in iso-octane using a He pressure setting of 32 psi. No change in day-to-day optimum Ar carrier gas flow rate was observed when using the same He pressure setting and similar transfer line length.

6.4.2. Transfer Line

In preliminary studies, PTFE tubing of various diameters (0.25, 0.508 and 1.5 mm i. d.) was tested for the best sensitivity and BT peak shapes. It was found that 0.25 mm i. d. PTFE tubing resulted in a high back pressure and therefore required a much higher He pressure setting. The broadening of peak shape was observed when 1.5 mm i. d. PTFE was used and a 0.508 mm i.d. was finally selected as a result of good peak shape and sensitivity being obtained.

It is worth noting that the length of the transfer line had little effect on either the butyltin peak shape or sensitivity due to the very short residence time of the analyte in the transfer line. Therefore, for ease of handling of the GC-ICP-MS, a 110 cm long PTFE tubing was used for the final work. It was necessary to change this PTFE tubing daily to minimize significant broadening of TBT peaks. Evidently, a surface residue accumulates over the

course of the day in this unheated line, but this was not verified

6.4.3. Transfer Line Position in the Injector

A significant influence of the distance between the injector tip and the end of the transfer line on the resulting sensitivity was observed. Optimum sensitivities for all three butyltins were achieved at distances between 10 and 20 mm, decreasing at greater or shorter distances. A 15 mm distance was therefore chosen for this study. As shown in Figure 6.2, good resolution and peak profiles for all three ethylated butyltins were obtained under optimized conditions using the home-made interface and transfer line. The peak widths for the butyltin compounds ranged between 2 and 4 seconds at 10 % height, comparable to those obtained using conventional GC detectors under the given temperature program.

6.5. Determination of BTs in PACS-2 Using GC-ICP-MS

The developed method was validated by the determination of TBT, DBT and MBT in PACS-2. All three butyltin species were determined by GC-ICP-MS using a standard additions calibration methodology with tripropyltin (TPrT) as the internal standard. All sample blanks, unspiked and spiked samples were injected for GC-ICP-MS analysis immediately after their preparation. Quantitation of all three butyltins was based on the ratios obtained from the intensity of the peak area for ^{118}Sn butyltin divided by the peak area for TPrT. Concentrations of 0.93 ± 0.03 , 1.14 ± 0.02 and $0.53 \pm 0.02 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=5$) as tin in PACS-2 with precisions of 3.2, 1.7 and 3.8 % RSD were obtained for TBT, DBT and MBT, respectively. TBT and DBT concentrations measured in PACS-2 using GC-ICP-MS are in good agreement with the certified values of 0.98 ± 0.13 and $1.09 \pm 0.15 \mu\text{g g}^{-1}$ (mean and 95 % confidence interval), whereas the measured MBT.

Figure 6.2. Chromatogram of 1 μl volume of iso-octane extract of PACS-2 obtained by GC-ICP-MS

concentration is slightly higher than the certified value of $0.45 \pm 0.05 \mu\text{g g}^{-1}$.

Method detection limits (three standard deviations), based on a 0.5 g sub-sample of sediment, were estimated to be 0.006, 0.001 and $0.002 \mu\text{g g}^{-1}$ for MBT, DBT and TBT, respectively, based on ten replicate sample blanks, superior to those obtained using the HPLC-ICP-MS method described in Chapter 3.

6.6. Conclusions

A sensitive method was developed for the determination of TBT, DBT and MBT in sediments using GC-ICP-MS. Butyltin compounds were extracted with acetic acid, ethylated in aqueous solution with sodium tetraethylborate and then extracted into iso-octane. All three butyltin compounds were separated in less than 10 minutes under optimum experimental conditions. Method detection limits (three standard deviations) for a 0.5 g sub-sample of sediment were estimated to be 0.006, 0.001 and $0.002 \mu\text{g g}^{-1}$ for MBT, DBT and TBT, respectively. Overall, GC-ICP-MS provides better separation of the butyltins and better detection limits, although sample preparation for GC is somewhat more time-consuming due to the extra derivatization step needed. On the other hand, only a relatively simple extraction of the butyltins is needed for HPLC-ICP-MS analysis. Slightly better precisions of 1.7 to 3.8 % RSD for the three butyltins were obtained with GC-ICP-MS using standard additions calibration compared to 3.1 to 6.5 % RSD obtained with the HPLC-ICP-MS method.

CHAPTER 7. SPECIES SPECIFIC ISOTOPE DILUTION FOR THE DETERMINATION OF TBT IN PACS-2

7.1. Introduction

As noted earlier, over the past decade ICP-MS has often been used as a sensitive and selective detector for determination of BTs when coupled to HPLC⁴⁰⁻⁴² and GC^{33-34, 43-49}. Because of its capability for accurate ratio measurement, species specific isotope dilution has been applied to the determination of butyltin^{41-42, 45-47, 75} and other organometallic species, including organolead^{41-42, 53}, methylmercury⁷⁶⁻⁷⁹, and selenomethionine⁸⁰, using synthesized species specific spikes. Species specific ID-MS was evaluated for the determination of TBT in sediment CRMs using both HPLC- and GC-ICP-MS methods. Optimization of HPLC- and GC-ICP-MS systems was performed following procedures described in Chapter 3 for HPLC-ICP-MS and in Chapter 6 for GC-ICP-MS.

7.2. Sample Preparation

7.2.1. Sample Preparation for the Determination of TBT in PACS-2 Using HPLC-ICP-MS

Three process blanks, triplicate sub-samples of PACS-2 and six reverse spike isotope dilution calibration samples were prepared at the same time. Each 0.5 g mass of PACS-2 was spiked with 0.400 ml of ¹¹⁷Sn enriched TBT spike solution (0.40 µg ml⁻¹ as tin), along with 5 ml acetic acid and was heated in a Prolabo microwave digester at 60 % power for 3 minutes. The contents were centrifuged at 2000 rpm for 5 minutes. A 1.00 ml volume of the supernatant was transferred to a reaction vial, 1 ml DIW added and the mixture adjusted to pH 5-6 with 1.2 ml of ammonium hydroxide. The contents were then buffered with 0.8 ml of

ammonium citrate (2 M) and diluted to 10 ml with methanol. Three process blanks were prepared in the same way.

Six reverse spike isotope dilution calibration samples were also prepared, as described below. A 0.150 ml volume of ^{117}Sn enriched TBT spike solution and 0.250 ml of $2.05\ \mu\text{g ml}^{-1}$ natural abundance TBT solution were accurately pipetted into a vial. After 1 ml acetic acid and 1 ml DIW were added, the pH was adjusted to 5-6 with 1.2 ml of ammonium hydroxide. The mixture was then buffered with 0.8 ml of ammonium citrate (2 M) and diluted to 10 ml with methanol. A mass bias correction solution was prepared in the same way, without microwave heating, using 0.50 ml of $2.05\ \mu\text{g ml}^{-1}$ natural abundance TBT standard.

7.2.2. Sample Preparation for the Determination of TBT in PACS-2 Using GC-ICP-MS

For isotope dilution calibration analysis, three process blanks, five replicate subsamples of PACS-2 and six reverse spike isotope dilution calibration samples were prepared. Each 0.5 g mass of PACS-2 was spiked with 0.400 ml of ^{117}Sn enriched TBT spike solution ($0.40\ \mu\text{g ml}^{-1}$ as tin) and, along with 10 ml acetic acid, was heated in the Prolabo microwave digester at 60 % power for 3 minutes. The contents were centrifuged at 2000 rpm for 5 minutes and a 5 ml volume of the supernatant was transferred to a 50 ml glass vial. After 10 ml of 1 M NaAc buffer solution, 5 ml ammonium hydroxide, 1 ml of 2 % NaBEt_4 and 2 ml iso-octane were added, the vial was capped and shaken manually for five minutes. The iso-octane layer was then transferred to a 2 ml glass vial for GC-ICP-MS analysis. Phase separation was facilitated by centrifuging at 2000 rpm for 5 minutes.

Six reverse spike ID calibration samples were prepared as described below. In brief,

a 0.150 ml volume of ^{117}Sn enriched TBT spike solution and 0.250 ml of $2.05 \mu\text{g ml}^{-1}$ (or $1.99 \mu\text{g ml}^{-1}$) natural abundance TBT solution were accurately pipetted into a vial. After 10 ml of 1 M NaAc buffer solution, 1 ml of 2 % NaBEt_4 and 2 ml of iso-octane were added, the mixture was manually shaken for 5 minutes. After centrifuging at 2000 rpm for 5 minutes, the iso-octane layer was transferred to a small glass vial. A mass bias correction solution was prepared in the same way using 0.25 ml of $2.05 \mu\text{g ml}^{-1}$ natural abundance TBT standard. Following extraction and derivatization, all samples were immediately injected for GC-ICP-MS analysis.

7.3. Results and Discussion

To achieve accurate ratio measurements using ICP-MS, detector dead time (τ) and mass bias must be corrected⁸¹. Detector dead time is the time for which the ion counting system is unable to count incoming ions after the impact of a previous ion and is a feature of both the detector itself and the ion counting electronics⁸¹. This leads to counting losses that increase in magnitude as the counting rate increases, resulting in inaccurate isotope ratio measurements, if not accounted for. The following equation is used for this purpose:

$$I_t = \frac{I_0}{1 - I_0 \cdot \tau} \quad (7.1)$$

where I_t is the count rate corrected for dead time losses, I_0 is the count rate obtained with no dead time correction. A dead time of 55 ns was obtained following the procedure recommended by the manufacturer.

Mass bias occurs in ICP-MS when ions of different mass are transmitted through the spectrometer with different efficiencies, resulting in non-uniform sensitivity across the mass range and inaccurate isotope ratio measurements. Note that mass bias is considered here to

be entirely due to instrumental effects. It was put forward some time ago that the majority of mass bias observed in a quadrupole ICP-MS system was due to processes occurring in the interface region, including the space charge effect. The non-analyte species present in the ion beam are also said to contribute to the total mass bias. Among other effects, high concentrations of matrix elements result in an increased ion beam density, leading to greater space charge effects that exacerbate the loss of lighter ions from the ion beam, thereby enhancing the mass bias. A mass bias correction factor was calculated from the expected ratio divided by the measured ratio obtained with a natural abundance TBT standard solution.

As noted earlier, ID ICP-MS is capable of compensating for any subsequent loss of analyte during sample manipulation, suppression of ion sensitivities by concomitant elements present in the sample matrix and for instrument drift, providing equilibration has been achieved between the endogenous analyte in the sample and the added spike. In order to achieve accurate and precise results, care must be taken to avoid any contamination during the process, an interference free isotope pair must be available for ratio measurements and an optimum measurement procedure must be used to achieve accurate ratio measurements.

In this study, contamination control was effected by preparing all samples in a clean room environment. In a subsequent experiment, the $^{118}\text{Sn}/^{117}\text{Sn}$ ratio from TBT was measured in an unspiked PACS-2 extract using both HPLC-ICP-MS and GC-ICP-MS methods to investigate possible interferences on the isotopes selected for measurement. Mass bias corrected ratios of 3.148 ± 0.020 and 3.150 ± 0.015 (mean and one standard deviation) were obtained using unspiked PACS-2 when determined by HPLC-ICP-MS and GC-ICP-MS, respectively, not significantly different from the expected natural abundance ratio of 3.154. This confirmed that no significant spectroscopic interference on either isotope

arose from the sample matrix, permitting accurate results to be obtained using the chosen isotope pair.

Equilibration between added spike and the endogenous analyte in the sample is a prerequisite for achieving accurate results using isotope dilution techniques. Results would be biased low if equilibration between the spike and the sample were not achieved during the ratio measurements (with the spike being recovered with greater efficiency). Although direct proof of achievement of equilibration between the added spike and the sample is still lacking at this stage, the effect of sample mass on spike recovery can be used to help elucidate this process. A series of experiments outlined in Chapter 4 was previously conducted to study the effect of mass of sediment sub-sample on the recovery of the butyltin species spike. Sample weights of 0.15, 0.5, 1.0 and 2.0 g of PACS-2 sediment were used and recoveries of the spikes calculated. It was noted that quantitative recoveries of TBT spikes were obtained for sample mass in the range of 0.15 to 0.5 g. Recovery of TBT spikes decreased from 100 % to 83 % when sample mass increased from 0.5 g to 2.0 g. Despite these differences, no significant change in measured TBT concentrations in PACS-2 was observed using standard additions calibration. These results suggest that the added spike mimics the analyte in the sample, as equilibration between the added spike and the endogenous TBT in the sample was likely achieved during microwave assisted extraction

7.3.1. Results for TBT in PACS-2 Using ID HPLC-ICP-MS

TBT in PACS-2 was determined using HPLC-ICP-MS with ID calibration using a ^{117}Sn enriched TBT spike. The concentration of ^{117}Sn enriched TBT in the spike was determined at the same time using a reverse spike ID-MS technique based on two natural abundance TBT standard solutions, which had been previously characterized (Chapter 5). To

achieve best accuracy and precision for the ratio measurement, a mass bias correction solution was repeatedly introduced between a spiked sample and reverse spike ID sample. The mass bias correction factor was calculated using the expected ratio divided by the ratio measured with the TBT mass bias correction solution. A mass bias correction factor of 0.986 ± 0.009 (mean and one standard deviation, $n = 6$) for $^{118}\text{Sn}/^{117}\text{Sn}$ was obtained.

The following equation was used for the quantitation of TBT in PACS-2:

$$C = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} - C_b \quad (7.2)$$

where C is the blank corrected TBT concentration as Sn ($\mu\text{g g}^{-1}$) based on dry sample mass; C_z is the concentration of natural abundance TBT standard ($\mu\text{g ml}^{-1}$); v_y is the volume (ml) of spike solution used to prepare the blend solution of sample and spike; m_x is the mass (g) of sample used; v_z is the volume (ml) of natural abundance TBT standard used; v'_y is the volume (ml) of spike used to prepare the blend solution of spike and natural abundance TBT standard solution; A_y is the abundance of the reference isotope (^{118}Sn) in the spike; B_y is the abundance of the spike isotope (^{117}Sn) in the spike; A_{xz} is the abundance of the reference isotope in the sample or in the standard; B_{xz} is the abundance of the spike isotope in the sample or in the standard; R_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike; R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance TBT standard and C_b is the analyte concentration in the blank ($\mu\text{g g}^{-1}$).

A concentration of $1.010 \pm 0.008 \mu\text{g g}^{-1}$ (one standard deviation, $n=5$) as tin was obtained for TBT in PACS-2, in agreement with the certified value of $0.98 \pm 0.13 \mu\text{g g}^{-1}$ (as 95 % confidence interval). For comparison, TBT was also measured by standard additions

calibration and a concentration of $0.97 \pm 0.03 \mu\text{g g}^{-1}$ (one standard deviation, $n=5$) as tin was obtained. It is evident that the precision of 0.79 % RSD ($n=5$) for TBT measured using isotope dilution was significantly better than the precision of 3.0 % RSD obtained using standard additions calibration.

7.3.3. Results for TBT in PACS-2 Using ID GC-ICP-MS

TBT was determined in PACS-2 by GC-ICP-MS based on ID quantitation with the ^{117}Sn enriched TBT spike. All sample blanks, spiked samples and reverse spike isotope dilution samples were analyzed by GC-ICP-MS in a manner similar to that described above for HPLC-ICP-MS. The mass bias correction solution was repeatedly introduced between a spiked sample and reverse spike ID sample. A TBT concentration of $0.921 \pm 0.008 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=5$) as tin with a precision of 0.87 % was obtained, in good agreement with the certified value. TBT was also measured by standard additions calibration and a concentration of $0.93 \pm 0.03 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=5$) as tin with a precision of 3.2 % in PACS-2 was obtained. As expected, the precision of 0.87 % RSD for TBT measured using ID was significantly better than the precision of 3.2 % RSD obtained using calibration by standard additions.

7.4. Conclusions

Accurate and precise methods have been developed for the determination of TBT in sediment using both ID HPLC-ICP-MS and ID GC-ICP-MS. A 4-fold improvement in the precision of determination of the TBT concentration was achieved using ID, clearly demonstrating its superiority over quantitation by the method of standard additions, making it ideal for certification work.

CHAPTER 8. A STUDY OF THE SHORT AND LONG TERM STABILITY OF BUTYLTINS IN PACS-2

8.1. Introduction

One of the most important properties of a CRM is that the analyte concentrations remain stable over a specified period of time⁸². It is incumbent on CRM producers to provide users with information concerning proper storage and sampling procedures to be used for determination of (speciated) analytes. Long-term storage may produce a significant alteration of the chemical species content because of chemical reactions between species, interaction with the container material, microbial activity, temperature, photolysis, etc. Thus, the stability of BT species in PACS-2 CRM during storage, handling or shipment is of great concern. In this context, a series of experiments was performed in an attempt to assess the impact of varying environmental conditions on the integrity of the certified data. Long-term stability studies of BTs in PACS-2 have been undertaken over the past six years to check the integrity of this CRM. HPLC-ICP-MS was used in the initial part of a long-term study; GC-ICP-MS was used more recently.

8.2. Stability of BTs in Opened Bottles

It is obligatory that CRM producers state the shelf life and expiration date of CRMs. With this in mind, the stability of BTs in opened bottles of PACS-2 was investigated. Four different scenarios were investigated: A – the PACS-2 bottle was “opened”, resealed and left on the shelf at room temperature for 2 years; B – the PACS-2 bottle was “opened”, closed and subsequently wrapped in aluminum foil to minimize exposure to light and left standing on the laboratory shelf at room temperature for 2 years; C – after the bottle was “opened”, it

was then resealed and refrigerated for 2 years at 4°C; D – a new bottle of PACS-2 was used as a control sample, obtained from refrigerated, dark storage (4 °C).

Sample preparation followed the method described in Chapter 4 using the Prolabo open vessel microwave digester. A 0.5 g sub-sample of PACS-2 along with 5 ml acetic acid was heated in the Prolabo microwave digester at 60 % power for 3 minutes. The contents were centrifuged at 2000 rpm for 5 minutes and a 1.00 ml volume of the supernatant was transferred to a reaction vial along with 1 ml DIW. The mixture was adjusted to pH 5-6 with 1.2 ml of ammonium hydroxide, buffered with 0.8 ml of ammonium citrate (2 M) and then diluted to 10 ml with methanol.

Quantitation of BTs was performed by standard additions calibration using HPLC-ICP-MS. Results obtained are summarized in Table 8.1.

Table 8.1. Effect of environmental factors on the stability of butyltin species in opened bottles

Sample type	Measured ($\mu\text{g g}^{-1}$ as Sn)*		
	MBT	DBT	TBT
Type A	0.79±0.03	0.83±0.04	0.72±0.04
Type B	0.75±0.03	0.80±0.01	0.72±0.02
Type C	0.44±0.02	1.06±0.04	0.97±0.02
Type D	0.45±0.02	1.11±0.03	0.96±0.02
Certified	0.45±0.05	1.09±0.15	0.98±0.13

* Results are reported as mean and one standard deviation, n=3.

It is clear that when the sample is stored at room temperature, in the absence or presence of light, the integrity of both DBT and TBT is degraded after 2 years. In contrast,

the relative level of MBT increased by more than 70% during this period of time. On the other hand, if the sample has been properly refrigerated, the species concentrations remain reliable even after 2 years. All these observations point to the need for proper storage of samples in the laboratory once samples are opened. In the case of speciated materials, their careful handling as well as proper refrigeration is needed to ensure reliable results.

8.3. Short-Term Stability Study of BTs in New Bottles

A short-term stability study is required to monitor the stability of BTs in PACS-2 during transport under possibly increased temperature conditions. The maximum real time duration of transport may be two to three weeks using normal surface transportation. Thus, a one month test period was used for this study. Nine bottles of unopened PACS-2, which were stored in - 20°C, were used. Three different scenarios were investigated: E – three new PACS-2 bottles were stored at - 2°C for one month; F – three new PACS-2 bottles were stored at room temperature for one month; G – three new PACS-2 bottles were stored at 40°C for one month (worst case scenario representing shipment to a tropical zone where temperatures may reach 40°C).

Sample preparation followed the procedure described in section 6.2 using the Prolabo open vessel microwave digester. Quantitation of BTs was performed by standard additions calibration using GC-ICP-MS in order to minimize consumption of the small quantity of enriched TBT and DBT spikes (despite more accurate and precise results achievable by ID). Results are summarized in Table 8.2.

Table 8.2. Results of short-term stability study of BTs in PACS-2

Sample type	Measured ($\mu\text{g g}^{-1}$ as Sn)*		
	MBT	DBT	TBT
Type E	0.559 $\pm$ 0.030	1.052 $\pm$ 0.013	0.910 $\pm$ 0.030
Type F	0.524 $\pm$ 0.080	1.032 $\pm$ 0.014	0.892 $\pm$ 0.029
Type G	0.728 $\pm$ 0.089	1.040 $\pm$ 0.029	0.823 $\pm$ 0.055
Certified	0.45 $\pm$ 0.05	1.09 $\pm$ 0.15	0.98 $\pm$ 0.13

* Results are reported as mean and one standard deviation, n=6.

No significant change in DBT concentrations occurs in PACS-2 bottles stored at these temperatures for one month. Degradation of TBT and increased concentration of MBT were observed in bottles stored at 40°C for one month. There were no significant changes in concentrations of BTs in bottles stored at room temperature, suggesting that they are stable during usual shipping periods (a few days). However, shipping to tropical countries may require that PACS-2 be maintained under cold conditions to guarantee its quality.

8.4. Long-term stability study of BTs in PACS-2

A study of long-term stability provides important information on the integrity of the product over the entire lifetime of the material, useful to CRM producers that need to determine expiry dates and establish the frequency at which recertification is required, especially for CRMs used for speciation. PACS-2 was γ -irradiated with a minimum dose of 2.5 Mrad to eliminate possible biological activity, initially stored at 4 °C for the first five years and then stored at -20 °C since October, 2003. The stability of the butyltins in PACS-2 was periodically examined over the past six years and the results are presented in Figure 8.1, wherein the error bars correspond to one standard deviation.

Early results (first 42 months, dots in Figure 8.1) were obtained using standard additions calibration with HPLC-ICP-MS detection. Owing to its high resolution and sensitivity, GC-ICP-MS methodology has also been applied to butyltin measurements (diamonds and triangles in Figure 8.1). Concentrations for TBT and DBT obtained by GC-ICP-MS agree with early results obtained by HPLC-ICP-MS. Access to enriched species specific TBT and DBT spikes has enhanced the precision and accuracy of recent data relating to this stability study. A significant improvement in precision of measurement of TBT and DBT (triangles in Figure 8.1) by ID is evident compared to their concentrations obtained by standard additions calibration.

Overall, no significant changes are evident for concentrations of DBT in new bottles of PACS-2 which have been stored at 4 °C for the first five years and then at -20 °C since October, 2003 (following irradiation treatment). It is evident that TBT in new bottles of PACS-2 has remained stable during the first five years. TBT in PACS-2 may have undergone some degradation during storage over the last year. More frequent monitoring of all BTs in PACS-2 CRM may be needed in the future and recertification of PACS-2 is recommended.

Figure 8.1. Long term stability of PACS-2. ●: HPLC-ICP-MS data; ♦: GC-ICP-MS data; ▲: isotope dilution GC-ICP-MS data; error bars represent one standard deviation characterizing at least 6 replicate measurements; solid line is PACS-2 CRM certificate value for each analyte whereas dashed line is the associated 95 % confidence interval

The first results (diamond in Figure 8.1 at year 4) obtained for MBT using the method of standard additions GC-ICP-MS are significantly different from the concentrations of MBT (dots) determined earlier using HPLC-ICP-MS. It was difficult to judge at that time whether the concentration of MBT had increased with storage time or if the difference was due to a bias between the methods used. Thus, a freshly prepared stock solution of MBT and a new bottle of PACS-2 were selected for the isochronous determination of MBT using both GC-ICP-MS and HPLC-ICP-MS. The reason for need of a fresh stock solution of MBT was due to an earlier observation that a $100 \mu\text{g ml}^{-1}$ solution of MBT in methanol suffered a 30 % decrease in concentration after approximately one year of storage at 4°C . A 10 % decrease in a $1 \mu\text{g ml}^{-1}$ solution of MBT in methanol stored in a freezer (-20°C) was also measured in preliminary studies. A concentration of $0.454 \pm 0.023 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=4$) as tin for MBT was determined in the new bottle of PACS-2, in good agreement with previous concentrations of MBT determined in PACS-2 using the HPLC-ICP-MS method (dots in Figure 8.1). By contrast, a concentration of $0.565 \pm 0.026 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=4$) as tin for MBT in the same bottle of PACS-2 was determined by GC-ICP-MS, in agreement with previous results for MBT (diamond at year 4) generated using this GC-ICP-MS methodology. Most likely, results obtained for MBT using HPLC-ICP-MS may be biased low; a much broader peak is obtained for MBT when the cation column is used for separation but the reason for the bias remains unknown at this time. Further investigations require an isotopically enriched spike of MBT to take advantage of the benefits offered by ID in overcoming any possible matrix effects in the sample and any potential losses of analyte during sample preparation following extraction and derivatization. Nevertheless, results obtained by both methods for MBT suggest that it has been stable in

PACS-2 over the first five years of storage but has increased significantly during the past year of storage.

The long-term stability data suggest that release of a set of revised certified values for TBT and MBT in PACS-2 sediment is needed. All remaining stocks of PACS-2 have been stored at -20 °C since October, 2003 to minimize any further changes in concentration of the BTs in this material.

8.5. Conclusions

A study of the stability of BTs in PACS-2 reveals that periodic monitoring of their concentrations permits CRM producers to track the integrity of such samples. Long-term stability data suggest that the material is stable for the first five years after its initial γ -irradiation, providing storage at 4 °C was achieved, although some degradation of TBT and an increase in MBT have occurred during this time. As colder storage conditions may be beneficial, in terms of shelf life for this CRM, remaining PACS-2 samples have been stored at -20 °C since October, 2003. Re-certification of the BT content may be required every few years. Once bottles have been opened, they should be placed in cold storage and can be used for at least another year. Shipping PACS-2 to tropical locations may require cold transit conditions be maintained to avoid any changes in the concentrations of the BTs.

CHAPTER 9. EFFECTS OF GAMMA-STERILIZATION ON HOMOGENEITY AND CONTENT OF BUTYLTIN IN SEDIMENTS

9.1. Introduction

As noted earlier, CRMs are produced to exacting standards (ISO Guide 34) and users must be guaranteed stability and homogeneity of analyte content. The preservation of homogenous materials and the stability of chemical species in CRMs during storage are of major concern to all CRM producers and users.

In general, a stabilization step is necessary to ensure the long-term integrity of CRMs, and in the case of sediment CRMs, this may be accomplished by a number of means. Simple deep-freezing is possible, but this approach complicates subsequent transport and storage. Pasteurisation and gamma irradiation have thus been preferred choices used to minimize possible biological activity that may be present prior to final storage under cold conditions (e. g., 4 °C or -20 °C). Pasteurization was used by the European Commission in the production of BCR 462 sediment in 1996 (certified for butyltins) followed by storage of the material at 4 °C⁸³. Both TBT and DBT were not stable under these conditions and the material required recertification in 1998, after which storage at -20 °C was recommended⁸³.

Gamma irradiation has been widely used to kill or reduce the numbers of pathogenic or spoilage organisms in food and to control infestation in stored products. Similarly, it can be used to minimize any biological activity in materials intended as CRMs, as was demonstrated for methylmercury in BCR 580 sediment⁸⁴ and is currently utilized by the NRC for production of their CRMs⁸⁵. More specifically, γ -irradiation (2.5 Mrad minimum dose) was used in the production of marine sediment CRM PACS-2 (certified for all three

butyltins) and for candidate sediment CRMs, HIPA-1 (for TBT) and SOPH-1 (for TBT and DBT) arising from recent international intercomparison programs⁸⁶.

Degradation of butyltin compounds due to irradiation has earlier been reported: 10% degradation of DBT in PVC polymers at 2.5 Mrad dosage was noted by Allen *et al.*⁸⁷. Similarly, some initial loss of TBT following irradiation (by comparison to non-irradiated samples) was also detected during the production of proposed NRC sediment CRM HIPA-1. Thus, the effects of γ -irradiation on degradation of butyltins in calibration solutions and sediment matrices were investigated in detail to elucidate the extent of loss and assess species inter-conversion. The GC-ICP-MS method was used for the determination of the BTs in these samples. Optimization of the GC-ICP-MS system followed the procedure described in Chapter 6 with similar operating conditions being used.

9.2. Sample Preparation

9.2.1. Sample Preparation for Irradiated Standard Solutions and Control Standard Solutions

Irradiation effects on butyltin standards, including any loss or species inter-conversions during irradiation, were investigated. At least six replicates of 5 ml of each standard solution of TBT, DBT and MBT from the same $1 \mu\text{g ml}^{-1}$ stock solution of TBT, DBT and MBT were prepared in brown glass bottles fitted with screw caps. Three bottles of each TBT, DBT and MBT standard solution were kept in a separate container without irradiation treatment and used as control samples, all other bottles were irradiated. After irradiation, both test and control samples were stored in a freezer at -20°C until analysis.

Sample preparation for GC-ICP-MS analysis of these solutions was as follows: six 0.100 ml sub-samples of each of the above standard solutions were pipetted into glass vials

along with 0.100 ml of $2 \mu\text{g ml}^{-1}$ TPrT internal standard. Into the last three vials, 0.200 ml each of $0.500 \mu\text{g ml}^{-1}$ of standard solutions of TBT, DBT and MBT was added. The vial was capped after 10 ml of 1 M NaAc buffer solution, 1 ml of 2 % NaBEt₄ and 2.0 ml iso-octane were added. Derivatization was complete after 5 minutes shaking. Samples were centrifuged at 2000 rpm for 5 minutes and the iso-octane layer was pipetted into a small glass vial and stored in the fridge at 4 °C overnight until GC-ICP-MS analysis the following day. Quantitation of the three butyltin compounds was accomplished using one point standard additions calibration based on peak area response. Three sample blanks were also prepared according to the above procedure.

9.2.2. Sample Preparation for Irradiated Spiked HISS-1 and Control HISS-1

Samples

Sediment CRM HISS-1 contains no detectable butyltins and was thus used to prepare spiked samples to investigate irradiation effects on individual butyltins spiked into the sediment matrix. Individual spiked HISS-1 samples were prepared by adding 0.100 ml of $10 \mu\text{g ml}^{-1}$ of each standard solution of TBT, DBT and MBT and 5 ml methanol to a 1 g sub-sample of HISS-1 in a brown glass bottle to form a homogeneous suspension. All samples were then air dried in a laminar flow fume hood. Half of the spiked HISS-1 samples were irradiated and the remainder served as control samples. After irradiation, both samples were stored in a freezer at -20 °C until analysis.

The sediment extraction procedure used has been described in Chapter 6. Three 5 ml volumes of acetic acid were added to the bottle and used to rinse a 1 g sediment sub-sample into the Prolabo microwave digestion vessel and 0.5 ml of $2 \mu\text{g ml}^{-1}$ TPrT internal standard was added. Following the digestion, two 5.00 ml volumes of the supernatant were

transferred to each of two glass vials and 0.050 ml of a $10 \mu\text{g ml}^{-1}$ standard solution of TBT, DBT and MBT was added to the second vial. The derivatization step was performed as described in section 9.2.1. The iso-octane layer was taken for analysis by GC-ICP-MS. Three sample blanks were prepared in an identical manner.

9.2.3. Sample Preparation for SOPH-1 Sediment

Preparation of the sediment sample was similar to that described above. TBT and DBT in PACS-2 and SOPH-1 were determined by ID calibration and MBT was determined by standard additions calibration. For determination of TBT and DBT, 0.5 g sub-samples of SOPH-1 were spiked with an appropriate amount of a ^{117}Sn enriched solution of TBT and DBT. Three sample blanks were also prepared and derivatized.

The concentrations of TBT and DBT in the ^{117}Sn enriched spike were measured by reverse spike isotope dilution against two independent natural abundance TBT and DBT standards. Due to the presence of DBT impurity in natural abundance TBT standard, and vice versa, a reverse spike isotope dilution was performed sequentially for TBT and DBT. For this purpose, an 0.100 ml volume of each ^{117}Sn enriched TBT and DBT spike solution was accurately pipetted into twelve vials. To the first six vials, 0.100 ml of $0.4896 \mu\text{g ml}^{-1}$ (or $0.5135 \mu\text{g ml}^{-1}$) natural abundance TBT solution was added, whereas 0.100 ml of $0.5063 \mu\text{g ml}^{-1}$ (or $0.5010 \mu\text{g ml}^{-1}$) natural abundance DBT solution was added to the last six vials. After 10 ml of 1 M NaAc buffer solution, 1 ml of 2 % NaBEt_4 and 2 ml of iso-octane were added, the vial was capped and the mixture was manually shaken for 5 minutes. Following separation of the phases, the iso-octane layer was transferred to a small glass vial. A mass bias correction solution was prepared in the same way using 0.10 ml of 0.5035 and $0.5010 \mu\text{g ml}^{-1}$ natural abundance TBT and DBT standards.

Standard additions calibration was used for the quantitation of MBT in both sediments because an enriched MBT spike was not available at the time. Sub-samples of 0.5 g PACS-2 or SOPH-1 (an appropriate amount of MBT standard was added for the spiked sample) along with 0.125 ml of $2 \mu\text{g ml}^{-1}$ natural TPrT internal standard and 10 ml acetic acid were heated in the Prolabo microwave digester at 60 % power for 3 minutes. Subsequent derivatization of the extract was performed as described above.

Following injection of the sample into the GC, data acquisition on the ELAN6000 was manually triggered. Simultaneous monitoring of ^{117}Sn , ^{118}Sn and ^{120}Sn and calculation of their peak areas were used to generate $^{118}\text{Sn}/^{117}\text{Sn}$ ratios, from which the analyte concentration in the sediment was calculated. The peak area of ^{120}Sn was used for calculating the concentration of MBT.

9.3. Results and Discussion

9.3.1. Homogeneity Study of SOPH-1 Sediment

SOPH-1 sediment was prepared from a blend of HISS-1 and PACS-2 CRMs, given a 2.5 Mrad dose of γ -irradiation and stored at -20°C . To investigate the effect of γ -irradiation on homogeneity of TBT and DBT in this sediment, twelve bottles of SOPH-1 were randomly selected and the TBT and DBT were quantitated in 0.5 g sub-samples by isotope dilution calibration with GC-ICP-MS using ^{117}Sn enriched TBT and DBT spikes for the best accuracy and precision. Equation 7.1 (Chapter 7) was used for quantitation of TBT. Better than 2% RSD in both TBT (1.8 % RSD) and DBT (1.6 % RSD) concentrations measured in twelve different bottles of SOPH-1 were obtained, indicating the material to be homogeneous at the 0.5 g level of sub-sampling. Furthermore, one may conclude that there was no inhomogeneity induced by γ -irradiation.

Figure 9.1. Chromatogram of a mixed natural abundance standard solution obtained by GC-ICP-MS; 100 pg each of TBT, DBT, TprT and MBT injected in 1 μl iso-octane

9.3.2. Effects of Irradiation on Standard Solutions

Quantitation of TBT, DBT and MBT in these standard solutions was undertaken using standard additions calibration with GC-ICP-MS detection. As shown in Figure 9.1, chromatographic separation of all three butyltin species and the TPrT internal standard was obtained under the chosen experimental conditions. The early peak appearing at 525s is an impurity artifact (unidentified) arising from use of the TPrT internal standard. Standard additions calibration was selected over isotope dilution calibration in an effort to minimize use of the limited amount of enriched TBT and DBT spikes available.

The linearity of the calibration curve was verified to encompass response from additions of approximately 1- and 2-fold the concentrations of TBT, DBT and MBT in the test solutions. Quantitation of all three butyltins was based on the calculated ratios of the measured intensities of the ^{120}Sn butyltin peak areas divided by the peak area for TPrT. The correlation coefficients for the standard additions calibration curves for each butyltin species were in the range of 0.9978 to 0.9998. No significant difference was found in the butyltin concentrations obtained using one point addition or multiple additions. For simplicity, subsequent butyltin concentrations in standard solutions were quantified using a single addition.

The concentrations of impurities (e. g., MBT and DBT as impurities in a TBT standard solution) obtained in each solution of non-irradiated butyltin standard were used for blank correction in both irradiated and non-irradiated test solutions. Blank corrected butyltin

Figure 9.2. Effects of irradiation on butyltin standards. Left bar: non-irradiated standard solutions; right bar: irradiated standard solutions; error bars represent one standard deviation characterizing at least three replicate measurements.

concentrations were then normalized to the standard concentration measured in the non-irradiated solution (e. g., normalized to TBT for TBT test solution). Results are presented in Figure 9.2.

From the data in Figure 9.2a, it is clear that only 10 % of the TBT present in the 1 $\mu\text{g ml}^{-1}$ solution of TBT remained after irradiation compared to that in the TBT control solution. No significant increase in DBT and MBT was detected in the irradiated TBT solution, suggesting TBT was degraded directly to inorganic tin. Less than 1 % of the initial DBT remained in the irradiated solution of DBT compared to that in the DBT control solution (Figure 9.2b). No significant increase in TBT or MBT was observed in the irradiated DBT solution, suggesting DBT was also degraded directly to inorganic tin. Similarly, approximately 80 % of the spiked MBT degraded to inorganic tin during the irradiation treatment (Figure 9.2c). It can be concluded that, in methanolic solutions, no butyltin species inter-conversions occur and all three butyltins degrade to inorganic tin during irradiation. The most severe effect occurs with DBT.

9.3.3. Effects of Irradiation on Spiked HISS-1 Sediment

Since CRM HISS-1 sediment contains no detectable amounts of TBT, DBT or MBT, it was used to prepare individual spiked samples to study the effects of γ -irradiation on the butyltin species present in a sediment matrix. Results were treated in a manner identical to the solutions described above, with data presented in Figure 9.3. Compared to an irradiated TBT solution (90 % loss in Figure 9.2a), significantly less degradation of TBT occurred (38 % loss) in the presence of the HISS-1 matrix, as shown in Figure 9.3a. No commensurate

Figure 9.3. Effect of irradiation on spiked HISS-1. Left bar: non-irradiated spiked HISS-1; right bar: irradiated spiked HISS-1; error bars represent one standard deviation characterizing at least three replicate measurements.

increase in DBT and MBT was detected in the irradiated TBT HISS-1 spiked with TBT, indicating that TBT degraded directly to inorganic tin, as was the case in the methanolic solutions. The sediment matrix appears to possess a shielding effect, which serves to preserve the TBT during irradiation.

Similarly, much less degradation of DBT (32 %) in HISS-1 spiked with DBT (Figure 9.3b) was observed in comparison to an irradiated DBT solution (close to 100 % loss; Figure 9.2b). A noteworthy conclusion can also be drawn: there is a significant increase in MBT in irradiated HISS-1 spiked with DBT as compared to the non-irradiated control samples, suggesting that a fraction of the DBT is converted to MBT during the irradiation treatment. Furthermore, no significant difference was detected in the sum of the three butyltin concentrations in the irradiated HISS-1 compared to the control samples, suggesting that the product of degradation of DBT is only MBT.

No significant change in concentration of MBT in the irradiated HISS-1 (Figure 9.3c) was detected compared to the 80 % loss of MBT which occurs upon irradiation of methanolic solution of MBT. Once again, this suggests that the sediment matrix serves to protect or minimize degradation of the butyltins or that irradiation of methanol induces a substantial population of radicals capable of homogeneous degradation of the analytes.

9.3.4. Effects of Irradiation on SOPH-1 Sediment

Figure 9.4 presents results for the concentrations of TBT, DBT and MBT in irradiated SOPH-1 normalized to their concentrations determined in the non-irradiated samples. Approximately 16 % of TBT and 10 % of DBT were lost following irradiation. By contrast,

Figure 9.4. Effects of irradiation on SOPH-1. Left bar: non-irradiated SOPH; right bar: irradiated SOPH-1; error bars represent one standard deviation characterizing at least six replicate measurements.

a 14 % increase in MBT concentration in the irradiated sample was measured. No significant difference was detected in the sum of concentrations of the DBT and MBT in irradiated and non-irradiated SOPH-1, suggesting that the loss of DBT in irradiated SOPH-1 results from its conversion to MBT, producing a 14 % gain in its content of MBT. TBT in SOPH-1 may be converted to inorganic Sn during irradiation, as was noted for the irradiated HISS-1 spiked with TBT. It is clear that irradiation should not be applied to real samples for the purpose of preserving butyltins *before* analysis in any environmental monitoring study, but will provide a useful approach for the preparation of CRMs.

9.4. Conclusions

Significant conversion of TBT (90 %), DBT (100 %) and MBT (80 %) to inorganic tin occurs during γ -irradiation of methanolic solutions of these standards. Some degradation of TBT (38 %) and DBT (32 %), but no significant change in MBT content was found using adulterated CRM HISS-1 sediment. Conversion of DBT to MBT in spiked HISS-1 was detected. Much smaller degradation of TBT (16 % loss) and 10% loss of DBT by conversion to MBT (14 % gain) were registered using SOPH-1 sediment. Despite some initial losses of TBT and DBT due to irradiation, better than 2% RSD in both TBT and DBT concentrations measured in twelve different bottles of blended sediment SOPH-1 (0.5 g sub-sample size) were obtained, indicating the material may be considered homogeneous.

CHAPTER 10. DEVELOPMENT OF A SPME-GC-ICP-MS METHOD FOR THE DETERMINATION OF TBT IN SEDIMENT

10.1. Introduction

During the past decade, GC-ICP-MS has become one of most popular and sensitive methods used for the determination of BTs. Unfortunately, sample preparation for GC analysis is typically time-consuming and organic solvents used for liquid-liquid extraction are frequently toxic. In an effort to simplify sample preparation for GC analysis, solid phase microextraction (SPME) was introduced by Pawliszyn and co-workers⁵⁴⁻⁵⁵ in the early 1990s. Since 1993, following the commercialization of SPME, this sampling technique has enjoyed widespread acceptance as a consequence of its simplicity, relatively low cost and ease with which analytes can be sampled and transferred to the GC column.

As shown in Figure 10.1, a small-diameter fused-silica fiber, coated with the extraction phase (usually less than 1 μ l), is mounted in a syringe-like device for protection and ease of handling. The needle serves to conveniently pierce septa during sample extraction and desorption operations. With the syringe-like mechanism of the holder unit, the fiber can be extruded from the needle to expose the extraction phase to the sample (headspace or liquid). After the sampling, the fiber can be withdrawn into the needle for transport.

Transfer of analytes from the matrix to the extraction medium begins as soon as the coated fiber has been placed in contact with the sample. In most cases, SPME extraction is considered to be terminated when the analyte concentration has reached distribution equilibrium between the sample matrix and the fiber coating.

Figure 10.1. SPME device

In practice, this means that the amount extracted is constant, within the limits of experimental error, and is independent of further increases in extraction time. When only three phases, the fiber coating, headspace and sample are considered, under the condition that the sample volume is significantly larger than the volume of the coating, the following equation can be used to calculate the total mass of analyte extracted by the coating^{55, 57}:

$$n = K_{cs} V_c C_0 \quad (10.1)$$

where n is the mass of the analyte absorbed on or in the coating, K_{cs} is the fibre coating/sample matrix distribution constant, V_c is the volume of coating and C_0 is the initial concentration of analyte in the sample matrix. From equation 10.1, it is evident that it is not

necessary to sample a well-defined volume of sample because the amount of analyte extracted by the fiber coating is independent of the sample volume. Therefore, SPME sampling can be easily adapted to field applications and can be used for direct sampling of unknown sample volumes. The amount of analyte extracted will correspond directly to its concentration in the matrix, without being dependent on the sample volume.

Since SPME is an equilibrium technique, and therefore does not extract target analytes exhaustively, its presence in a system should not result in any significant disturbance. It should be noted, however, that the fraction of analyte extracted increases as the ratio of coating volume to sample volume increases. Complete extraction can be achieved for small sample volumes when distribution constants are large and analyte concentrations are low. This can be used to advantage if exhaustive extraction is required. When equilibration times are excessively long, shorter extraction times can be used but the extraction time and mass transfer conditions have to be strictly controlled to assure good precision. Small variations in the extraction time do not affect the amount of analyte extracted by the fiber at equilibrium, whereas even small variations in the extraction time may result in significant variations in the amount extracted when non-equilibrium sampling is selected. The relative error becomes larger the shorter the extraction time.

Two types of extraction can be performed using SPME: direct and headspace extraction. In the direct extraction mode, the coated fiber is inserted into the sample medium and the analytes are transferred directly to the extraction phase. Usually some level of agitation is required to enhance transport of the analytes from the bulk of the solution to the vicinity of the fiber to facilitate rapid extraction. In the headspace mode, the SPME fiber is located above the sample and the analytes are transported through the gas phase before they

can reach the coating. This approach serves primarily to protect the fiber coating from damage by high molecular-weight species and other non-volatile concomitants present in a liquid sample matrix. Headspace sampling also allows modification of the matrix, such as a change of the pH, without damaging the fiber. Amounts of analyte extracted into the coating from the same vial at equilibrium using direct and headspace sampling are identical, as long as sample and gaseous headspace volumes are the same and equilibrium is achieved. This is a result of the fact that the equilibrium concentration is independent of location of the fiber in the system.

The application of solid phase microextraction as an elegant, solvent-free sample preparation technique for determination of TBT was therefore investigated with the objective of taking advantage of ID to overcome generally poor precision associated with this technique (typically 10 % RSD). The method was applied to the determination of TBT in PACS-2 marine sediment CRM to demonstrate the high accuracy and enhanced precision offered by ID SPME GC-ICP-MS.

10.2. Sample Preparation and Procedure for Analysis

The determination of TBT in PACS-2 and its quantitation in the ^{117}Sn enriched TBT solution by ID were conducted on two consecutive days. During day 1, six reverse spike isotope dilution calibration samples were prepared in a class-100 clean room and subsequently analyzed using GC-ICP-MS. For this purpose, a 0.150 ml volume of ^{117}Sn enriched TBT spike solution and 0.250 ml of $2.05\ \mu\text{g ml}^{-1}$ (or $1.99\ \mu\text{g ml}^{-1}$) natural abundance TBT solution were accurately pipetted into a vial. After 10 ml of 1 M NaAc buffer solution, 1 ml of 2 % NaBEt_4 and 2 ml of iso-octane were added, the mixture was manually shaken for 5 minutes. After separation of the phases, the iso-octane layer was

transferred to a small glass vial. A mass bias correction solution was prepared in the same way using 0.25 ml of $2.05 \mu\text{g ml}^{-1}$ natural abundance TBT standard. All samples were injected onto the GC for ICP-MS analysis immediately following extraction and derivatization.

Three sample blanks and five samples of PACS-2 were prepared at the same time. Sub-samples of 0.5 g PACS-2 spiked with 0.250 ml of a ^{117}Sn enriched TBT and 10 ml of acetic acid were heated in a Prolabo microwave digester at 60 % power for 3 minutes. The contents were transferred to centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. Only a 50 μl volume of the supernatant (due to the high sensitivity of SPME GC-ICP-MS) was transferred to a 50 ml glass vial for quantitation. After 10 ml of 1 M NaAc buffer solution, 0.05 ml ammonium hydroxide, 10 ml of DIW and 1 ml of 2 % NaBEt_4 were added, the vial was capped with a PTFE coated silicone rubber septum. The SPME needle was inserted through the septum and headspace sampling was performed for 15 minutes. During the extraction, the solution was vigorously stirred with a Teflon coated magnetic stir bar. The collected analyte was then desorbed from the SPME fiber onto the GC column. A one minute desorption time at an injector temperature of 250 $^{\circ}\text{C}$ ensured complete desorption from the fiber.

Following injection of the sample onto the GC, data acquisition on the ELAN6000 was manually triggered. Both ^{117}Sn and ^{118}Sn were simultaneously monitored. The mass bias solution was introduced between samples to permit a mass bias correction factor to be determined.

10.3 Results and discussion

10.3.1. Reverse Isotope Dilution for the Quantitation of ^{117}Sn enriched TBT Spike

Solution

More accurate and precise results can be obtained if isotope dilution and reverse ID experiments are performed on the same day following the sequence developed by Watters *et al.*⁷², wherein a spiked sample and reverse spike isotope dilution sample are bracketed between mass bias drift correction solutions. Unfortunately, this generally cannot be achieved on the same day due to the time required for all measurements. Thus, reverse ID analysis was conducted first on the first day. To achieve best accuracy and precision for the ratio measurement, the mass bias correction solution was repeatedly injected onto the GC between reverse spike ID samples. The following equation was used to calculate the concentration of the ¹¹⁷Sn enriched TBT spike:

$$C_y = C_z \cdot \frac{m_z}{m'_y} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot \frac{AW_y}{AW_{xz}} \quad (10.2)$$

where C_y is the concentration of the ¹¹⁷Sn enriched TBT ($\mu\text{g ml}^{-1}$) in the spike; C_z is the concentration of natural abundance TBT standard ($\mu\text{g ml}^{-1}$); m_z is the volume (ml) of natural abundance TBT standard used; m'_y is the volume (ml) of spike used to prepare the blend solution of spike and natural abundance TBT standard solution; A_y is the abundance of the reference isotope (¹¹⁸Sn) in the spike; B_y is the abundance of the spike isotope (¹¹⁷Sn) in the spike; A_{xz} is the abundance of the reference isotope in the sample or in the standard; B_{xz} is the abundance of the spike isotope in the sample or in the standard; R'_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance TBT standard; AW_y is the atomic weight of the analyte in the spike; and AW_{xz} is the atomic weight of analyte in the sample or standard. A concentration of $0.4075 \pm 0.0034 \mu\text{g ml}^{-1}$ as tin (one standard deviation, $n=6$) was obtained for the ¹¹⁷Sn enriched TBT spike

solution. This value was later used to quantify the concentration of TBT in PACS-2 sediment.

10.3.2. Optimisation of SPME Sampling for Determination of TBT.

For convenience, SPME sampling was conducted in a regular fumehood. Headspace sampling was chosen to minimize exposure of the SPME fiber to the sample matrix, thereby enhancing the life time of the fiber. As SPME extraction can be influenced by a number of factors, including extraction temperature, extraction time, pH of the solution and the concentration of derivatization reagent, derivatization of BT species and SPME sampling were performed at room temperature for simplicity.

Equilibration time, which is the time required for the amount of analyte extracted to reach a steady-state, is one of the most important parameters influencing SPME extraction. At equilibrium, variations in the extraction time will not affect the amount of analyte extracted by the fiber, thereby providing the best measurement precision⁵⁵. Thus, the effect of extraction time in the range of 2 to 30 minutes was investigated by sampling 0.100 ml of a 20 ng ml⁻¹ standard solution of TBT.

As shown in Figure 10.1a, the ¹¹⁸Sn signal for TBT increased sharply as extraction time increased from 2 to 8 minutes and thereafter slowly increased after 10 minutes. However, even a 30 minute extraction time was not sufficient to achieve an equilibrium distribution of derivatized TBT between the matrix and the PDMS fiber. A compromise condition of fifteen minutes extraction time was therefore chosen to shorten the analysis time

Figure 10.2. Effects of extraction time and sample pH on TBT response using SPME sampling; a: extraction time effect; b: pH effect.

as much as possible but retain enough sensitivity for precise measurements.

The effect of sample pH in the range of 2 to 8 on response was investigated. Highest response from 0.1 ml of a 20 ng ml⁻¹ solution of TBT was obtained over the range of pH 4 to 6, as shown in Figure 10.1b, with sensitivity decreasing at both lower and higher pH. A pH of 5, obtained using a 1 M NaAc buffer solution, was selected for use.

The influence of amount of NaBEt₄ used on response was evaluated; no significant effect was detected using 0.2 to 1.5 ml of 1 % NaBEt₄. A one ml volume of 1 % NaBEt₄ was used to ensure complete ethylation of analyte in real sample matrices.

10.3.3. Results for TBT in PACS-2 Using ID SPME GC-ICP-MS

Application of ID with SPME sampling, GC separation and ICP-MS detection provided good separation of all three butyltin species present in the PACS-2 sediment, as shown in Figure 10.2. All sample blanks and spiked samples were analyzed immediately after they were prepared. An average concentration of $0.00041 \pm 0.00003 \mu\text{g g}^{-1}$ (one standard deviation, n=3), obtained from three sample blanks, was insignificant compared to the concentration of TBT in PACS-2, confirming that contamination was effectively under control during sample preparation. The blank was subtracted from the gross TBT concentration measured in PACS-2.

To achieve optimum accuracy and precision for the ratio measurement, the mass bias correction solution was repeatedly introduced between spiked samples. Equation 10.3 was used for the quantitation of TBT in PACS-2:

$$C_x = C_y \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{AW_{xz}}{AW_y} \quad (10.3)$$

Figure 10.3. Chromatogram obtained by SPME sampling of ^{117}Sn TBT spiked PACS-2 with GC-ICP-MS detection. Black trace: ^{118}Sn , gray trace: ^{117}Sn (shifted for clarity).

where C_x is the concentration ($\mu\text{g g}^{-1}$) of TBT as tin based on dry mass of sample; C_y is the concentration ($\mu\text{g ml}^{-1}$) as tin of ^{117}Sn enriched TBT in the spike; m_y is the volume (ml) of spike used to prepare the blend solution of sample and spike; m_x is the mass (g) of sample used; w is the dry weight correction factor; A_y is the abundance of the reference isotope (^{118}Sn) in the spike; B_y is the abundance of the spike isotope (^{117}Sn) in the spike; A_{xz} is the abundance of the reference isotope in the sample or in the standard; B_{xz} is the abundance of the spike isotope in the sample or in the standard; R_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike; AW_y is the atomic weight of the analyte in the spike; and AW_{xz} is the atomic weight of the analyte in the sample or in the standard.

A concentration of TBT of $0.923 \pm 0.005 \mu\text{g g}^{-1}$ as tin (mean and one standard deviation, $n=5$) was obtained, in agreement with the certified value of $0.98 \pm 0.13 \mu\text{g g}^{-1}$ (mean and 95 % interval).

The detection limit for the ID SPME GC-ICP-MS technique was calculated based on measurement of three ^{117}Sn TBT spiked blanks. A value of 0.09 ng g^{-1} was estimated, based on three times the standard deviation of the measured concentrations normalized to a 0.5 g sub-sample and a 0.05 ml sub-sample of the acetic acid extract. It is worth noting that this detection limit could, in principle, be improved 200-fold if the entire 10 ml extract was used. The detection limit obtained with ID SPME GC-ICP-MS is superior to those obtained using ID with HPLC- or direct GC-ICP-MS methods.

It is of interest to determine the absolute mass of TBT sampled by the SPME fiber under the chosen experimental conditions. Based on a comparison of intensities obtained by injection of a standard of TBT in iso-octane and the SPME sampling of a PACS-2 extract,

this was estimated to be 250 pg. In other words, about 10 % of the total TBT in the extract (2450 pg) was absorbed by the SPME fiber.

10.3.4. Results for TBT in PACS-2 Using Standard Additions Calibration With SPME GC-ICP-MS

A subsequent comparative analysis of PACS-2 sediment was undertaken using the method of standard additions for calibration with SPME GC-ICP-MS. A 15 minute headspace sampling time did not permit extraction equilibrium to be reached, but was sufficient to yield acceptable intensities. The linearity of the calibration curve was tested under the chosen experimental conditions, permitting additions of approximately 1- and 2-fold of the concentration of TBT in the sediment. A spike of 50 ng of TPrT was added to all samples as an internal standard. Quantitation of TBT was based on the ratios obtained from the intensity of ^{118}Sn for the TBT peak area divided by the peak area for TPrT. The correlation coefficient of the standard additions calibration curve for TBT in the concentration range $0 \sim 3 \mu\text{g g}^{-1}$ was 0.99998. The final analysis of the PACS-2 sediment was performed using five replicate samples based on only one addition. Each spiked sample was prepared by adding approximately the same mass of TBT as expected in the PACS-2 sub-sample. A mean concentration of $0.93 \pm 0.09 \mu\text{g g}^{-1}$ (one standard deviation, $n=5$) was obtained, in good agreement with the certified value.

As expected, the precision of 0.54 % RSD for TBT measured in PACS-2 using isotope dilution SPME GC-ICP-MS was significantly better than the precision of 9.7 % RSD obtained using standard additions calibration. This 18-fold improvement demonstrates the superior capability of the ID technique for improving the precision of SPME sampling for determination of TBT in PACS-2.

10.4. Conclusions

An accurate and precise method was developed for the determination of TBT in sediment using ID SPME GC-ICP-MS. A significant improvement in the precision of measurement using ID, as opposed to standard additions calibration, was obtained, overcoming the generally poor precision associated with the SPME sampling technique. The approach is well suited not only for certification of BTs, but for improving the methodology used for organometallic speciation in general when SPME sampling is used.

CHAPTER 11. COMPARISON OF SECTOR FIELD- AND QUADRUPOLE-ICP-MS FOR THE QUANTITATION OF DBT AND TBT IN SEDIMENT

11.1. Introduction

The isotope ratio precision measured by qICP-MS⁸⁸⁻⁹¹ usually lies in a range of 0.1 to 1 %. The recent introduction of sector field ICP-MS (SF-ICP-MS) with single or multi-collector detection onto the market has brought a new dimension to this analytical field. In addition to its high mass resolution and high sensitivity, its unique flat topped peaks produced in low resolution mode provide for a more accurate and precise isotope ratio measurement⁹²⁻⁹⁶. This has been reported to be better than 0.04 % precision with a single detector and as low as 0.002 % on instruments equipped with multi-collector detectors.

To achieve more accurate and precise determination of BTs, a species specific ID technique has been used with HPLC-ICP-MS and GC-ICP-MS detection. qICP-MS has frequently been the choice as a detector due to its relatively fast sampling speed for acquiring transient signals and its simplicity in use compared to early SF-ICP-MS instruments. Recent improvements in new magnet technology for SF-ICP-MS (e. g., Element2) have significantly enhanced its scan speed to the point where it is competitive with that of q-ICP-MS⁹⁷.

There are very few applications wherein GC⁹⁸⁻¹⁰⁰ or HPLC^{97, 102-103} have been coupled to SF-ICP-MS for elemental speciation, and none highlighting the advantage of precise ratio measurements offered by SF-ICP-MS when using species specific isotope dilution for calibration. An investigation of the relative performance of GC for separation coupled to SF-ICP-MS and qICP-MS for the determination of TBT and DBT in a sediment using species specific isotope dilution was deemed appropriate. Enriched (¹¹⁷Sn) TBT and DBT spikes

were used for this purpose and a reverse spike ID approach was performed to quantify enriched TBT and DBT concentrations in the mixed spike solution, as delineated in Chapter 7. The method was validated by the determination of TBT and DBT in PACS-2 marine sediment CRM.

11.2. Sample Preparation

Quantitation of TBT and DBT in sediments and application of reverse spike isotope dilution for quantitation of the ^{117}Sn enriched TBT and DBT spike solution were conducted on the same days. The sediment extraction procedure was similar to that described in Chapter 7. Three sample blanks, four samples of PACS-2 and six replicate samples of test sediment were prepared. Sub-samples of 0.25 g PACS-2 or 0.5 g test sediment along with 0.4560 or 0.1250 ml of ^{117}Sn enriched TBT and DBT (0.12 and $0.18\ \mu\text{g ml}^{-1}$ as tin) spike solution and 10 ml of acetic acid were heated in a Prolabo microwave digester at 60 % power for 3 minutes. Derivatization of analytes in the extract was performed in a manner similar to that described in Chapter 7.

The concentrations of TBT and DBT in the ^{117}Sn enriched spike were measured by reverse spike isotope dilution against two independently prepared natural abundance TBT and DBT standards. Due to the presence of DBT impurity in the natural abundance TBT standard, and vice versa, a reverse spike isotope dilution was performed sequentially for TBT and DBT. For this purpose, a 0.125 ml volume of ^{117}Sn enriched TBT and DBT spike solution was accurately pipetted into each of twelve vials. To the first six vials, 0.1285 ml of $0.538\ \mu\text{g ml}^{-1}$ (or $0.525\ \mu\text{g ml}^{-1}$) natural abundance TBT solution was added whereas 0.1488 ml of $0.629\ \mu\text{g ml}^{-1}$ (or $0.619\ \mu\text{g ml}^{-1}$) natural abundance DBT solution was added to the last six vials. Derivatization was performed as described earlier in Chapter 7.

11.3. Results and Discussion

11.3.1. Optimization of GC With SF-ICP-MS

The interface and transfer line previously used for qICP-MS and described in Chapter 6 were used to couple the GC to the SF-ICP-MS instrument. Optimization of the GC-qICP-MS system is also detailed in Chapter 6. Initial optimization of the SF-ICP-MS system was undertaken as recommended by the manufacturer using liquid sample introduction of a 1 ppb multi-element standard to achieve stable and high sensitivity for Li, In and U. Low resolution (300) was used to achieve best sensitivity with flat topped peaks. Dead time correction (18 ns) was performed as recommended by the manufacturer, based on use of three different concentrations of U. The value of dead time, which generates same $^{235}\text{U}/^{238}\text{U}$ ratios measured from three U solutions, was chosen. Mass calibration was only performed once a week as a result of the stability of the Element2. The spray chamber and nebulizer assembly were replaced with the transfer line and its ball joint adapter. The final optimization of lens voltages, torch position and RF power for dry plasma conditions was performed by monitoring ^{129}Xe signal arising from the blending of a small amount of Xe to the Ar carrier gas through a T-connection. As more than one lens in the system requires optimization, a steady-state signal is required. No significant difference in parameters for lens voltages, torch position and RF power was evident between wet and dry plasma conditions.

The optimum Ar carrier gas flow rate is dependent on that of the He effluent from the GC column as the Ar was introduced through the side arm of the interface. Therefore, the approach based on monitoring ^{129}Xe under dry plasma conditions was not successful for the optimization of Ar carrier gas flow. Thus Ar carrier gas flow rate was optimized in the same

manner as for the ELAN6000 system, described in Chapter 6 (section 6.4.1). An optimum Ar carrier gas flow rate of 0.30 l min^{-1} was found for the dry plasma conditions, resulting in a He flow rate of 1.2 ml min^{-1} at 32 psi. This optimum is significantly different from the 1.20 l min^{-1} used with wet plasma conditions. A further study was conducted to determine whether a make-up gas, introduced through a T connection at the end of transfer line to the torch (to avoid any pressure build up at the end of GC column at the interface), would improve sensitivity. No significant improvement was observed as make up gas flow rate was increased from 0.1 to 1.1 l min^{-1} , producing a total sample Ar flow rate of 0.4 to 1.4 l min^{-1} .

As a result of information from the above studies, optimization of the GC-SF-ICP-MS system was simplified for ease of operation in the final study. The Element2 liquid sample introduction system was brought on line once a week to optimize the instrument and conduct mass calibration. It was then removed to permit GC sample introduction. The Ar carrier gas flow rate was then optimized by injection of a 25 ng ml^{-1} butyltin standard in iso-octane using a He pressure of 32 psi. No change in day-to-day optimum Ar carrier gas flow rate was observed when using the same He pressure setting and similar transfer line length. Optimized operating conditions for the GC SF-ICP-MS system are presented in Table 11.1.

As noted in Chapter 6, the length of the transfer line had little effect on either the butyltin peak shape or sensitivity, due to the short analyte residence time and, for flexibility and ease of handling of the tandem instrumentation, a 100 cm long PTFE line was used for the final work. It was necessary to change this PTFE tubing daily, to minimize significant broadening of TBT peaks. Evidently, a surface residue accumulates over the course of the day in this unheated line. No significant influence of the distance between the injector tip and the end of the transfer line on the resulting sensitivity was observed in the range of 0 to

12 mm. However, plasma instability was noted at distances greater than 15 mm when iso-octane extract was injected. A 5 mm distance was selected for this study.

Table 11.1. GC-SF-ICP-MS operating conditions

	GC
Injection mode	Splitless
Injection volume	1 μ l
Injector temperature	250 °C
Column	MXT-5 (20 m x 0.28 mm x 0.5 μ m)
Carrier gas	He at 32 psi, 1.3 ml min ⁻¹
Oven program	60 °C (1 min) to 200 °C at 20 °C min ⁻¹ to 270 °C at 30 °C min ⁻¹ (2 min)
Detector temperature	300 °C
	Element2
Rf power	1150 W
Plasma Ar gas flow rate	15.0 l min ⁻¹
Auxiliary Ar gas flow rate	1.05 l min ⁻¹
Ar carrier gas flow rate	0.30 l min ⁻¹
Sampler cone (nickel)	1.1 mm
Skimmer cone (nickel)	0.8 mm
Lens voltage	Focus: -870 V; x deflection: -2.75 V; y deflection: -0.40 V; Shape: 105 V
Dead Time	18 ns
Resolution	300
Data acquisition	E-scan, 4000 passes, 5 % mass window, 0.0050s sample time

Figure 11.1. Chromatogram of an extract of unspiked SOPH-1 obtained by GC-SF-ICP-MS (with ^{117}Sn , ^{118}Sn and ^{120}Sn traces, ^{118}Sn and ^{120}Sn traces shifted for clarity).

As evident in Figure 11.1, good resolution and peak profiles for all three ethylated butyltin species were obtained under optimized conditions using this home-made interface and transfer line. The peak widths ranged from 2 to 5 seconds at 10 % height, comparable to those obtained using conventional GC detectors with the given temperature program. Chromatograms obtained using qICP-MS for detection suffered from a 10-fold poorer sensitivity than those obtained with SF-ICP-MS. With the data acquisition parameters used, all peaks could be reliably recorded using SF-ICP-MS without distortion, despite their narrow half widths

An example of this is presented in Figure 11.3, wherein a segment of the recorded chromatograms illustrating the narrow DBT ^{120}Sn peak is presented for both detection systems. More than 40 data points characterize the peak in the SF-ICP-MS chromatogram, which has a full width at half maximum (FWHM) of only 2.0 seconds. It is clear that the temporal characteristics of this peak are identical to those recorded using the qICP-MS. The rapid mass scanning capability of the SF-ICP-MS (Element2) thus offers no impediment to the accurate recording of these transient signals.

11.3.2. Ratio Measurements for TBT and DBT Using GC-SF-ICP-MS and GC-qICP-MS

Two major effects, detector dead time^{87, 104-107} and instrument mass bias¹⁰⁸⁻¹⁰⁹, must be compensated for to achieve accurate isotope ratio measurements when using an ion counting detection system with single detector ICP-MS systems. Counting losses due to detector dead time increase in magnitude as the counting rate increases, resulting in inaccurate isotope ratio measurements if not accounted for^{87, 104-107}. A recent publication

Figure 11.2. Segment of chromatograms from an extract of SOPH-1 showing the DBT (^{120}Sn) peak obtained by GC-SF-ICP-MS (solid line) and GC-qICP-MS (dashed line). For comparative purposes, the qICP-MS signal intensity has been enhanced 9.5-fold and has been shifted 4.2s to achieve a retention time match so as to permit temporal overlap of the peaks.

by Nelms *et al.*¹⁰⁶ evaluated four popular dead time calculation models used for ICP-MS and concluded that all four models gave similar results for the estimation of this parameter. A method in which the dead time was derived from a plot of the measured $^{204}\text{Pb}/^{208}\text{Pb}$ ratio versus Pb concentration was recommended as it generated a clear, unambiguous value for the dead time and a systematic approach to derive the uncertainty in the resulting value could also be obtained. This model was thus used in this study. Detector dead time was derived from plots of measured analyte isotope ratio versus concentration. To determine detector dead time, a $^{238}\text{U}/^{235}\text{U}$ ratio was measured in three U standard solutions at concentrations of 0.5, 1.0 and 2.5 ng ml⁻¹. A dead time of 18 ns was derived from a plot of the measured $^{238}\text{U}/^{235}\text{U}$ ratios versus U concentration at slope of zero.

Mass bias correction factors were determined based on the assumed natural abundance ratio of $^{120}\text{Sn}/^{117}\text{Sn}$ (4.246) divided by the mean value of $^{120}\text{Sn}/^{117}\text{Sn}$ obtained from inorganic Sn peaks, calculated for all injected standards. A mass bias correction factor of 0.9942 ± 0.0047 (one standard deviation, n=16) for $^{120}\text{Sn}/^{117}\text{Sn}$ was obtained using the Element2, significantly smaller than 0.970 ± 0.011 (one standard deviation, n=16) obtained with the ELAN6000.

Ratios of $^{120}\text{Sn}/^{117}\text{Sn}$ measured with the butyltin standards in the concentration range of 10 to 200 ng ml⁻¹ in iso-octane were obtained with both GC-SF-ICP-MS and GC-qICP-MS to investigate if dead time corrections were adequate for determinations of BTs. Mass bias corrected $^{120}\text{Sn}/^{117}\text{Sn}$ ratios for TBT and DBT in standard solutions over the concentration range 10 to 200 ng ml⁻¹ are reported in Table 11.2. No significant difference was found for calculated ratios at different concentrations, suggesting that the dead time corrections used are adequate for both instruments.

Table 11.2. $^{120}\text{Sn}/^{117}\text{Sn}$ ratios obtained with GC-SF-ICP-MS and GC-qICP-MS

Measured ratios, $^{120}\text{Sn}/^{117}\text{Sn}$ (n=4)				
Sample name	GC-SF-ICP-MS		GC-qICP-MS	
	DBT	TBT	DBT	TBT
10 ppb	4.231±0.025	4.244±0.032	4.241±0.060	4.244±0.051
50 ppb	4.237±0.010	4.254±0.024	4.241±0.067	4.253±0.039
100 ppb	4.250±0.020	4.247±0.016	4.242±0.054	4.248±0.035
200 PPb	4.242±0.020	4.245±0.016	4.246±0.040	4.246±0.031
test sediment	4.248±0.038	4.245±0.024	4.250±0.060	4.247±0.058

As noted earlier, the Element2 produces flat topped peaks at low resolution, thereby permitting more accurate and precise ratio measurements. As expected, precisions of 0.25 to 0.75 % RSD in the calculated $^{120}\text{Sn}/^{117}\text{Sn}$ ratios obtained using GC-SF-ICP-MS are significantly better than the 0.70 to 1.6 % RSD obtained using GC-qICP-MS. This is also a direct consequence of the enhanced sensitivity improving the counting statistics.

11.3.3. Determination of TBT and DBT in Sediment Using ID GC-SF-ICP-MS and GC-qICP-MS

Although both isotope pairs of $^{120}\text{Sn}/^{117}\text{Sn}$ and $^{118}\text{Sn}/^{117}\text{Sn}$ can be used for the quantitation of butyltins, $^{120}\text{Sn}/^{117}\text{Sn}$ was selected because of enhanced sensitivity. As shown in Table 11.2, both mass bias corrected $^{120}\text{Sn}/^{117}\text{Sn}$ ratios measured in the unspiked SOPH-1 using GC-SF-ICP-MS and GC-qICP-MS are not significantly different from the expected value of 4.246, confirming that no significant spectroscopic interference arises on either

isotope from sample matrix components. This permits accurate results to be obtained using the chosen isotope pair.

Mass bias correction can be achieved based on data derived either by injection of natural abundance butyltin standards or by using the inorganic Sn peak in the chromatogram of each sample. The latter approach not only saves analysis time, but also provides better correction since instrument drift is accounted for with each sample. A $^{120}\text{Sn}/^{117}\text{Sn}$ ratio of 4.266 ± 0.028 (one standard deviation, $n=22$) was obtained based on inorganic Sn peaks in chromatograms of all injected samples, in good agreement with ratios of 4.267 ± 0.025 and 4.271 ± 0.017 (one standard deviation, $n=6$) obtained for TBT and DBT in the natural abundance butyltin standards. This suggests that the inorganic Sn peak in the chromatogram of each sample can be used to calculate the mass bias correction factor. A value of 0.9952 ± 0.0065 (mean and one standard deviation, $n=22$) for $^{120}\text{Sn}/^{117}\text{Sn}$ was obtained, indicating no significant mass bias drift during a run sequence. Equation 7.2 in Chapter 7 was used for the quantitation of TBT and DBT in the test sediment. Concentrations of 0.1244 ± 0.0020 and $0.1813 \pm 0.0029 \mu\text{g g}^{-1}$ (one standard deviation, $n=6$) as Sn for TBT and DBT, respectively, were obtained using the average mass bias correction factor of 0.9952, in good agreement with values of 0.1243 ± 0.0022 and $0.1812 \pm 0.0032 \mu\text{g g}^{-1}$ generated using individual mass bias correction factors for each sample.

A subsequent comparative analysis of this sediment was performed using GC-qICP-MS. Concentrations of 0.1248 ± 0.0021 and $0.1794 \pm 0.0059 \mu\text{g g}^{-1}$ (one standard deviation, $n=6$) as Sn for TBT and DBT, respectively, were obtained using an average mass bias correction factor of 0.9696 ± 0.0089 (mean and one standard deviation, $n=22$) obtained from the inorganic Sn peaks. As expected, slightly better precisions of 1.59 to 1.62 % RSD for

measured butyltin concentrations were obtained by GC-SF-ICP-MS compared to 1.64 to 3.31 % RSD generated with the GC-qICP-MS approach.

Method detection limits (LODs, based on three times the standard deviation of the blanks) for ID GC-SF-ICP-MS and GC-qICP-MS techniques were calculated based on three measurements of ^{117}Sn TBT and DBT spiked sample blanks. Values of 0.4 and 0.3 ng g^{-1} for TBT and DBT, respectively, were obtained using GC-SF-ICP-MS, based on a 0.5 g sample. These are slightly better than the LODs of 0.9 and 1.0 ng g^{-1} obtained using GC-qICP-MS. It should be noted that detection limits could, in principle, be enhanced 5-fold if the entire 10 ml volume of extract was taken for derivatization. Although sensitivities for TBT and DBT obtained using GC-SF-ICP-MS were 10-fold better than those arising with GC-qICP-MS, the 2-fold improvement in method LODs most likely arises as a result of a constant and high, but unidentified, background observed at m/z ^{120}Sn using the SF-ICP-MS instrument.

11.3.4. Validation of Isotope Dilution GC-SF-ICP-MS and GC-qICP-MS Methods for the Determination of TBT and DBT

Sediment CRM PACS-2 was used to validate the proposed method. Concentrations of 0.883 ± 0.013 and 1.126 ± 0.013 $\mu\text{g g}^{-1}$ (mean and one standard deviation, $n=4$) as tin were obtained for TBT and DBT, respectively, by GC-SF-ICP-MS, in agreement with certified values of 0.98 ± 0.13 and 1.09 ± 0.15 $\mu\text{g g}^{-1}$ (95 % confidence interval) for these species. Concentrations of 0.883 ± 0.019 and 1.116 ± 0.014 $\mu\text{g g}^{-1}$ (mean and one standard deviation, $n=4$) as tin were obtained for TBT and DBT, respectively, by GC-qICP-MS.

More than a 2-fold improvement in precision of measured $^{120}\text{Sn}/^{117}\text{Sn}$ ratios for both TBT and DBT in the butyltin standards was obtained using GC-SF-ICP-MS, as compared to GC-qICP-MS. However, a similar improvement in the precision of measurement of resulting

TBT and DBT concentrations in both SOPH-1 sediment and PACS-2 CRM was not realized, possibly due to limitations in the homogeneity of the samples or variability contributed by the sample preparation procedures.

11. 4. Conclusions

A sensitive method was developed for the accurate and precise determination of TBT and DBT in sediments by species specific isotope dilution using both qICP-MS and GC-SF-ICP-MS. A 2-fold enhancement in the precision of Sn isotope ratios measured in standard solutions of TBT and DBT by GC-SF-ICP-MS, as opposed to GC-qICP-MS, was obtained. Unfortunately, this advantage is not realized when real samples are processed because the variability introduced with the additional steps of sample preparation and inhomogeneity become the major contributors to the overall imprecision of the results. A 2-fold improvement in their LODs was obtained as a consequence of the enhanced sensitivity realized with the sector field instrument. The rapid mass scanning capability of the SF-ICP-MS, contrary to currently perceived notions, offered no impediment to rapid peak hopping for the accurate recording of transient signals having FWHM of only a few seconds.

CHAPTER 12. DETERMINATION OF TBT, DBT AND MBT FOR RECERTIFICATION OF PACS-2 USING GC-SF-ICP-MS WITH A HEATED TRANSFER LINE AND EVALUATION OF ITS COMBINED UNCERTAINTY

12.1. Introduction

The custom designed easily removable interface and transfer line used to couple GC to the SF-ICP-MS and quadrupole ICP-MS instruments produced peak broadening for TBT that increased significantly as the number of determinations performed with the same transfer line accumulated. The peak width of 5 seconds at baseline for TBT characterizing the first injection with a new PTFE transfer line increased to 25 seconds after 20 injections, as shown in Figure 12.1. As noted in Chapter 6, it was necessary to change the PTFE tube daily to minimize significant broadening of TBT peaks. Evidently, a surface residue accumulates over the course of the day in this unheated line.

A modification was thus made to the transfer line in order to overcome this problem, it being shrouded in a flexible heating cable to permit control of the temperature over the range of 30 to 300 °C. The shroud extends from the GC interface to the base of the torch. Thereafter, the inner PTFE transfer line is not heated and terminates 5 mm below the injector tip. Parameters affecting sensitivity and peak shape using this heated transfer line were investigated and the optimized GC-SF-ICP-MS system was used for the determination of TBT, DBT and MBT in PACS-2 by ID for the purposes of recertifying this CRM.

Figure 12.1. Chromatograms obtained from 1 μl injections of standard solutions containing 10 ng ml^{-1} BTs and TPrT with GC-SF-ICP-MS using a new unheated PTFE transfer line a: first injection; b: after 20 injections.

12.2. Sample Preparation

Isotope dilution and reverse spike isotope dilution calibration were used for analyte quantitation. The sediment extraction procedure was the same as that described in Chapter 6 and the reverse spike determination of the concentrations of TBT and DBT in the ^{117}Sn enriched spikes was achieved using the procedure outlined in Chapter 11.

A small volume of ^{116}Sn enriched MBT solution was made available by Dr. Wolfgang Frech (Department of Chemistry, Umeå University, Sweden). MBT in PACS-2 was also determined by species specific ID GC-SF-ICP-MS. Sample preparation was similar to that described earlier for determination of TBT and DBT. Duplicate samples from each of three bottles of PACS-2 were prepared. Sub-samples of 0.5 g of PACS-2 were spiked with 0.100 ml of ^{116}Sn enriched MBT solution ($0.65\ \mu\text{g ml}^{-1}$ as tin) and 10 ml acetic acid. The mixture was heated in the Prolabo microwave digester at 60 % power for 3 minutes, the contents centrifuged at 2000 rpm for 5 minutes and a 5 ml volume of the supernatant transferred to a 50 ml glass vial. After 10 ml of 1 M NaAc buffer solution, 5 ml ammonium hydroxide, 1 ml of 2 % NaBeT_4 and 2 ml iso-octane were added, the vial was capped and shaken manually for five minutes. The iso-octane layer, separated by centrifuging at 2000 rpm for 5 minutes, was then transferred to a 2 ml glass vial for quantitation by GC-SF-ICP-MS.

Six reverse spike isotope dilution calibration samples were prepared. A 0.100 ml volume of ^{116}Sn enriched MBT spike solution and 0.100 ml of $1.704\ \mu\text{g ml}^{-1}$ (or $1.744\ \mu\text{g ml}^{-1}$) natural abundance MBT solution were accurately pipetted into a vial. Derivatization was performed in the same manner as the samples. A mass bias correction solution was prepared in the same way using $0.10\ \text{ml}$ of $1.704\ \mu\text{g ml}^{-1}$ natural abundance MBT standard. All samples were immediately injected for analyses by GC-SF-ICP-MS following extraction and

derivatization.

Isotopes of ^{120}Sn , ^{117}Sn and ^{116}Sn were simultaneously monitored with the Element2. At the end of the chromatographic run, the acquired data were transferred to an off-line computer for further processing using in-house software to yield $^{120}\text{Sn}/^{117}\text{Sn}$ and $^{120}\text{Sn}/^{116}\text{Sn}$ ratios based on peak area data, from which the analytes concentrations in the sediment were calculated.

12.3. Optimization of GC-SF-ICP-MS Using a Heated Transfer Line

Optimization of the SF-ICP-MS system was undertaken as described in Chapter 11. Earlier results (Chapter 11) have shown that there is no significant influence on the resulting sensitivity of the distance between the injector tip and the end of the transfer in the range 0 to 12 mm; a 5 mm distance was thus used. It was also found that the length of the transfer line had little effect on either the analyte peak shape or sensitivity, due to the short analyte residence time. Therefore, for flexibility and ease of handling, a 150 cm long PTFE line was used in the heating cable.

With the heated transfer line, the He pressure on the GC had to be increased to 40 psi to enable separation of the analytes from the column as the back pressure from Ar admitted to the interface side arm was higher in the heated transfer line. Thus, corresponding optimized Ar carrier gas flow rate, established by injection of 1 μl of a 10 ng ml^{-1} mixed standard solution of the BTs with transfer line temperature at 200 $^{\circ}\text{C}$, was 0.275 l min^{-1} . Sensitivities decreased gradually at either lower or higher flow rates.

The effect of transfer line temperature on the sensitivity and peak shape for the BTs was investigated in the range of 60 to 270 $^{\circ}\text{C}$. A 1 μl volume of a 10 ng ml^{-1} solution of the mixed BTs was injected for this purpose. No significant difference in either sensitivity or

peak shape was observed over the temperature range 60 to 250 °C, but sensitivity for TBT decreased slightly as transfer line temperature increased above 250 °C. A compromise transfer line temperature of 220 °C was selected for final measurements in an effort to maintain sharp peak shape for TBT as well as avoid its decomposition.

12.4. Determination of TBT, DBT and MBT in PACS-2 Using ID GC-SF-ICP-MS and a Heated Transfer Line

As shown in Figure 12.2, good separation of the BTs and sharp peaks were obtained for an extract of PACS-2 using the heated transfer line interface. Mass bias correction was implemented using the inorganic Sn peak in the chromatogram of each sample, as outlined in Chapter 11. A mass bias correction factor of 0.9942 ± 0.0065 (mean and one standard deviation, $n=6$) for $^{120}\text{Sn}/^{117}\text{Sn}$ based on inorganic Sn response in all injected samples was obtained. Equation 7.2 was used for calculation of the analyte concentrations. Concentrations of 0.894 ± 0.012 and $1.115 \pm 0.015 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=6$) as tin were obtained for TBT and DBT, respectively. No significant change in peak widths for TBT and DBT was evident when using the heated transfer line, even after three weeks of use.

The concentration of MBT in PACS-2, calculated using equation 7.2 and based on the ^{116}Sn isotope spike, was determined to be $0.639 \pm 0.017 \mu\text{g g}^{-1}$ (one standard deviation, $n=6$) as tin, in agreement with the value of $0.629 \pm 0.03 \mu\text{g g}^{-1}$ measured by standard additions calibration (shown in Figure 8.1 of the long-term stability study at year six). It is evident that the precision of 2.7 % RSD ($n=6$) characterizing measurement of MBT using isotope dilution is better than the precision of 4.7 % RSD obtained using standard additions

Figure 12.3. Chromatograms obtained from a 1 μl injection of an extract of PACS-2 with GC-SF-ICP-MS using a heated PTFE transfer line.

calibration. Despite the agreement between measured concentrations of MBT by both calibration strategies, these values are significantly higher than previous results obtained by GC-ICP-MS a year earlier and are significantly higher than the certified value of $0.45 \pm 0.05 \mu\text{g g}^{-1}$ determined in 1999, confirming that recertification is necessary.

12.5. Total Combined Standard Uncertainty Calculations

In recent years, the subject of the evaluation of measurement uncertainty in analytical chemistry has generated significant interest and discussion. It is recognized that the fitness for purpose of an analytical method cannot be assessed without some estimate of the measurement uncertainty to be compared with the level of confidence required. Well characterized and controlled uncertainties are also fundamental to the implementation of traceability as a means of ensuring comparability of results, large uncertainties imply poor comparability. Accounting for all possible sources of uncertainty arising from the measurement process is of fundamental importance for producing an accurate value for a measurement. The total combined standard uncertainty and its expanded uncertainty are now required for the characterization of CRMs, in agreement with ISO17025.

12.5.1. Definitions for Total Combined Standard Uncertainty Calculations

To better understand and implement total combined standard uncertainty, a few related definitions must first be introduced. According to ISO International Vocabulary of Basic and General Terms in Metrology¹¹⁰, measurand is defined as the particular quantity subject to measurement. Uncertainty is defined as a parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand; or simply the number after the plus/minus sign. Standard

uncertainty (u_{x_i}) is the uncertainty of a result, x_i , of a measurement, expressed as a standard deviation. Combined standard uncertainty [$u_c(y)$] is defined as the standard uncertainty of the result, y , of a measurement when the result is obtained from the values of a number of other quantities. It can be calculated by use of equation 12.1 (below). Expanded uncertainty (U) is the quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand. U is obtained by multiplying $u_c(y)$, the combined standard uncertainty, by a coverage factor, k . Coverage factor (k) is the numerical factor used as a multiplier of the combined standard uncertainty to obtain an expanded uncertainty. The value of k is usually 2 or 3, and depends on the level of confidence desired. Type A uncertainties are defined as those uncertainties which are evaluated by statistical analysis of a series of observations; for example, the standard uncertainty of the mean of a series of independent observations is obtained by using the standard deviation divided by the square root of number of measurements. Type B uncertainties are defined as those which are evaluated by means other than the statistical analysis of a series of observations; for example, the standard uncertainty associated with the purity of a metal can be calculated by use of the manufacturer's specification of its uncertainty value (at 95 % confidence interval) divided by 1.96.

12.5.2. Equations Needed for Total Combined Standard Uncertainty Calculations

According to the ISO Guide to the Expression of Uncertainty in Measurement¹¹¹, the combined standard uncertainty of a measurement result y (measurand), designated $u_c(y)$, can be obtained from the following equation 12.1:

$$u_c^2(y) = \sum_{i=1}^N \left(\frac{\partial f}{\partial x_i} \right)^2 u^2(x_i) + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^N \left(\frac{\partial f}{\partial x_i} \right) \cdot \left(\frac{\partial f}{\partial x_j} \right) \cdot u(x_i, x_j) \quad (12.1)$$

where $y = f(x_1, x_2, \dots, x_N)$ and is conveniently referred to as the law of propagation of uncertainty. The partial derivatives, $\partial f / \partial x_i$, are often referred to as sensitivity coefficients; $u(x_i)$ is the standard uncertainty associated with the input x_i , and $u(x_i, x_j)$ is the estimated covariance associated with x_i and x_j . Equation 12.1 can also be expressed in the form:

$$u_c^2(y) = \sum_{i=1}^N \left(\frac{\partial f}{\partial x_i} \right)^2 u^2(x_i) + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^N \left(\frac{\partial f}{\partial x_i} \right) \cdot \left(\frac{\partial f}{\partial x_j} \right) \cdot u(x_i) \cdot u(x_j) \cdot r_{(i,j)} \quad (12.2)$$

where $r_{(i,j)}$ is correlation coefficient, $-1 < r_{(i,j)} < 1$. When $x_1, x_2, \dots, x_N$ are independent parameters, equation 12.2 can be simplified to equation 12.3:

$$u_c^2(y) = \sum_{i=1}^N \left(\frac{\partial f}{\partial x_i} \right)^2 u_{x_i}^2 \quad (12.3)$$

It is worth noting the following two simplified equations for calculation of the combined standard uncertainty:

$$\left(\frac{u_y}{y} \right)^2 = \left(\frac{u_a}{a} \right)^2 + \left(\frac{u_b}{b} \right)^2 \quad (12.4)$$

when $y = r \cdot a \cdot b$ or $y = \frac{r \cdot a}{b}$ with a and b being independent parameters and r is a

constant and:

$$(u_y)^2 = (r_a u_a)^2 + (r_b u_b)^2 \quad (12.5)$$

when $y = r \pm r_a a \pm r_b b$ where r, r_a, r_b are constants and a and b are independent parameters.

Equations 12.4 and 12.5 can only be applied when dealing with either an exclusive mix of only additions and subtractions or with a combination of only divisions and

multiplications. For example, equation 12.5 can be used to calculate the combined standard uncertainty of the concentration of a stock solution of Sn obtained by quantitative dissolution of pure Sn metal in HCl. The concentration can be expressed as $C_{\text{std}} = wp/m$, where w is the mass of Sn metal used, p is the purity of the Sn metal and m is the final mass of the solution. The combined standard uncertainty (u_c) in such case can be calculated using the following equation:

$$\left(\frac{u_c}{c}\right)^2 = \left(\frac{u_w}{w}\right)^2 + \left(\frac{u_p}{p}\right)^2 + \left(\frac{u_m}{m}\right)^2 \quad (12.6)$$

Equation 7.2 used for calculation of the concentrations of BTs is much too complicated and equations 12.4 and 12.5 are no longer adequate to calculate the resulting combined standard uncertainty in the concentration of BTs in PACS-2 obtained by isotope dilution and reverse spike isotope dilution techniques. The natural complexity of equation 7.2 makes calculation of the combined standard uncertainty a difficult and time-consuming task.

Kragten¹¹³ reported a relatively simple spreadsheet method to calculate combined standard uncertainty and the EURACHEM/ CITAC Guide¹¹¹ has adopted this approach for application to isotope dilution analysis. This spreadsheet method is based on the following principle: when $y(x_1, x_2, \dots, x_N)$ is either linear in x_i or $u(x_i)$ is small compared to x_i , the partial derivatives ($\partial f/\partial x_i$) can be approximated by equation 12.7. If conditions do not meet these requirements, equation 12.2 must be used:

$$\frac{\partial f}{\partial x_i} \approx \frac{f(x_i + u(x_i)) - f(x_i)}{u(x_i)} \quad (12.7)$$

Thus:

$$\frac{\partial f}{\partial x_i} \cdot u(x_i) \approx f(x_i + u(x_i)) - f(x_i) \quad (12.8)$$

By substitution of equation 12.8 into equation 12.2, the combined standard uncertainty in C can now be obtained using equation 12.8:

$$u_c^2 \approx \sum (f(x_i + u(x_i)) - f(x_i))^2 + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^N (f(x_i + u(x_i)) - f(x_i)) \cdot (f(x_j + u(x_j)) - f(x_j)) \cdot r_{(i,j)} \quad (12.9)$$

In this study, the universal equation 12.2 was used for calculation of the combined standard uncertainty in order to achieve an accurate estimation of total uncertainty in the concentrations of the BTs measured in PACS-2 using ID GC-SF-ICP-MS. Although the extraction efficiency (E) of the method does not affect the final concentrations of the BTs because equilibration between the added spike and the endogenous analyte was achieved, the total combined uncertainty calculation must still account for the uncertainty contributed by the extraction process. To fully express the measurement process using ID and reverse ID with blank correction for the determination of the BTs, equation 7.2 used for the calculation of the concentrations of the BTs is thus modified to equation 12.10:

$$C = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E - C_b \quad (12.10)$$

To obtain an estimate of the total combined standard uncertainty in analyte concentration measured by ID GC-SF-ICP-MS, individual partial derivatives were derived for all variables in equation 12.10, as follows:

$$\frac{\partial C}{\partial C_z} = \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.11)$$

$$\frac{\partial C}{\partial v_y} = C_z \cdot \frac{1}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.12)$$

$$\frac{\partial C}{\partial w} = -C_z \cdot \frac{v_y}{w^2 \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.13)$$

$$\frac{\partial C}{\partial m_x} = -C_z \cdot \frac{v_y}{w \cdot m_x^2} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.14)$$

$$\frac{\partial C}{\partial v_z} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{1}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.15)$$

$$\frac{\partial C}{\partial v'_y} = -C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'^2_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.16)$$

$$\frac{\partial C}{\partial A_y} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_y \cdot R_n - B_y \cdot R'_n}{(A_y - B_y \cdot R'_n)^2} \cdot E \quad (12.17)$$

$$\frac{\partial C}{\partial B_y} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{A_y \cdot R'_n - A_y \cdot R_n}{(A_y - B_y \cdot R'_n)^2} \cdot E \quad (12.18)$$

$$\frac{\partial C}{\partial A_{xz}} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{A_y - B_y \cdot R'_n} \cdot \frac{B_{xz} \cdot R'_n - B_{xz} \cdot R_n}{(B_{xz} \cdot R_n - A_{xz})^2} \cdot E \quad (12.19)$$

$$\frac{\partial C}{\partial B_{xz}} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{A_y - B_y \cdot R'_n} \cdot \frac{A_{xz} \cdot R_n - A_{xz} \cdot R'_n}{(B_{xz} \cdot R_n - A_{xz})^2} \cdot E \quad (12.20)$$

$$\frac{\partial C}{\partial R_n} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_{xz} \cdot B_y - A_y \cdot B_{xz}}{(B_{xz} \cdot R_n - A_{xz})^2} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \cdot E \quad (12.21)$$

$$\frac{\partial C}{\partial R'_n} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot A_y - B_y \cdot A_{xz}}{(A_y - B_y \cdot R'_n)^2} \cdot E \quad (12.22)$$

$$\frac{\partial C}{\partial E} = C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} \quad (12.23)$$

$$\frac{\partial C}{\partial C_b} = -1 \quad (12.24)$$

12.5.3. Standard Uncertainty of Individual Parameters

Calculation procedures used for individual parameters were in line with ISO guide¹¹¹ and EURACHEM/ CITAC Guide¹¹³. Calculation of these standard uncertainties is outlined here only for TBT as the same calculation procedure was used for DBT and MBT.

The standard uncertainty of m_x was calculated using the equation 12.25:

$$u_{m_x}^2 = u_r^2 + u_l^2 + u_l^2 \quad (12.25)$$

where u_r is standard uncertainty of repeatability of the balance, as given by the vendor's specification and based on 5 sets of tare and gross weight, a value of 0.0001 g. The standard uncertainty of linearity of the balance (u_l) was calculated using the vendor's specification of 0.0002 divided by $\sqrt{3}$ for an assumed rectangular distribution, yielding 0.00011547 g. Because one weight was obtained by weighing twice, u_l was used twice to calculate the standard uncertainty of m_x .

$$u_{m_x} = \sqrt{0.0001^2 + 0.00011547^2 + 0.00011547^2} = 0.000191 \text{ g}$$

Standard uncertainties of v_y , v_z , and v'_y were calculated in the same manner, as these volumes were obtained by accurately pipetting a methanolic solution. Prior to use, a Gilson Microman M250 piston pipette (Mandel Scientific Company LTD, Guelph, Ontario, Canada) was gravimetrically calibrated using DIW to calculate the accurate delivery volume from the mean of repeated weightings (accounting for the density of DIW). In general, the uncertainty contribution from volume measurements is usually larger than the uncertainty arising from

mass, but the overall uncertainty contributions from dilutions by volume remain insignificant compared to the total combined uncertainty characterising the overall procedure (as shown below in Table 12.1). Thus, for simplicity in sample preparation, all dilutions were conducted by volume. Volumes of v_y , v_z or v'_y , were obtained using the mean value of several repeated weighings of DIW divided by the density of DIW. The uncertainties of v_y , v_z , and v'_y were calculated by use of the following equation 12.26:

$$(u_{v_i})^2 = v_i^2 \cdot \left(\frac{u_m}{m}\right)^2 + v_i^2 \left(\frac{u_d}{d}\right)^2 + (u_r)^2 \quad (12.26)$$

where $v_i = v_y, v_z$ or v'_y ; u_m is the standard uncertainty of weight of DIW, which equals 0.000191 g; m is the weight of DIW; u_d is the standard uncertainty of the density of DIW and was estimated by using half of the estimated lower and upper limits of $(a_- + a_+)/2$ divided by $\sqrt{3}$ assuming a uniform distribution and yielding 0.000002887 g ml⁻¹, d is the density of DIW (0.99823 g ml⁻¹) and u_r is the repeatability of the pipette (which was needed for calculation of uncertainty) and estimated from the calculated standard deviation arising from the weighting of several repeated pipettings of methanol into a beaker containing 10 ml DIW (to minimize vaporization of methanol during the weighing). Values of standard uncertainties of 0.000243, 0.000225 and 0.000243 ml were obtained for v_y , v_z , and v'_y , respectively.

C_z was prepared by serial dilution of a 5000 µg ml⁻¹ stock solution which was itself prepared by dissolving 0.5200 g of 98.3 % pure TBT in methanol in a 100 ml volumetric flask. The standard uncertainty of C_z was obtained using the following equation 12.27:

$$C_z = \left(\frac{0.5200 \text{ g} \cdot 0.983}{100 \text{ ml}} \cdot 10^6\right) \cdot \left(\frac{1.0026 \text{ ml}}{50 \text{ ml}}\right) \cdot \left(\frac{0.9970 \text{ ml}}{50 \text{ ml}}\right) = 2.0438 \text{ µg ml}^{-1} \quad (12.27)$$

The standard uncertainty in the volume of the volumetric flask was calculated using equation

12.28:

$$u_{v(\text{volumetric flask})}^2 = u_c^2 + u_r^2 + u_T^2 \quad 12.28$$

where u_c is standard uncertainty of calibration of the volumetric flask and was obtained by using the manufacturer's specification of 0.08 ml (for 100 ml) divided by $\sqrt{3}$, assuming a rectangular distribution; u_r is the standard uncertainty of repeatability of use of the volumetric flask and was obtained from the standard deviation of 10 fill and weigh experiments; u_T is the standard uncertainty of the effect of temperature on the volume and was obtained using the coefficient of volume expansion for water ($2.1 \times 10^{-4} \text{ }^\circ\text{C}^{-1}$) times the volume of the flask times $4 \text{ }^\circ\text{C}$ (assuming variation in T was $4 \text{ }^\circ\text{C}$ in the lab) divided by $\sqrt{3}$, assuming a rectangular distribution. Standard uncertainties of 0.0699 and 0.0390 ml were obtained for 100 and 50 ml volumetric flasks, respectively. The standard uncertainty associated with volume of pipetting was calculated in the same way as for the standard uncertainties for v_y , v_z , and v'_y and values of 0.000969 and 0.000920 ml were obtained for 1.0026 and 0.9970 ml of stocks, respectively. The standard uncertainty in the purity (0.001155) was obtained based on the vendor's specification of 0.002 divided by $\sqrt{3}$, assuming a rectangular distribution. Thus, the standard uncertainty of C_z of $0.004573 \text{ } \mu\text{g ml}^{-1}$ was obtained as below:

$$\left(\frac{u_{C_z}}{2.0438}\right)^2 = \left(\frac{0.000191}{0.5200}\right)^2 + \left(\frac{0.001155}{0.983}\right)^2 + \left(\frac{0.0699}{100}\right)^2 + \left(\frac{0.000969}{1.0026}\right)^2 + \left(\frac{0.0390}{50}\right)^2 + \left(\frac{0.000920}{0.9970}\right)^2 + \left(\frac{0.0390}{50}\right)^2$$

The standard uncertainty in the dry weight correction (u_w) was obtained from the standard deviation of multiple measurements of dry weight divided by the square root of the number of replicates.

The standard uncertainties of A_y , B_y , A_{xz} and B_{xz} were obtained by using half of the

estimated lower and upper limits of $(a+a_+)/2$ divided by $\sqrt{3}$, assuming a uniform distribution and values of 0.00002887, 0.0002887, 0.000002887 and 0.0000002887 were obtained, respectively.

The standard uncertainties in R_n and R'_n were calculated in a similar manner. The mass bias corrected ratio of R_n was obtained:

$$R_n = \frac{R_{\text{expected}}}{R_{\text{measured in std}}} \cdot R_{\text{measured in sample}} \quad (12.29)$$

where R_{expected} is the expected ratio obtained from A_{xz} divided by B_{xz} and its standard uncertainty can be calculated using equation 12.4; $R_{\text{measured in std}}$ is the measured ratio of reference and spike isotopes in a natural abundance standard solution and its uncertainty was obtained from the standard deviation of several measurements divided by the square root of the number of replicates; $R_{\text{measured in sample}}$ is the measured ratio of reference and spike isotopes in a spiked sample and its uncertainty was calculated the same way as for $R_{\text{measured in std}}$. Thus, standard uncertainties of R_n and R'_n were calculated using equation 12.4 and values of 0.007801 and 0.007229 were obtained, respectively.

A typical value of 1.00 for E (100 %, based on spike recovery data) was used in equation 12.2 for the calculation of uncertainty. The standard uncertainty of E was estimated from spike recovery data and was calculated from the standard deviation of replicate spike recovery results divided by the square root of the number of replicates.

The standard uncertainty in C_b was obtained using the standard deviation of measured concentrations in three sample blanks divided by the square root of three.

12.5.4. Total Combined Standard Uncertainty for TBT

Using the information on each standard uncertainty of the individual parameters

and individual partial derivatives in equations 12.11 to 12.24, the total combined standard uncertainty associated with the concentration of TBT determined in PACS-2 using the ID GC-SF-ICP-MS can be calculated by use of equation 12.2. Results are summarized in Table 12.1.

Table 12.1. Results for TBT, DBT and MBT in PACS-2 ($\mu\text{g g}^{-1}$ as Sn)

	TBT	DBT	MBT
Recommended value	0.894	1.115	0.639
Combined uncertainty, u_c	0.0152	0.0175	0.0140
Coverage factor, k	2	2	2
Expanded uncertainty, U	0.030	0.035	0.028
Mean	0.894	1.115	0.639
Standard deviation (n=6) S_i	0.012	0.015	0.017

The components of uncertainty for the determination of TBT in PACS-2 by ID GC-SF-ICP-MS are summarized in Table 12.2. It was noted that values contributed from co-related parameters of A_y and B_y , as well as A_{xz} and B_{zx} , calculated from the second term in equation 12.2 were at least six orders smaller than any value calculated from the first term and thus contributed negligibly to the total combined standard uncertainty. It is clear that the uncertainty associated with the measurement of the isotopic ratio in the spiked sample and in the reverse ID calibration solutions and the uncertainty associated with the extraction efficiency are the principal sources contributing to the total combined standard uncertainty. This elucidation provides important information for the design of future experiments aimed at reducing the overall uncertainty of the methodology, thereby

achieving more precise and accurate results.

Table 12.2. Components of Uncertainty for Determination of TBT in PACS-2

Sediment					
Parameter	Typical value	Type A/B	u_{xi}	$\partial f/\partial x_i$	$(\partial f/\partial x_i)u_{xi}$
C_z ($\mu\text{g ml}^{-1}$)	2.0438	B	0.00457	0.4394	0.002009
v_y (ml)	0.2500	B	0.000243	3.5924	0.000872
m_x (g)	0.5021	B	0.000191	-1.7887	-0.000342
w	0.98960	A	0.000225	-0.9076	-0.000204
v_z (ml)	0.1250	B	0.000216	7.1849	0.001549
v'_y (ml)	0.1500	B	0.000243	-5.9874	-0.001454
A_y	0.0004	B	0.0000289	0.0351	0.000001
B_y	0.921	B	0.000289	-0.000015	-0.000000004
A_{xz}	0.3259	B	0.0000029	-0.0398	-0.000000115
B_{xz}	0.0768	B	0.0000029	0.1688	0.000000
R_n	1.01643	B	0.007801	1.1621	0.009065
R'_n	0.98060	B	0.00723	-1.1913	-0.008612
E	1.000	A	0.00894	0.898	0.008033
C_b ($\mu\text{g g}^{-1}$)	0.0011	A	0.00058	-1.0000	-0.000577
Combined uncertainty ($\mu\text{g g}^{-1}$), u_c					0.0152

12.5.5. Total Combined Standard Uncertainty for DBT and MBT

Total combined standard uncertainty for DBT and MBT was calculated using equation 12.2, similar to that described above for TBT. Results are also presented in Table 12.1. The components of uncertainty for determination of DBT and MBT in PACS-2 by ID GC-SF-ICP-MS are summarized in Table 12.3 and 12.4, respectively.

Table 12.3. Components of Uncertainty for Determination of DBT in PACS-2

Sediment					
Parameter	Typical value	Type A/B	u_{xi}	$\partial f/\partial x_i$	$(\partial f/\partial x_i)u_{xi}$
C_z ($\mu\text{g ml}^{-1}$)	5.0475	B	0.01034	0.2203	0.002279
v_y (ml)	0.1248	B	0.000216	8.9115	0.001921
m_x (g)	0.5021	B	0.000191	-2.2150	-0.000423
w	0.98960	A	0.000225	-1.1238	-0.000253
v_z (ml)	0.1016	B	0.000216	10.9464	0.002360
v'_y (ml)	0.1251	B	0.000243	-8.8902	-0.002159
A_y	0.0004	B	0.0000289	0.0708	0.000002
B_y	0.921	B	0.000289	-0.000031	-0.000000009
A_{xz}	0.3259	B	0.0000029	-0.0816	-0.000000235
B_{xz}	0.0768	B	0.0000029	0.3463	0.000001
R_n	1.03465	B	0.006722	1.4217	0.009557
R'_n	0.97553	B	0.00660	-1.4806	-0.009774
E	1.00000	A	0.00894	1.1122	0.009947
C_b ($\mu\text{g g}^{-1}$)	0.0014	A	0.00058	-1.0000	-0.000577
Combined uncertainty ($\mu\text{g g}^{-1}$), u_c					0.0175

Table 12.4. Components of Uncertainty for Determination of MBT in PACS-2

Sediment					
Parameter	Typical value	Type A/B	u_{xi}	$\partial f/\partial x_i$	$(\partial f/\partial x_i)u_{xi}$
C_z ($\mu\text{g ml}^{-1}$)	1.7278	B	0.00412	0.3697	0.001522
v_y (ml)	0.1008	B	0.000216	6.3366	0.001366
m_x (g)	0.5021	B	0.000191	-1.2721	-0.000243
w	0.98960	A	0.000225	-0.6454	-0.000145
v_z (ml)	0.1007	B	0.000216	6.3429	0.001367
v'_y (ml)	0.1008	B	0.000243	-6.3366	-0.001539
A_y	0.0111	B	0.0000289	0.3556	0.000010
B_y	0.955	B	0.000029	-0.004134	-0.000000119
A_{xz}	0.3259	B	0.0000029	-0.6189	-0.000001786
B_{xz}	0.1454	B	0.0000289	1.3872	0.000040
R_n	0.92626	B	0.006949	1.1839	0.008227
R'_n	0.62709	B	0.00652	-1.4334	-0.009341
E	1.00000	A	0.00894	0.6387	0.005713
C_b ($\mu\text{g g}^{-1}$)	0.0006	A	0.00035	-1.0000	-0.000352
Combined uncertainty ($\mu\text{g g}^{-1}$), u_c					0.0140

It is evident from Tables 12.3 and 12.4 that uncertainty associated with the measurement of the isotopic ratio in the spiked sample and in the reverse ID calibration solutions and the uncertainty associated with extraction efficiency are the principal sources contributing to the total combined standard uncertainty for both DBT and MBT in PACS-2. In the case of MBT, the standard deviation, S_i , of the six replicates is larger than the combined uncertainty. This is indicative of either some major underestimation of parameters in the uncertainty budget, or of sample inhomogeneity. A careful re-evaluation of the

uncertainty estimation revealed no major underestimation of parameters. An ANOVA test was then conducted on the measured MBT concentrations from Bottle 1 (0.623 and 0.623 $\mu\text{g g}^{-1}$) and from Bottle 2 (0.659 and 0.669 $\mu\text{g g}^{-1}$). Based on this test, a significant difference in the concentrations of MBT between the two bottles was indicated, confirming inhomogeneity between bottles was the source of the problem.

12.6. Conclusions

A PTFE transfer line, heated to 220 °C, eliminates peak broadening for the less volatile TBT analyte. No significant change in peak widths for TBT and DBT was obtained with the heated transfer line, despite three weeks of constant use. Accurate and precise results for the concentrations of all three BTs in PACS-2 were obtained using species specific ID GC-SF-ICP-MS with this arrangement.

An analysis of total combined standard uncertainty associated ID GC-SF-ICP-MS for determination of TBT, DBT and MBT revealed that the uncertainty arising from the measurement of the isotope ratio in the spiked sample and in the reverse ID calibration solutions, and from estimation of the extraction efficiency were the main contributors. This calculation exercise is important for the design of future experiments aimed at reducing the overall uncertainty of the methodology, permitting achievement of yet more precise and accurate results.

CHAPTER 13. DEVELOPMENT OF AN ALTERNATIVE GC-MS METHOD FOR THE DETERMINATION OF BUTYLtin CONTENT

13.1. Introduction

Gas chromatography mass spectrometry (GC-MS) has been used as a powerful technique for characterizing organic molecules, including organometallic compounds, in various sample matrices due to its capability for structure elucidation. Moreover, the cost of GC-MS instrumentation is much lower than that for GC-ICP-MS. On the other hand, the spectra generated by GC-MS can be complex. Despite the advantages offered by GC-MS, isotope dilution calibration has rarely been applied, due to the complex molecular spectrum generated and the need to calculate relative isotopic abundances for any molecular ions used for quantitation.

An evaluation of the application of isotope dilution with GC-MS for the accurate and precise determination of TBT, DBT and MBT in PACS-2 sediment was thus undertaken so as to make available an independent method for these measurements.

13.2. Sample Preparation

Isotope dilution and reverse spike isotope dilution calibration were based on use of ^{117}Sn enriched TBT and DBT spikes and ^{116}Sn enriched MBT spike. Sample preparation was as described in Chapter 12 (section 12.2). Parameters for GC separation were carefully optimized as described earlier (Chapter 6) and were adapted for GC-MS. Typical operating conditions are summarized in Table 13.1.

Table 13.1. GC-MS operating conditions used for the determination of butyltins

Column	DB-5MS 30 m x 0.25 mm id x 0.25 μm d_f
Injection system	split/splitless injector- splitless mode
Injector temperature	250 °C
Oven temperature program	60°C to 260 °C at 20°C/min (temperature hold 1 min)
Carrier gas; flow rate	Helium; 1.2 ml min ⁻¹
Transfer line temperature	260 °C
MS	HP model 5973 mass selective detector
SIM parameters	Measured ions: $m/z = 232; 235$, Dwell times: 25 ms for each m/z
MS quad temperature	150 °C
MS source temperature	250 °C

13.3. Determination of TBT in PACS-2 Using Different ID Calibration Strategies

As shown by equation 7.2, only the isotopic ratios (mass bias corrected) in the spiked samples and reverse ID calibration samples need to be measured to derive the final analyte concentration, since abundances of selected isotopes are known.

The spectrum generated by GC-MS is complicated due to fragmentation patterns such that the measured isotopic pattern of molecular ions is skewed from that of the naturally occurring isotopic pattern for elemental tin. All species arising from combinations of various isotopes for the reference and spike ions must thus be included in calculations of isotope ratios for the real abundance of the reference and spike ions to permit equation 7.2 to be used for the final quantitation.

Equation 13.1 can be used for quantitation of BTs using isotope dilution GC-MS without requiring the relative abundance of selected ions:

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

$$= C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{k_y \cdot R_y - k_b \cdot R_b}{k_b \cdot R_b - k_x \cdot R_x} \cdot \frac{k'_b \cdot R'_b - k_z \cdot R_z}{k_y \cdot R_y - k'_b \cdot R'_b} \quad (13.1)$$

where C_x is the analyte concentration ($\mu\text{g g}^{-1}$) based on dry mass; C_z is the concentration ($\mu\text{g ml}^{-1}$) of the natural abundance BT standard solution; v_y is the volume (ml) of spike used to prepare the blend solution of sample and spike; m_x is the mass (g) of sample used; w is the dry weight correction factor; v_z is the volume (ml) of natural abundance BT standard used to prepare the blend solution of spike and standard; v'_y is the volume (ml) of spike used to prepare the blend solution of spike and natural abundance BT standard; k_y , k_b , k_x , k'_b , and k_z , are the mass bias correction factors for the measured reference/spike ion ratios in the enriched spike solution, the blend solution of sample and spike, the unspiked sample, the blend solution of spike and natural abundance BT standard and the natural abundance BT standard solution, respectively; R_y , R_b , R_x , R'_b , and R_z , are the measured reference/spike ion ratios in the enriched spike solution, the blend solution of sample and spike, the unspiked sample, the blend solution of spike and natural abundance BT standard and the natural abundance BT standard solution; $\sum(k_{ix} \cdot R_{ix})$ and $\sum(k_{iz} \cdot R_{iz})$ are the sums of the ratios of all isotope abundances to one isotope abundance chosen as a reference in the sample and in the standard, respectively. Note that $\sum(k_{ix} \cdot R_{ix}) = \sum(k_{iz} \cdot R_{iz})$ for all elements whose isotopic abundance is invariant in nature. As shown in Figure 13.1 the $(\text{C}_4\text{H}_9)_2\text{SnH}^+$, fragment ion

Figure 13.1. Chromatogram for an extract of spiked PACS-2 obtained using GC-MS: a: total ion chromatogram; b: isotope pattern for TBT.

was selected for quantitation as a result of its better signal-to-noise ratio as well as the fact that it presents as a fragment from all three derivatized butyltins. The ion pair m/z 235(^{120}Sn) and 232 (^{117}Sn) was used for determination of TBT and DBT whereas the ion pair m/z 235(^{120}Sn) and 231 (^{116}Sn) was used for determination of MBT as a result of use of the ^{117}Sn enriched spikes for TBT and DBT and ^{116}Sn for MBT.

To achieve best accuracy and precision for the ratio measurement, a mass bias drift correction solution (which was prepared the same way as the reverse spike ID sample) was repeatedly introduced to bracket every four sample solutions in a run sequence, shown in Table 13.2. The initial mass bias correction factor of k_0 for the 235/232 ratio and a mass bias drift factor of 1.000 ($f_{t=0}$) were used. The mass bias drift correction solution was injected at the beginning of the run sequence and the 235/232 ratio ($R_{\text{mass bias drift-0}}$) was measured. The mass bias drift factor for measurement “t” of the mass bias drift solution in the measurement sequence can be obtained from the following equation:

$$f_{t=t} = f_{t=0} \cdot \frac{R_{\text{mass-bias-drift-t}}}{R_{\text{mass-bias-drift-0}}} \quad (13.2)$$

where $R_{\text{mass-bias-drift-t}}$ is the ratio measured at number t in the mass bias drift correction solution. The mass bias drift correction factors for the bracketed four samples can be calculated using $f_{t=i}$ and $f_{t=i+1}$, assuming a linear drift. During the sequence shown in Table 13.2, the mass bias drift factor obtained was 1.010 ± 0.004 (mean and one standard deviation, $n=6$). It is apparent that no significant mass bias drift occurs during a GC-MS run sequence. These observations suggest that the mass bias drift solution can be injected less frequently to decrease the analysis time. The initial mass bias correction factor of k_0 for the 235/232 ratio

Table 13.2. Injection sequence

1. mass bias drift solution	16. mass bias drift solution
2. unspiked PACS-2	17. spiked sample #1
3. unspiked PACS-2	18. reverse ID solution #1 -stock A
4. natural TBT standard	19. spiked sample #2
5. natural TBT standard	20. reverse ID solution #2- stock A
6. mass bias drift solution	21. mass bias drift solution
7. enriched spiked TBT standard	22. spiked sample #3
8. enriched spiked TBT standard	23. reverse ID solution #3 -stock A
9. blank #1	24. spiked sample #4
10. blank #2	25. reverse ID solution #4-stock B
11. mass bias drift solution	26. mass bias drift solution
12. blank #3	27. spiked sample #5
13. blank #1	28. reverse ID solution #5-stock B
14. blank #2	29. spiked sample #6
15. blank #3	30. reverse ID solution #6-stock B
	31. mass bias drift solution

was therefore used for all measured ratios in the samples. Equation 13.2 can therefore be simplified to:

$$\begin{aligned}
 C_x &= C_z \cdot \frac{v_y}{w \cdot v_x} \cdot \frac{v_z}{v'_y} \cdot \frac{k_0 \cdot R_y - k_0 \cdot R_b}{k_0 \cdot R_b - k_0 \cdot R_x} \cdot \frac{k_0 \cdot R'_b - k_0 \cdot R_z}{k_0 \cdot R_y - k_0 \cdot R'_b} \\
 &= C_z \cdot \frac{v_y}{w \cdot m_x} \cdot \frac{v_z}{v'_y} \cdot \frac{R_y - R_b}{R_b - R_x} \cdot \frac{R'_b - R_z}{R_y - R'_b} \quad (13.3)
 \end{aligned}$$

Equation 13.3 was used to calculate the concentration of TBT (C_x) in PACS-2, yielding $0.895 \pm 0.015 \mu\text{g g}^{-1}$ (one standard deviation, $n=4$) as tin, in good agreement with the

value of 0.894 ± 0.012 obtained using GC ICP-MS (Chapter 12). Evidence for the absence of interferences on selected ions was confirmed to assure accuracy; measurements were also conducted by monitoring different masses. In addition to m/z 232 and 235, the concentration of TBT (C_x) was calculated using the m/z 204 and 207 pair of ions. The concentration of TBT obtained was in good agreement with the value obtained using the m/z 235 to 232 ratio. However, poorer signal-to-background ratios and poorer precision were also realized.

As noted earlier, quantitation of TBT using equation 7.2, which is based on the relative abundance of isotope compositions of molecular ions, is a difficult task due to the complicated calculations of relative abundances of selected ions. It is of interest to compare results obtained using equation 7.2 and equation 13.3. A software program (Isotope Pattern Calculator v 4.0) developed by Yan¹¹⁴ was used to calculate contributions for $(C_4H_9)_2SnH^+$ at m/z 235 and 232 from all species. Relative abundances of 30.60 % for m/z 235 and 8.21 % for m/z 232 were obtained in the samples and natural abundance standard based on the natural isotopic abundance of Sn, C and H. Relative abundances of 0.052 % for m/z 235 and 84.81% for m/z 232 were calculated for the ^{117}Sn enriched TBT spike based on the provided isotope composition of Sn. With this information, equation 7.2 can be used for the quantitation of TBT in PACS-2.

A mass bias correction factor was obtained from the calculated ratio of 3.727 (30.60 % / 8.21%) divided by the measured 235/232 ratio in a natural abundance standard solution. A TBT concentration of $0.895 \pm 0.015 \mu g\ g^{-1}$ (one standard deviation, $n=4$) as tin in PACS-2 was obtained using equation 7.2. This result is in an excellent agreement with the value obtained using equation 13.3.

13.4. Final Quantitation of TBT, DBT and MBT in PACS-2

For simplicity, equation 13.3 was used for the final quantitation of TBT, DBT and MBT in PACS-2 sediment. Concentrations of 0.894 ± 0.017 and $1.059 \pm 0.023 \mu\text{g g}^{-1}$ as tin (one standard deviation, $n=6$) for TBT and DBT, respectively, using ^{117}Sn enriched spikes were obtained. These results are in agreement with those obtained by GC ICP-MS (Chapter 12).

A concentration of $0.629 \pm 0.014 \mu\text{g g}^{-1}$ (one standard deviation, $n=6$) as tin was obtained for MBT in PACS-2 using a ^{116}Sn enriched spike, in agreement with the value of $0.639 \pm 0.017 \mu\text{g g}^{-1}$ (one standard deviation, $n=4$) obtained by GC ICP-MS.

The detection limit for the ID GC-MS technique was evaluated using three spiked blank samples. Values of 0.008, 0.010 and $0.011 \mu\text{g g}^{-1}$ were estimated for TBT, DBT and MBT, respectively, based on three times the standard deviation of the calculated concentrations normalized to a 0.50 g sub-sample with derivatization of a 5 ml volume of extract.

13.5. Conclusions

An alternative accurate and precise method has been developed for the determination of TBT, DBT and MBT in PACS-2 sediment CRM using species specific ID GC-MS. Results obtained for all three BTs using this method are in good agreement with those obtained by GC ICP-MS, fulfilling the certification requirement of applying at least two independent analytical methodologies. Lower sensitivity and slightly poorer detection limits were obtained with GC-MS compared to GC ICP-MS, but still adequate to produce accurate and precise results for the concentrations of the BTs in PACS-2.

CHAPTER 14. SUMMARY AND FUTURE WORK

14.1. Summary

Several analytical methods for the determination of BTs in sediments using ICP-MS as a sensitive and selective detector in combination with HPLC or GC separation were developed. An early method based on HPLC separation with ICP-MS detection using a Whatman SCX strong cation-exchange column and an ammonium citrate buffer in a methanol-water as mobile phase achieved chromatographic separation of MBT, DBT and TBT in 8 minutes. Detection limits (3 SD), based on a 0.5 g sample, of 0.08, 0.05 and 0.03 $\mu\text{g g}^{-1}$ for MBT, DBT and TBT, respectively, were obtained. Concentrations of 0.96 ± 0.03 , 1.08 ± 0.07 and $0.46 \pm 0.03 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=5$) as tin were measured for TBT, DBT and MBT with precisions of 3.1, 6.5 and 6.5 %, respectively, based on standard additions calibration. These values were in agreement with the certified values.

A second GC-ICP-MS method was developed with which chromatographic separation of MBT, DBT and TBT could be achieved in 10 minutes with method detection limits (3 SD) of 0.006, 0.001 and 0.002 $\mu\text{g g}^{-1}$, respectively, based on a 0.5 g sub-sample. Concentrations of 0.53 ± 0.02 , 1.14 ± 0.02 and $0.93 \pm 0.03 \mu\text{g g}^{-1}$ (mean and one standard deviation, $n=5$) for MBT, DBT and TBT, respectively as tin were obtained in PACS-2 using standard additions calibration with tripropyltin (TPrT) as an internal standard. Slightly better precisions of 1.7 to 3.8 % RSD for the three butyltins were obtained by GC-ICP-MS using standard additions calibration compared to 3.1 to 6.5 % RSD obtained with the HPLC-ICP-MS method. In addition, resolution and sensitivity obtained for all butyltin species by GC-ICP-MS were far better than could be obtained using HPLC-ICP-MS.

Methodologies having the highest accuracy and precision for determination of these

species in sediments using species specific ID ICP-MS in combination with separation by either HPLC or GC were also developed and validated. Significant improvements (close to 4-fold) in precision of measurement of TBT with ID were obtained with both HPLC- and GC-ICP-MS methods, clearly demonstrating its superiority in providing more precise results as compared to the method of standard additions. Furthermore, a significant 18-fold improvement in the precision of measurement of TBT in PACS-2 using ID SPME GC-ICP-MS detection clearly demonstrated its superior capability to overcome the generally poor precision associated with the SPME sampling technique. The approach is well suited not only for certification of BTs, but for improving the methodology used for organometallic speciation in general when SPME sampling is used. Amongst the developed methods, ID SPME-GC-ICP-MS is the most sensitive, possessing a superior detection limit of 0.09 ng g^{-1} for TBT.

A performance comparison was made between SF-ICP-MS detection and qICP-MS detection using GC separation for the determination of TBT and DBT in sediments. Accurate and precise determination of TBT and DBT in sediments by species specific isotope dilution using both qICP-MS and GC-SF-ICP-MS was obtained. A 2-fold enhancement in the precision of Sn isotope ratios measured in standard solutions of TBT and DBT by GC-SF-ICP-MS, as opposed to GC-qICP-MS, was realized. A 2-fold improvement in their LODs was obtained as a consequence of the enhanced sensitivity with the sector field instrument. The rapid mass scanning capability of the SF-ICP-MS offered no impediment to rapid peak hopping for the accurate recording of transient signals having FWHM of only a few seconds.

Robust and quantitative extraction methods using glacial acetic acid with both open

and closed-vessel microwave assisted systems were investigated and used to monitor the short- and long-term stability of BTs under various conditions. Sample homogeneity, the effects of γ -irradiation on the stability of BTs during the production of CRMs, as well as production of accurate and precise results for concentrations of BTs in the certification of several candidate CRMs were studied.

As a result of the study of long-term stability of BTs in PACS-2, its recertification is currently underway. Concentrations of 0.894 ± 0.030 , 1.115 ± 0.035 and $0.639 \pm 0.028 \mu\text{g g}^{-1}$ (mean and expanded uncertainty, $k=2$) were obtained for TBT, DBT and MBT for the recertification, respectively, using species specific ID GC-SF-ICP-MS methodology. An alternative ID GC-MS method was also developed for the determination of these species for this purpose and values generated are in good agreement with those obtained by ID GC-SF-ICP-MS.

14.2. Future Work and Conclusions

The bias noted in the concentrations of MBT determined in PACS-2 using HPLC- and GC-ICP-MS methods with standard additions calibration during the long-term stability study remains unresolved. Further investigations require an isotopically enriched spike of MBT to take advantage of the benefits offered by ID in overcoming any possible matrix effects in the sample and any potential losses during sample extraction as well as derivatization. Thus, for future studies, an enriched MBT should be synthesized for this purpose. Recently, a triple spike methodology was developed¹¹⁵⁻¹¹⁶ for the study of different extraction procedures for the determination of BTs in a mussel tissue and was able to correct conversion between BTs and inorganic Sn during the sample digestion. This methodology may also be used to investigate the bias noted earlier in the concentrations of MBT.

During the work undertaken in the past four years for this study, the significance of the analytical problem relating to the determination of organotin speciation in environmental samples of sediment and biological tissue became evident by the dramatic increase in publications addressing this concern. No attempt was made in this study to comprehensively cite all of the literature relating to the quantitation of butyltins in various samples, but rather to focus only on a core group of international laboratories seeking to enhance the accuracy and precision of these measurements, the objective of this research. Despite targeting BTs, the procedures devised here are universally applicable to other organometallic compounds and the contributions of this research to the field of speciation are thus broader than originally anticipated. Methodologies based on species specific isotope dilution developed in this study have served to fulfill the research objectives of generating more accurate and precise results for quantitation of butyltins in sediment CRMs for the purpose of their certification, to monitor their short- and long-term stability under various storage conditions, as well as overcome the generally poor precision associated with SPME sampling. These methodologies can also be applied to the speciation of other organometallic compounds in the related fields of toxicology, environmental monitoring and for any work requiring high accuracy and precision, such as quantitation of methylmercury in biological CRM matrices and selenomethionine in yeast CRM. There is no doubt that species specific isotope dilution techniques will be applied to achieve high accuracy and precision in many more research fields in the future.

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