DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR THE EXTRACTION, SEPARATION AND DETERMINATION OF ORGANOMETALLIC AND INORGANIC METAL SPECIES IN ENVIRONMENTAL AND BIOLOGICAL MATRICES

Raimund Wahlen

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ABSTRACT

DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR THE EXTRACTION, SEPARATION AND DETERMINATION OF ORGANOMETALLIC AND INORGANIC METAL SPECIES IN ENVIRONMENTAL AND BIOLOGICAL MATRICES

Raimund Wahlen (BSc, MSc)

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Existing methodologies for the separation and detection of organotin species by liquid- and gas-chromatography coupled to inductively coupled plasma mass-spectrometry (ICP-MS) have been improved significantly. The LC separation of dibutyltin (DBT), triphenyltin (TPhT) and unidentified species in mussel tissue has been enhanced in terms of peak shape and resolution. GC-ICP-MS detection limits for organotin species are significantly reduced by addition of oxygen or nitrogen to the ICP-MS carrier gas. A comparison of LC-ICP-MS and GC-ICP-MS for the species-specific isotope dilution determination of tributyltin (TBT) in sediment extracts has shown that both methods deliver accurate results. However, GC-ICP-MS is significantly more sensitive (absolute detection limit of 0.03 pg TBT as Sn compared to 3 pg TBT as Sn by LC-ICP-MS) and provides superior isotope ratio measurement precision, which is linked to more reproducible peak integration and greater analyte signals by this technique. ^{117}Sn enriched DBT and TBT have been used to investigate the stability of species during extraction and the derivatisation efficiency of ethylation.

The speciation of arsenic (As) has been performed using anion-exchange chromatography coupled to ICP-MS detection. Good separations have been achieved for up to seven species including monomethyl- and dimethylarsinic acid (MMA and DMA) and especially between arsenobetaine (AsB) and arsenite (As(III)). The accuracy and precision of the methods have been tested by analysis of a range of biological certified reference materials.

Extraction methods for organotin and arsenic species have been developed using accelerated solvent extraction (ASE), and this technique has been assessed for the simultaneous co-extraction of Sn, As and Hg species from environmental samples. Accurate data were obtained for DBT, TBT, AsB and DMA in a certified oyster tissue, and promising results were also obtained for methylmercury. The combined extraction, separation and detection methods have been used to determine the vertical profiles of organotin and arsenic species in sediments from the Thames estuary (UK). Up to nine organotin species were detected, with DBT being the predominant at levels ranging from ~ 12 - 160 ng/g (as Sn). The main As species were As(III) and As(V) at concentrations between ~ 160 - 4200 ng/g (as As). Methylated arsenicals were also detected throughout the core.

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GLOSSARY

AES	Atomic Emission Spectrometer
Ar	Argon
As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
AsB	Arsenobetaine
AsC	Arsenocholine
ASE	Accelerated Solvent Extraction
BFD	Blackfoot disease
BR	Boundary Region
Ce	Cerium
CE	Capillary Electrophoresis
Cl	Chlorine
CO_2	Carbon dioxide
CRM	Certified Reference Material
CH_3COOH	Acetic Acid
DBT	Dibutyltin
DMA	Dimethylarsinic acid
DOT	Dioctyltin
DPhT	Diphenyltin
ECD	Electron Capture Detector
ESI-MS	Electrospray Ionisation-Mass Spectrometry
FID	Flame Ionisation Detector
GC	Gas Chromatography
GC-ICP-MS	Gas Chromatography coupled to ICP-MS
HCl	Hydrochloric Acid
Hg	Mercury
HNO_3	Nitric Acid
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICP	Inductively Coupled Plasma

ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IDMS	Isotope Dilution Mass Spectrometry
IRZ	Initial Radiation Zone
IUPAC	International Union of Pure and Applied Chemistry
kPa	kilo Pascal
LC	Liquid Chromatography
LC-ICP-MS	Liquid Chromatography coupled to ICP-MS
Li	Lithium
LOD	Limit of Detection
MAE	Microwave assisted extraction
MBT	Monobutyltin
MeOH	Methanol
MMAA	Monomethyl arsonic acid
MPhT	Monophenyltin
MS	Mass Spectrometer
MS-MS	Tandem mass spectrometer
N	Nitrogen
N_2	Nitrogen gas
Na_2SO_4	Sodium Sulphate
$NaBEt_4$	Sodium tetraethylborate
NAZ	Normal Analytical Zone
NH_4HCO_3	Ammonium Hydrogen Carbonate
O_2	Oxygen
OD	Ordinance Datum
OES	Optical Emission Spectrometry
P	Phosphorus
PEEK	Polyether Ether Ketone
PFE	Pressurized Fluid Extraction
PHZ	Pre-Heat Zone
psi	pounds per square inch
PTFE	Polytrifluoroethylene
PTWI	Provisional Tolerable Weekly Intake
Rh	Rhodium

RSD	Relative Standard Deviation
SD	Standard Deviation
Sn	Tin
SPE	Solid-phase Extraction
SS-IDMS	Species-Specific Isotope Dilution Mass Spectrometry
TBT	Tributyltin
TEA	Triethylamine
TeBT	Tetrabutyltin
Tl	Thallium
TOF-MS	Time-Of-Flight Mass Spectrometry
TPhT	Triphenyltin
TPrT	Tripropyltin
UV	Ultra-violet
W	Watts
WHO	World Health Organisation
Xe	Xenon
Y	Yttrium

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AIMS & OBJECTIVES

- To develop robust and reliable separation and detection methods based on hyphenated chromatographic ICP-MS for the determination of organotin and arsenic species in environmental matrices.

- To try and improve the performance of currently used chromatographic ICP-MS techniques in terms of detection limits, analyte resolution or analysis time so as to make them more amenable for environmental monitoring applications.

- To investigate existing extraction methodologies for organometallic species of toxicological significance (mainly arsenic and organotin species) and to improve such methods by either increasing analyte capabilities, overall resource efficiency and sample through-put or by combining separate extraction approaches for species of different elements into a single simultaneous extraction.

- If successful, to apply the combined extraction, separation and detection methodologies to a range of sample matrices, both in the form of matrix certified reference materials (CRMs) and 'real-world' environmental samples.

- The performance benchmark for the developed methodologies should be that they are suitable in terms of accuracy and precision for providing data to the certification of new certified reference materials for organometallic speciation for environmental applications.

CHAPTER 1

INTRODUCTION

The toxicity of some trace metals has been known since ancient times. Arsenic, for example was often used as a poison in assassination attempts and the neurotoxic effects of mercury on people working in the hat manufacture in Victorian England led to the phrase 'mad as a hatter'. However, the fact that the toxicity of a metal depends primarily on its chemical form or 'species' is a relatively recent discovery.

One of the earliest environmental pollution incidents in which fatal consequences of a trace metal have subsequently been linked to one of its organometallic species took place in the Japanese coastal town of Minamata in the early 1950s. Initially, mostly fishermen and their families were affected by neurotoxic and teratogenic symptoms, which were not immediately diagnosed as metal poisoning (Clark, 1992). The source of the problem was not conclusively identified until the late 1950s when it was shown that a nearby vinyl chloride and acetaldehyde production plant had discharged effluents containing mercuric sulphate ($HgSO_4$), which was used as a catalyst and contained some monomethylmercury (CH_3Hg^+). It was subsequently demonstrated that the mercuric sulphate precipitated in the sea as insoluble HgS, which was transformed by bacteria in the sediments *via* biomethylation into CH_3Hg^+ (Alloway and Ayres, 1997).

Monomethylmercury is lipophilic, volatile and far more toxic than inorganic mercury and has since been shown to bio-accumulate in the marine food-chain leading to part-per million (ppm) level concentrations in some predatory fish species such as tuna, marlin or swordfish. The neurotoxic effects observed in the population at Minamata were directly linked to this species and the exposure source was determined as locally caught fish. The total number of cases of what has become known as 'Minamata Disease' from this single pollution incident are estimated as ~2000 of which 43 were fatalities and ~700 people were left with permanent neurological dysfunction (Plate 1.1). The World Health Organisation (WHO) as well as a number of individual states have since introduced maximum permitted levels of mercury and methylmercury in food (Clark, 1992) and this is particularly highlighted

in countries with a high sea-food consumption such as Japan and Sweden (Clark, 1992). The European Economic Community (EEC) introduced a level of 0.3 μg/g Hg in seafood in 1986. The fish from Minamata contained mercury levels of 10 – 55 μg/g of which most was in the form of methylmercury (Clark, 1992).

Plate 1.1 Tomoko Uemura in her Bath, Minamata 1972 (Photograph by W. Eugene Smith, © The Heirs of W. Eugene Smith).

Although only some of the initial effluent contained the more toxic methylmercury, in-situ natural processes led to a serious environmental pollution incident by an organometallic molecule. Most natural processes tend to act as de-toxifying mechanisms in areas of exposure to toxic metal compounds. In contrast to the biomethylation processes in Minamata, the biomethylation of inorganic arsenicals, such as arsenite (AsIII) and arsenate (AsV) in exposed sediments leads to the conversion to less toxic organic arsenicals of monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB). Several fungi, yeasts and bacterial strains have been shown to be able to transform different arsenic species, and the mechanisms are primarily reduction, oxidation, methylation and degradation reactions (Fodor, 2001). Although some micro-organisms appear to convert more toxic forms into methylated and volatile arsines and this is seen as a detoxification step allowing the organisms to persist in sediments containing up to 48000 mg/L of AsV (Xu et al., 1991), there is also some evidence that degradation of the less toxic AsB back to AsV is possible (Hanaoka et al., 1995).

Arsenic is present in the environment due to both natural and anthropogenic processes. The weathering of As-containing minerals from rocks, iron deposits and sedimentary iron ores is a significant natural input into the global geochemical As-cycle. Anthropogenic inputs are mainly due to arsenic containing pesticides and the use of arsenic compounds in the pottery, glass, wood preservative, and electronics industries. The relative proportion of natural and anthropogenic inputs is estimated as ~ 60:40 respectively (Chilvers and Peterson, 1987). Arsenic pollution has also affected human populations in areas such as Bangladesh, Taiwan and West Bengal, where drinking water from deep wells was contaminated by mostly inorganic arsenicals due to the weathering of the locally As-rich geology. A survey in West Bengal (Chatterjee et al., 1995) determined that in this area about 800,00 people were drinking As- contaminated water and ~ 175,000 people showed long-term exposure effects such as conjunctivitis, hyperkeratosis, hyperpigmentation, cardiovascular disease, disturbances in the peripheral vascular and nervous systems, skin cancers and gangrene of the limbs, also referred to as Blackfoot Disease (BFD, Plate 1.2).

Plate 1.2 Photograph showing a person affected by skin lesions on his hands and feet referred to as Blackfoot Disease due to chronic consumption of As-contaminated drinking water.

A third example of environmental pollution due to an organometallic species rather than the inorganic metal was the discovery that Tributyltin (TBT), an antifouling compound applied to ships' hulls in order to minimise algal and other biological growth, had lethal and sub-lethal effects on a variety of non-target organisms at concentrations as low as 0.02 μg/L (Alloway and Ayres, 1997). TBT is present in the

environment solely due to anthropogenic use, and it has been shown to accumulate in sediments particularly around harbours and marinas. Sublethal effects of this compound include reduced tissue growth and enhanced shell growth in bivalves and it was by these mechanisms that TBT was identified as the agent responsible for near collapse of the oyster fisheries in the Bay of Arcachon, France in the early 1980s (Alzieu et al., 1986). In addition TBT has been shown to be an endocrine disrupter leading to the development of male sexual features and reduced fertility in female marine gastropods leading to a decline in the populations of affected species even in areas where TBT concentrations are below the lethal threshold levels. In humans the toxic effects of TBT are thought to include a disruption of the mitochondrial function (Manahan, 1991). Bivalves such as mussels and oysters that are benthic filter feeders have been shown to accumulate TBT due to direct contact with polluted sediments and the ingestion of sediment particles during the feeding process. The consumption of such seafood is obviously an exposure route for humans to this toxic compound. Due to the economic impact on shellfish fisheries and the subsequent studies on the sub-lethal effects of TBT, a number of countries have introduced legislation (e.g. EEC directive 75/437/EEC) banning the use of TBT in antifouling paints applied to vessels shorter than 25m in length (Appendix 1). This has significantly ameliorated the pollution effects in some previously affected areas.

Although the examples described above highlight the negative impacts of some organometallic species in the environment and to humans, it is important to bear in the mind that such toxicity is species dependent. As shown by the examples in Table 1.1, different species of the same elements can range in toxicity from very toxic to being classified as non-toxic or even beneficial. Therefore, the analysis of the total inorganic metal content, which has traditionally been used for environmental heavy metal monitoring, can often only reveal a limited amount of information of toxicological relevance. This is illustrated by the fact that most seafood contains relatively high levels of arsenic (high part-per million levels are common), which would indicate a toxicological risk to consumers if only the total As levels were used as the basis for a risk assessment. However, the use of speciation methodologies, where the actual form of the arsenic in the sample is revealed, usually indicates that the majority of the total As (up to 92 - 99%) is in the form of the less or non-toxic

organic species indicating a low toxicological risk (e.g. Branch et al., 1994; Kirby and Maher, 2002).

Table 1.1 Examples of some toxic and non-toxic species of the metals tin, mercury, and arsenic.

Element	Symbol	Toxic Species	Symptoms	Non/less toxic species	Comment
Tin	Sn	TBT, TPhT, DBT, DPhT	Acute toxicity, Endocrine disruptors	MBT, MPhT, Sn	Breakdown product of toxic species in environment
Mercury	Hg	Hg, MeHg	Damage to CNS "Minamata disease"	None	
Arsenic	As	As(III), As(V)	Acute toxicity, "Blackfoot disease"	Arsenosugars, AsB, DMA, MMAA	Species synthesised by natural processes in environment

Although this is now widely accepted, and the field of organometallic speciation research has grown increasingly in recent years (Figure 1.1), even quite recent textbooks on marine and environmental pollution rarely address this topic in significant detail, except for referring to pollution incidents such as those mentioned above. Most environmental legislation is also still based on the levels of total metal concentrations, and the determination of their individual compounds is often only vaguely defined in legislative texts (Donard, 2001).

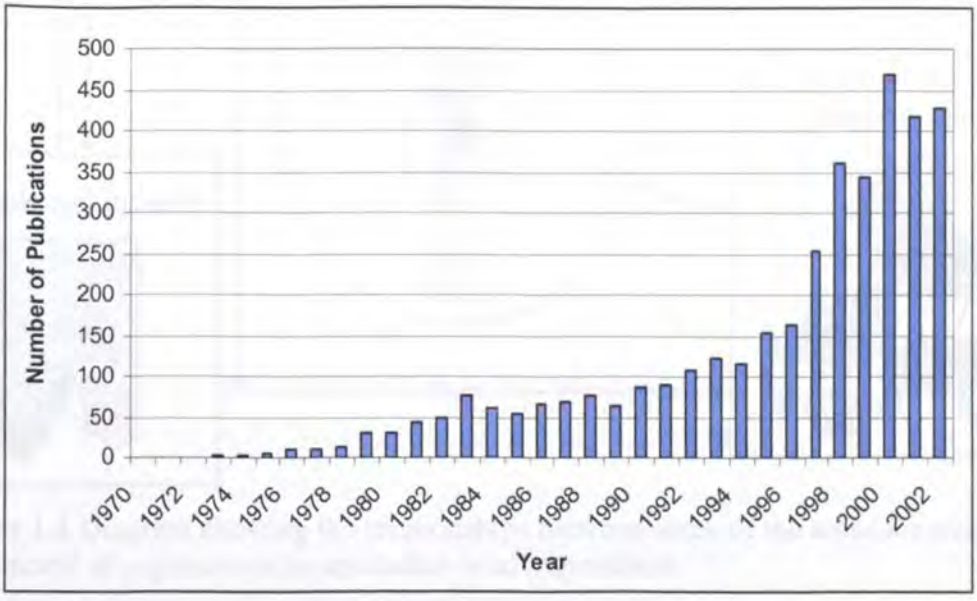

Figure 1.1 Diagram of the number of publications per year between 1970 - 2002 relating to 'metal speciation' or containing 'organometallic species' in their title. (Search performed by SciFinder 2002- Chemical Abstracts on your desktop)

It is evident that the concept of metal speciation is beginning to play an increasing part in scientific research both in furthering our understanding of environmental processes and how mankind affects these, as well as from an analytical point of view. Some of the possible interactions between this research area and everyday life are shown in Figure 1.2. Although the diagram is a gross simplification, it attempts to highlight some of the areas which are or will be affected by our knowledge about organometallic species. Metal species in the environment, be they of anthopogenic or natural origin, have an impact on the environment itself, the food commodities that humans exploit and other products such as medicines, which are derived from natural resources. Usually these three areas are subject to legislation, which is aimed at protecting human health and the environment itself. Such legislation often has a direct impact on trade and industry, which in turn has a knock-on effect on the environment.

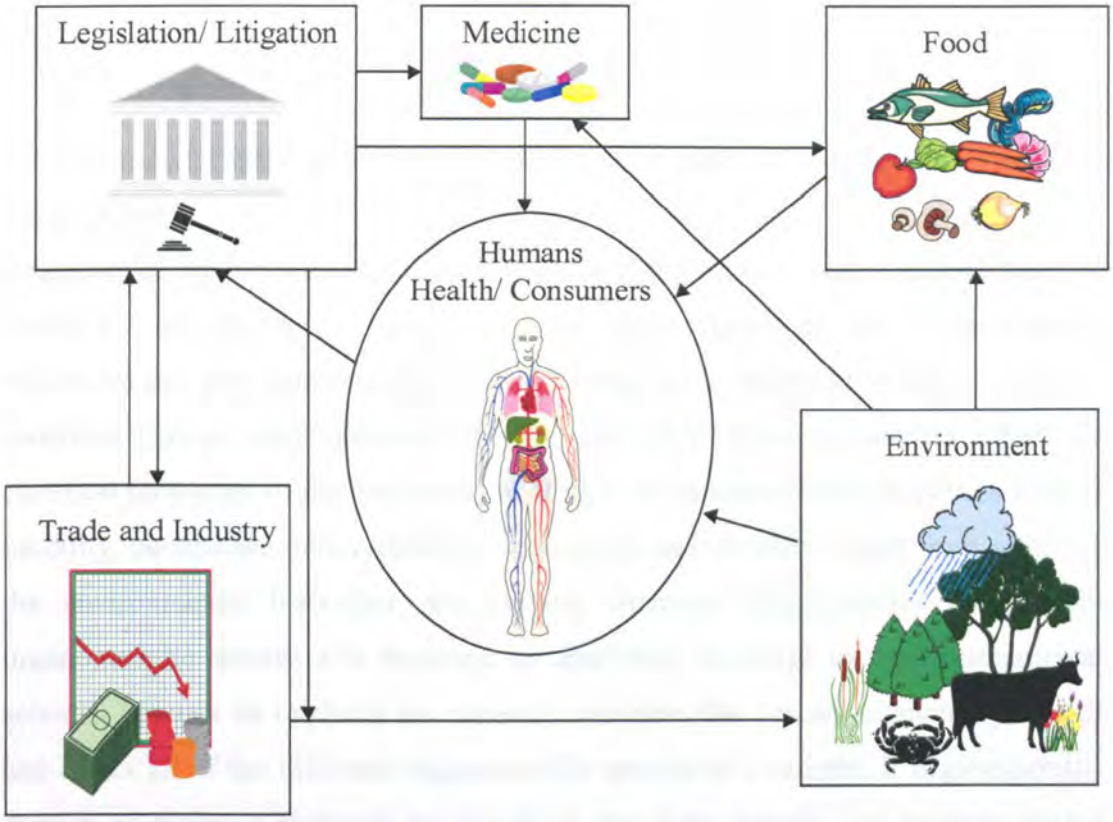

Figure 1.2 Diagram showing the relationships between some of the areas for which the concept of organometallic speciation is of importance.

1.1 DEFINITIONS

The definitions given by the International Union for Pure and Applied Chemistry (IUPAC) for the terms 'Chemical species' and 'Speciation analysis' are as follows:

> *Chemical species.*
> *<chemical elements> specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.*

> *Speciation analysis.*
> *<analytical chemistry> analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.*

The term 'organometallic species' is usually used to refer to molecules that contain a metal as a hetero-atom in their structure.

1.2 CHEMICAL PROPERTIES OF ORGANOMETALLIC SPECIES AND ANALYTICAL CHALLENGES

Examples of the molecular structures of some of the organometallic species listed in Table 1.1 are shown in Figure 1.3. The figure illustrates that organometallic molecules can vary significantly in terms of their size, molecular structure, polarity, functional groups and molecular weight, and all of these parameters affect the chemical properties of the compound relating to its environmental properties such as mobility, persistence, bioavailability, ecotoxicity and stability. Apart from affecting the environmental behaviour, the varying chemical characteristics of different organometallic species also represent an analytical challenge to the environmental scientist because no methods are currently available that can simultaneously extract and detect all of the different organometallic species in a sample. If organometallic species of different elements are sought in the same sample, for example during environmental monitoring surveys, it is therefore necessary to use several different analytical protocols to obtain the information requested.

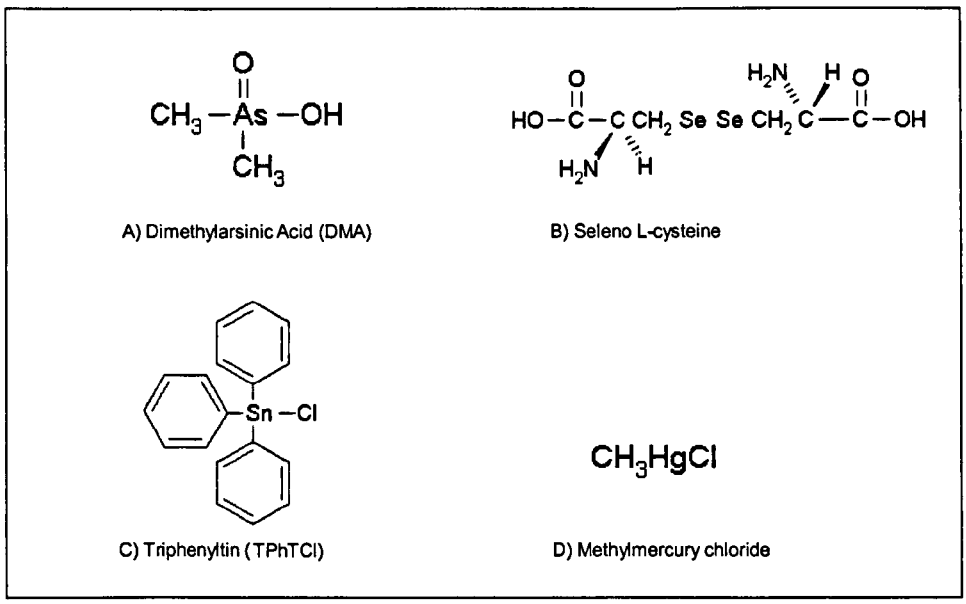

Figure 1.3 Examples of molecular structures of some organometallic species of different elements A) As (DMA), B) Se (Seleno L-cysteine), C) Sn (TPhTCl) and D) Hg (Methylmercury chloride).

The analysis of organometallic species in a particular sample requires a series of analytical steps. The most important aspects are highlighted as a flowchart in Figure 1.4 and comprise steps a) to e) below.

a) *The sample of interest*: The characteristics of the sample, such as organic carbon content, fat content, particle size, moisture content etc. have a significant influence on the performance of the analytical method. Therefore, the more that is known about the sample the better the analytical approach can be adapted to overcome potential difficulties caused by the physical, chemical and biological properties of the sample matrix. Depending on the physical characteristics of the sample, some pre-treatment such as fat removal, peeling, drying, grinding or sieving may be required to prepare the sample for the extraction step.

b) *Extraction of the analyte(s) of interest from the sample*: The extraction methodology is the first and often the most crucial step in the analytical process. In order to obtain accurate results it is essential that the sample preparation is both quantitative, i.e. all of the analyte is extracted from the matrix and representative i.e. the chemical or physical form of the target analyte is not

altered during the extraction process. In addition it should be reproducible in order to allow comparisons between different samples to be made.

c) *Clean up/ Derivatisation/ Pre-concentration steps*: At present no extraction methodologies exist that extract the target analyte exclusively whilst leaving interfering matrix components in the residue. Therefore, a clean-up step is sometimes necessary in order to filter out larger particles or remove non-analyte molecules that have been co-extracted and could interfere with the subsequent analytical steps. Derivatisation is used to change non-volatile compounds into a volatile form and vice versa and this enables the use of either gas or liquid chromatography with compounds that can be treated in this way. If the device used for detection of the target analyte is not sensitive enough to detect the analyte at the normal concentration level in the sample, a pre-concentration step can facilitate the detection. Not all speciation methodologies require these steps (as indicated by the dashed arrows in Figure 1.4), but the performance of some can be significantly improved if additional sample pre-treatment is used.

d) *Separation*: The separation of the different species is an important step in the analytical procedure because, if successful, it allows the identification and quantitation of individual compounds. It is usually achieved by means of chromatography and a variety of different separation mechanisms can be used according to the chemical and physical properties of the analytes. In addition, the separation of the analyte of interest from matrix components that have not been eliminated from the sample at this point by the extraction and clean-up steps, can be achieved in this way.

e) *Detection*: The instrument used for the detection of the compounds that have been separated in step d) has a significant impact on the performance of the method as a whole. The choice of instrument has an influence on the achievable detection limit, the analytes that can be determined, and the impact of interfering components, which may be minimised by the right detector choice for a given application. Due to the fact that some species can result in lethal or sub-lethal effects on organisms at concentration ranges of part per billion to part per million

levels, the detector of choice has to provide sufficient sensitivity to detect such species in the sample extracts.

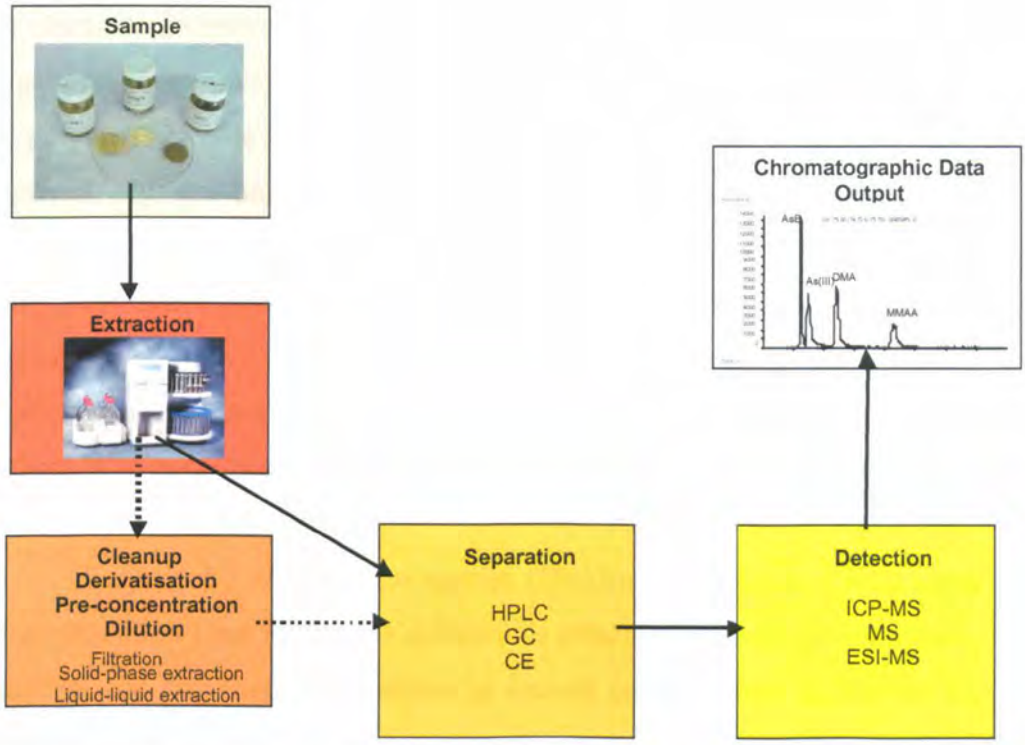

Figure 1.4 Schematic flow-chart of the analytical steps involved in organometallic speciation analysis.

All of these processes together result in the final signal output, and thus data are obtained for a given analyte or sets of analytes determined in the sample in question. It is of vital importance throughout the whole procedure that the integrity of the species is preserved, and verifying this is an important part of the method development and validation. Specific examples of currently used instrumentation and methodologies for organotin and arsenic speciation will be discussed in the literature review in Chapter 2, and in Chapters 4 and 5 of this work.

CHAPTER 2

LITERATURE REVIEW

2.1 ORGANOTIN SPECIATION

Organotin compounds have been widely used since the mid-1950s in the production of polyvinylchloride (PVC) materials as heat and light stabilisers and as anti-bacterial and anti-fungal agents in pesticides and anti-fouling applications. It is estimated that ~ 20,000 tons of organotin compounds are currently manufactured per year, and that 12,000 - 13,000 tons are used for PVC stabilisation purposes (Reference 50). Other uses include wood preservation treatment, pesticide applications and glass coatings. The half-life of antifouling compounds such as TBT is thought to range from days in the water-column, compared to months or years after deposition in sediments.

The toxicity of organotin species (Nicklin and Robson, 1998), especially of tributyltin (TBT) has resulted in deleterious effects on non-target organisms, such as shellfish and dogwhelk populations in coastal environments (Alzieu et al., 1986). Organotin compounds have been detected in most marine and fresh-water sediment and water samples (Tao et al., 1999) as well as seafood destined for human consumption (Keithly et al., 1999). As a result, a wide range of methods has been developed over the years for extraction, separation and detection of organotin compounds. Due to the fact that the toxicity of organotin species in environmental systems has been known for more than two decades, a great variety of methodologies have been developed with the aim of providing fast, sensitive and accurate analytical methods for their determination. However, significant variations in results obtained by different methodologies in the literature have been highlighted (Zhang et al., 1991; Pellegrino et al., 2000) and some routine methods require extraction durations in excess of several hours (EDANA, 2000). At present there is still a scarcity of suitable reference materials for quality control purposes.

2.1.1 Extraction Techniques

As stated by Quevauviller and Morabito (2000), the extraction of organometallics '*should be performed in such a way that the analyte is separated from the interfering matrix without loss, contamination or change of speciation, and with the minimum of interferences*'. In addition, the extraction recovery should be verified in one of several ways. This can be done by using a certified reference material (CRM) if the relevant combination of target analyte and matrix is available as a CRM, or by evaluating the recovery of a spiked standard from the matrix in question. The evaluation of the recovery by spike addition is controversial, because it grossly simplifies the interaction between the analyte and the matrix, which may be more complex than a superficial contact, but this is sometimes the only alternative if no suitable CRM is available.

A wide variety of extraction methods have been developed over the years for organotin speciation. Initial methodologies made use of classical extraction techniques such as solvent extraction aided by manual shaking or ultrasonication and soxlet extraction. More recently, the use of semi-automated techniques such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) has been increasingly applied by a number of research groups. Gómez-Ariza et al. (2001) reviewed the respective benefits of solvent extraction based approaches versus fast semi-automated techniques for the applications of organometallic speciation of Sn as well as other elements. The authors concluded that the use of the fast extraction techniques highlighted improvements over the classical approaches in terms of time-efficiency and reagent and sample consumption. However, it was also stated that the more rigorous extraction conditions usually employed by these techniques can lead to analyte instability and potentially hazardous conditions with high-energy sources such as microwaves.

Pellegrino et al. (2000) compared twelve selected extraction methods for the determination of butyl- and phenyltin compounds in mussel samples. The methods ranged from mechanical shaking to ultrasonication and microwave extraction, and the target species determined were monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPhT), diphenyltin (DPhT) and triphenyltin (TPhT). In general it was concluded that there was significant variation between the

recoveries obtained by the different approaches for different analytes (Figure 2.1). It was shown that the application of acidic conditions combined with polar organic solvents and the use of the chelating agent tropolone (2-hydroxycyclohepta-2,4,6-trienone) enhanced the recovery of organotin compounds from this matrix. However, acidic conditions above 1M concentration resulted in lower recoveries of the species, which was linked to degradation of the tri- and mono-substituted species in particular. The use of more polar solvents also increased recoveries probably due to better interaction with the sample matrix. Tropolone was shown to increase the extraction efficiency of mono- and di-substituted species such as MBT and DPhT.

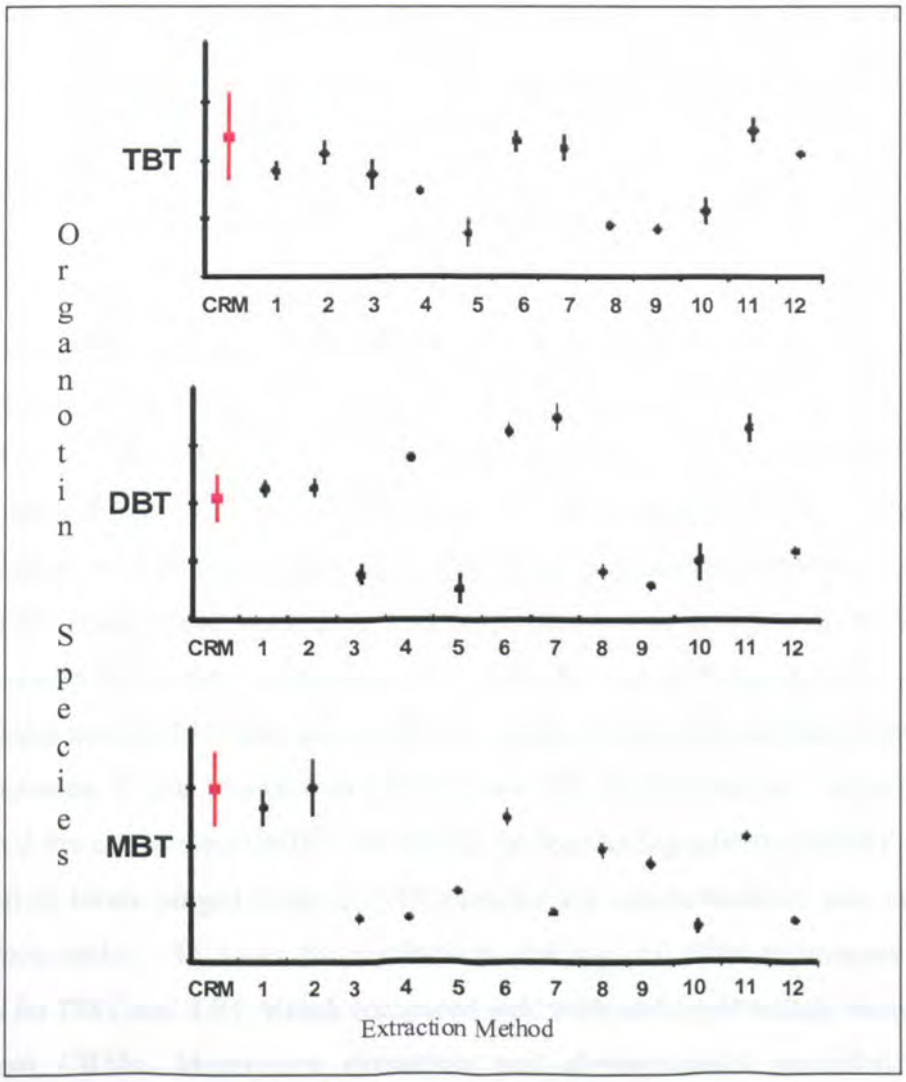

Figure 2.1 Diagram showing the significant variation in results obtained for TBT, DBT and MBT in comparison to the certified value (red) of a mussel tissue CRM by twelve different extraction techniques (1-12) chosen from the literature. (From Pellegrino et al., 2000)

Supercritical fluid extraction (SFE) was first investigated for organotin extraction in the early 1990s. Different authors have reported variable recoveries for soil and fish samples. Kumar et al. (1993) used SFE for the extraction of TBT and TPhT from fish tissues. Optimum recoveries for TBT and TPhT from a fish tissue CRM (NIES-11) were 44% and 23% respectively. Due to low recoveries the authors concluded that further work using complexing agents or different modifiers needed to be carried out to make this a viable technique. Liu et al. (1993) applied SFE to the extraction of six tetraalkyltin and seven ionic organotin compounds from spiked topsoil samples. They found that tetraalkyltin compounds were extracted with efficiencies between 90 - 110% at pressures of 100 atm and temperatures of ~ 40°C, whereas most ionic species were only recovered by 70% to 90% when a complexing agent, diethyldithiocarbamate was added to the topsoil prior to the extraction. Extraction durations were in the range of 10 - 50 minutes.

One of the first publications on the use of microwave extractions for organotin species was the work by Donard et al. (1995) who showed that quantitative recoveries of DBT and TBT could be achieved by using 0.5M acetic acid in methanol with extraction power of 60W and a duration of 3 minutes. Some degradation of TBT and TPhT under microwave conditions was shown in aqueous solutions, and exposure times of more than 10 minutes at powers above 100W led to degradation of butyl- and phenyltin species. A comparison between microwave extraction, mechanical shaking and ultrasonication was carried out for organotin speciation in sediments by Encinar et al. (2002 b). The stability of MBT, DBT and TBT were monitored under all conditions using isotopically labelled analogues of these species. It was shown that strong extraction conditions and longer durations favoured the extraction of MBT, but could also lead to degradation of DBT and TBT. Extraction times ranged from 2 - 15 minutes for ultrasonication and microwave extraction and 0 - 15 hours for mechanical shaking. All three techniques provided results for DBT and TBT, which compared well with certificate values from different sediment CRMs. Microwave extraction and ultrasonication provided the best recoveries for MBT with the shortest extraction times and no degradation was observed with ultrasonication.

Rajendran et al. (2000) carried out a comparison of a mechanical shaking method, which was developed in their laboratory with microwave and ultrasonication

methodologies for organotins in sediments. They determined that the mechanical shaking method, which employed an extraction of 60 minutes in the presence of toluene with 0.1% tropolone, showed significantly greater recoveries of MPhT, DPhT and TPhT than either sonication or microwave extraction. Lower recoveries of the phenyltin species by sonication and microwave extraction were associated with their degradation. The accuracy of the method for DBT and TBT was shown by extraction of certified reference materials (PACS-2 sediment and NIES-11 fish tissue).

Arnold et al. (1998) first reported accelerated solvent extraction (ASE) also known as pressurised fluid extraction (PFE) for the extraction of organotin species from sediments. The authors used a mixture of acetic acid and sodium acetate in methanol as the extractant and the extractions were optimised with different concentrations of the reagents in the solvent. The duration of the extraction depended on the number of 5-minute extraction cycles, which were used and these ranged from 3 to 5. The method was validated by the extraction and analysis of a sediment CRM (PACS-2) for MBT, DBT and TBT and then applied to lake sediments from Lake Zürich, Switzerland. The recovery of phenyltin species (MPhT, DPhT, TPhT) was assessed using spike recovery experiments and fell within 80 - 104%.

Looser et al. (2000) recently compared this ASE method with results obtained by cold-methanolic digestion (CMD) of biological samples, and concluded that both methods compared well for DBT and TBT, although the recovery of MBT by CMD was lower than by ASE. CMD was therefore highlighted as a cheaper alternative extraction compared to ASE, although the extraction duration was significantly longer (2 hours) than the ASE method. Chiron et al. (2000) modified the solvent used with the ASE method described by Arnold et al. by adding 0.2% w/v tropolone to enhance the extraction of MBT and MPhT from sediments. Recoveries of butyl- and phenyltin species ranged from 72 - 102%. Encinar et al. (2002 a) also evaluated this technique for the extraction of butyltins from sediment and observed quantitative recoveries in good agreement with certificate values for DBT and TBT at a temperature of 110°C with extraction times as short as 10 minutes. The extraction solvent used for this work contained neither sodium acetate nor tropolone and was made up as 10% acetic acid in methanol.

2.1.2 Separation and Detection Techniques

Generally, the separation method of choice has been gas chromatography (GC), which allows for the analysis of many different groups of organotin compounds (e.g. butyl-, phenyl-, octyl- and propyl) in a single analysis after derivatisation (Rajendran et al., 2000). GC separation has been successfully coupled to a variety of detectors such as atomic absorption spectroscopy (AAS) (Astruc et al., 1989; Bergmann and Neidhart, 2001), atomic emission detection (AED) (Tutschku et al. 2002), microwave induced plasma atomic emission detection (MIP-AED) (Girousi et al. 1997; Aguerre et al. 2001) and, more recently, inductively coupled plasma mass-spectrometry (ICP-MS) (Hill, 1992; Encinar et al. 2001); all of these detectors can offer sufficient detection limits for organotin analysis. However, the derivatisation required for GC analysis can result in variation in yields between species and in terms of efficiency depending on matrix components.

Organotin separations by high performance liquid chromatography (HPLC) (e.g. Praet et al., 1990; Chiron et al., 2000) analysis have also been used and a comprehensive review of HPLC techniques was given by Ebdon et al. (1998 b). This approach offers the advantage that sample preparation does not involve a derivatisation step, which can eliminate a potential source of uncertainty in the final result. However, the range of compounds that can be analysed in a single run, and the number of detectors that give comparable sensitivity are limited compared to GC. Common HPLC detectors for organotin analysis include fluorescence (Shum et al., 1992), ultra-violet (UV) (Praet et al., 1990) and more recently inductively coupled plasma optical emission spectrometry (ICP-OES) (Rivaro et al., 1995), inductively coupled plasma mass-spectrometry (ICP-MS) (White et al., 1998; Chiron et al. 2000) and molecular mass spectrometry detectors such as atmospheric pressure chemical ionisation mass spectrometry (APCI-MS-MS) (O'Connor et al., 2001) and electrospray ionisation mass spectrometry (ESI-MS) (Wu et al., 2001).

HPLC separations can be prone to insufficient resolution between analyte peaks (Rivaro et al., 1995), because the peak-widths are generally broader than those encountered with GC. Therefore, it is imperative that efforts are made to maximise the chromatographic resolution for the HPLC based methods. This need has become more evident as detection systems with improved sensitivity have revealed additional organotin components in matrix samples.

2.1.3 Quantitation of Organotin Compounds by Species-specific Isotope Dilution Analysis (SS-IDMS)

Isotope dilution mass-spectrometry (IDMS) has been considered a 'primary' method in trace metal analysis for many years due to the capacity to deliver accurate and precise results. IDMS can be applied if the target analyte is not mono-isotopic and artificially enriched isotopes are available. Ideally none of the isotopes used should be affected by spectroscopic interferences. Different types of mass spectrometers can be used for IDMS quantitation, and due to the increasing use of ICP-MS in laboratories world-wide, there are a significant number of IDMS methodologies which have been developed for this technique. The effects of calibration drift, variations in signal intensity and matrix related problems are largely overcome by this technique (Heumann et al., 1998), because quantitation is based on the ratio measurements between two isotopes rather than signal intensities. The accuracy of IDMS is affected by parameters such as spectroscopic interferences, instrumental components and space charge effects if ICP-MS is used. The precision of such measurements on the other hand is influenced by signal counting statistics and the stability of the ion current (Heumann et al., 1998). Catterick et al. (1998) showed that the propagation of errors during IDMS measurements could be negated by matching the ratio between the enriched spike and the inherent analyte as closely as possible to 1:1.

An on-line isotope dilution approach for the determination of elemental species by HPLC-ICP-MS was shown by Rottmann and Heumann (1994). Such a species-unspecific ID approach is useful where the exact structure of the target analyte is unknown in a sample or a matching molecule containing an enriched spike isotope cannot be synthesised. Species-specific isotope dilution mass-spectrometry (SS-IDMS) for organometallic speciation studies has been made possible by the synthesis of organometallic molecules containing an isotopically enriched hetero-atom (e.g. Sutton et al., 2000; Kumar et al., 2003). The application of IDMS to speciation methodologies can help reduce the uncertainties associated with such methods because IDMS has the capability to overcome some shortfalls of conventional calibration methods such as analyte losses, analyte breakdown or incomplete recovery provided that complete equilibration between the spike and the inherent analyte has been achieved. Bancon-Montigny et al. (2002) showed this for the

measurement of TBT by solid-phase micro extraction gas chromatography (SPME-GC). A comparison of conventional calibration with and without internal standards and SS-IDMS showed that the measurement precision could be reduced from 18% (no internal standard) to 12% using tripropyltin (TPrT) as an internal standard, and further to 4% using SS-IDMS with a ^{117}Sn enriched TBT. The resulting improvements in the measurement precision have therefore been used to overcome poor measurement precision as one of the main drawbacks of SPME, which as a result has shown further potential as a powerful sample preparation tool. In addition, the improvement in the measurement precision directly influences the uncertainty associated with the analytical result, as shown by Yang et al. (2003). The authors compared standard addition calibration (SA) with SS-IDMS for TBT in sediments and although the accuracy of both approaches was in good agreement, the comparative expanded uncertainties were significantly reduced from 11.3% RSD by SA to 6.2% by SS-IDMS. The application of this technique to the certification of speciated reference materials has an obvious benefit, by reducing the uncertainty of certificate values, which should be as small as possible to be of use to the analytical community. For the first time, the certification of TBT in a reference sediment has been carried out with the majority of participating laboratories using SS-IDMS (Sturgeon et al., 2003). The resulting relative expanded uncertainty for TBT of 13.1% at a level of 80.6 ng/g is equivalent to the 95% confidence interval quoted for TBT in PACS-2 of 13.2% at a level of 980 ng/g. SS-IDMS combined with GC-ICP-MS has also been applied to the speciation of organotin compounds by Encinar et al. (2001) and Monperrus et al. (2003 b).

2.2 ARSENIC SPECIATION

The element Arsenic (As) has for a long time been thought of as poisonous and highly toxic. However, it has been shown that the form or 'species' of As largely determines its toxicity. Arsenic is ubiquitous in the environment due to natural and anthropogenic sources and the relative contribution of these factors is estimated as roughly 60% and 40% respectively (Chilvers and Peterson, 1987). The natural input is due to weathering of As containing minerals from rocks and volcanic emissions, whereas the anthropogenic component is made up of insecticide and herbicide use as well as contributions from the pottery, glass wood and feed additive industries. In the

environment As behaves in similar ways to the Group V elements nitrogen (N) and phosphorus (P). As a result of these similarities arsenic gets taken into the biochemical pathways of N and P, resulting in the formation of compounds such as arsenobetaine (AsB) in fish, and arseno-sugars, which are found in marine algae. Arsenic is present in seawater at concentrations around 1 microgram per litre (μg/L) (Fodor, 2001). In UK coastal waters this arsenic appears to overwinter as arsenate but during the summer months it is partially converted to methylated arsenic species.

In the environment, arsenic is subject to transformations from inorganic to organic forms and vice-versa, and the process is dependent on factors such as redox potential, pH and microbial activity *in-situ*. For example, arsenite is converted to arsenate in oxidative environments, whereas under reductive conditions As(V) is converted to the more mobile, soluble and toxic As(III) (Xu et al., 1991). Microbial activity by fungi, yeast and certain bacterial strains has been shown to yield bio-transformation of inorganic As to the methylated organic forms, as well as de-methylation of organic arsenicals to inorganic species. Some methylated arsenicals are volatile, and biotransformations are therefore an important pathway of volatile As fluxes to the atmosphere. More than 20 different species of bacteria have been shown to be capable of methylating inorganic arsenicals (Cullen and Reimer, 1989). In reductive environments such as domestic waste deposits or sewage treatment works volatile arsine compounds can be released, although a concurrent release of gases such as methane or carbon dioxide in much greater quantities makes their detection difficult. Smelting processes and fossil fuel combustion are also a significant source of As to the atmosphere, and it is estimated that more than 90% of As in the atmosphere is in particulate form (Cullen and Reimer, 1989). Fly ash contains predominantly As(V) depending on the material combusted.

The toxicity of the inorganic As species such as arsenite, As(III) and arsenate As(V), is far greater than the organic forms, such as momomethyl- and dimethylarsinic acid (MMAA and DMA) and AsB (Kaise and Fukui, 1992). The International Agency for Research on Cancer (IARC) has classified inorganic arsenic as a human carcinogen, whereas AsB, the predominant form of As in most marine organisms is considered non-toxic to humans. Although AsB is the major form of As in many marine organisms, it is not present in all fish species (Edmonds et al., 1997), and therefore an evaluation of the proportion of AsB to the total As determined can

give a useful estimate of the toxicological significance of a seafood sample. In order to determine the toxicity of food, the determination of the total As alone is of limited value, and the different species of As have to be extracted, separated and determined. Fast, reliable and practical methods are therefore required that can provide speciation information for the screening of large batches of samples.

The World Health Organisation (WHO) has decreased its recommended maximum permissible arsenic level in drinking water from 200 μg/L in 1958 to the current level of 10 μg/L (see Figure 2.2). This value reflects total As, but currently no maximum levels are defined for individual species. In some geological areas, the natural levels of As in groundwater are significantly higher than the recommended values. Such areas include parts of India, Bangladesh, Hungary, France, USA, Bulgaria and Taiwan. In parts of Taiwan the average concentration of As in water from wells was 674 ± 149 μg/L. In such areas the occurrence of Blackfoot Disease (BFD), which manifests itself by discolouration of the skin on the extremities and can necessitate amputation of affected limbs has been linked to As exposure of patients from drinking water collected from deep wells (Chatterjee et al., 1995).

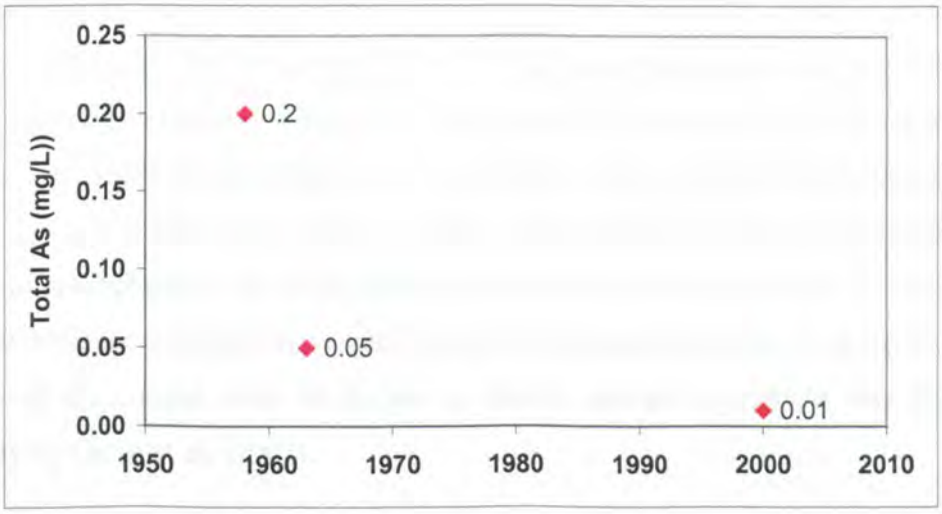

Figure 2.2 Maximum Permissible Levels of Arsenic in drinking water since 1958 as recommended by the World Health Organisation (WHO).

The main route for human exposure to As apart from drinking water is food. Mushrooms can contain high concentrations of different arsenicals and depending on the type of mushroom there is variability in the form of arsenic accumulated as well as the concentrations present (Londesborough et al., 1999). Seafood represents the

major source of arsenic for humans, followed by poultry products and cereals. The proportion of the toxic inorganic As species to the total consumed during a total diet survey carried out by the Basque regional government in northern Spain was shown to be low (0.3% - 1.8%) (Urieta et al., 2001). The total dietary intake of inorganic arsenic during this study was estimated as 0.22 μg/kg body weight/week and this was considered to be low compared to the provisional tolerable weekly intake (PTWI) of 15 μg/kg body weight/week set by the WHO in 1988. In other countries with high seafood consumption such as Japan, algae are also a dominant source. Marine algae have been shown to contain the greatest number of As species in the marine environment with about half of the total arsenic provided by As(III) and As(V) and the remainder by arsenosugars and arsenolipids (Francesconi and Edmonds, 1998). Little data on the toxicological implications of many arsenosugars has been obtained to date, so the implications of these species to human health are relatively unknown at present.

Inorganic arsenicals are readily absorbed by the gastro-intestinal tract and can be converted to methylated form such as MMAA and DMA in the liver (Fodor, 2001). A fraction of inorganic arsenic can also accumulate in hair, nails and the skin and this is why these tissues are sometime used as forensic samples in suspected cases of poisoning. The absorption rates of organic arsenicals are also high, and the main route of elimination of inorganic and organic arsenicals is by excretion in urine (Fodor, 2001). There are presently no indications that arsenobetaine is transformed during migration through the human body, whereas the oxidation of arsenocholine (AsC) to arsenobetaine has been shown after consumption of seafood. In total more than 20 different arsenicals have been identified in natural systems. A comprehensive review of the current state of the art in arsenic speciation analysis was prepared recently by Gong et al. (2002).

2.2.1 Extraction Methodologies for Arsenic Species

The majority of papers reporting on extraction and analysis of arsenic species from a variety of matrices have focussed on water-soluble arsenic fraction and de-ionised water or mixtures with methanol are frequently used (Alberti et al. 1995; Branch et al., 1994; Lai et al., 1997). According to the literature (e.g. Alberti et al., 1995), the

solubilisation of arsenicals can be achieved with relative ease, for example by shaking or ultrasonication, and more rigorous extraction techniques such as microwave extraction or accelerated solvent extraction have only been reported fairly recently. The following section aims to give an overview of extraction techniques reported in the literature since the early 1990s and covers a variety of approaches.

2.2.1 a) Shaking/Ultrasonication

Mechanical shaking and/or ultrasonication have been widely reported for the extraction of arsenic species in a variety of matrices. Both methods are reasonably cheap because they do not require specialised or expensive equipment. A study by Branch et al. (1994) compared two extraction methods for AsB, DMA and arsenate in different fish species. Method A involved sonication of 1g of sample with several aliquots of 20 mL of methanol (MeOH) and 10 mL of chloroform for 1 hour. The resulting sample slurry was centrifuged for 10 minutes at 2500 rpm and the supernatants were combined in a separation funnel. The chloroform layer was separated and the remaining solution was filtered, evaporated to dryness and re-dissolved in 10 mL of deionised water for HPLC-ICP-MS analysis. Method B was based on the enzymatic digestion proposed by Crews et al. (1985) and involved a tryptic digest of 1g sample with 100 mg of trypsin and 20 mL of 0.1 mol/L ammonium carbonate. The slurry was shaken for 1 hour at 37°C in a water bath. The main species recovered by both methods from different fish samples was AsB and only mackerel was found to contain detectable amounts of DMA and arsenate although at toxicologically insignificant levels.

The results of Branch et al. (1994) for AsB by both methods agreed well for the certified reference material DORM-1 and for Atlantic cod, mackerel and whiting, but were significantly different for 10 other samples tested. A comparison between the total arsenic determined and the arsenic species measured showed that both methods had recovered the majority of As present. With most of the fish samples, AsB constituted almost 100% of the total arsenic determined. The highest levels of As were determined in bottom feeding flatfish, such as sole and plaice. It is thought that AsB is not synthesised within higher trophic levels of marine animals, but derived from microbial action at the seabed and subsequently released into the water column. This would lead to a greater exposure of bottom-feeding species to AsB. It

was thought that the trypsin digestion should yield greater recoveries than Method A, because the protease disrupts the lipid-protein membrane and thereby releases the cell content. However, the mackerel sample showed that a significant proportion of As was contained in the chloroform fraction suggesting that this As was bound to lipids. The trypsin digest could not break down the lipid component in the sample, which was indicated by lower recoveries compared to Method A. The authors concluded that the As species recovered should be seen as the 'water-soluble' fraction of As species in the sample.

Alberti et al. (1995) reported a comparison of 1:1 mixtures of methanol with chloroform and water for extraction of As-species using sonication of marine tissue samples. 1g of sample (mussel tissue, cockle, sole and flounder) was ultrasonicated with several aliquots of 10 mL of the extraction solvent. The effects of sonication time, ratio of solvent volume: sample weight and clean-up of the raw extracts were then studied by LC-UV-HG-ICP-OES analysis of the extracts. It was shown that the MeOH: H_2O solvent provided the best recovery of AsB and DMA. The final extraction parameters proposed consisted of three consecutive extractions of 20 minutes duration. This study also showed that a significant proportion of As was present in the chloroform phase, suggesting As bound to lipids. The use of a C-18 solid-phase extraction (SPE) clean-up removed some of the matrix components which lead to better resolution of the chromatographic peaks.

McSheehy and Szpunar (1999) also used a sonication method based on the methods proposed by Lai et al. (1997) and Shibata and Morita (1992). 1g of dried algae was sonicated for 3 hours with 10 mL of 1:1 MeOH: H_2O, followed by centrifugation for 20 min at 2500 rpm. The supernatant was removed and the residue washed repeatedly (three times) with 10 mL of 9:1 MeOH: H_2O under sonication for 20 minutes. The supernatant and wash fractions were combined and evaporated to dryness, then redissolved in 10 mL of water and analysed by size exclusion/ anion exchange chromatography coupled to ICP-MS and tandem mass–spectrometry detection. Tandem mass spectrometry was used for the identification of arsenosugars, for which no standards for retention time matching were available. Tandem MS also proved useful where insufficient resolution between analyte peaks made their quantitation by HPLC-ICP-MS difficult or impossible. Marine algae contain a great variety of still unidentified arseno-sugars, and the possibility that two

or more of these can co-elute cannot be excluded. The authors concluded that tandem electrospray MS is mandatory for the absolute confirmation of the identity of arsenosugars present in algae samples.

Jackson and Bertsch (2001) used a shaking method with 10mL of water for the extraction of arsenicals from poultry litter. Poultry litter was chosen as a sample as the arsenic compounds 4-hydroxy 3 nitrobenzenearsenic acid (Roxarsane, ROX) and 4-aminobenzenearsenic acid (p-arsanilic acid, p-ASA) are used as feed additives in the poultry industry for disease control and enhanced feed efficiency. The sample (1g) was shaken with water for 2 hours and then centrifuged, filtered and diluted prior to analysis by HPLC-ICP-MS. A C-18 solid-phase extraction was found to be a suitable sample clean-up step for the compounds tested (As(III), MMAA, DMA, As(V), ROX and p-ASA). The main form of As identified in the poultry litter was ROX but As(V), DMA and other non-identified species were also detected.

2.2.1 b) Microwave Extraction Techniques

Microwave extraction has not been used as extensively as shaking and ultrasonication methods, but publications describing the use of the technique have shown its suitability, particularly for the extraction of arsenicals from soils and sediments. Thomas et al. (1997) studied the extraction of arsenicals from soils and sediments using an open vessel microwave system with phosphoric acid and HPLC-ICP-MS detection. They found that the main analytes present in a variety of soil and sediment types were As(III) (2.5 - 21.3 mg/kg) and As(V) (4.0 - 39 mg/kg), whilst DMA and MMAA were not detected. At microwave power settings above 40W and extraction durations above 20 minutes using 1M phosphoric acid there was a decrease in the amount of arsenite and an increase in arsenate suggesting oxidation of As(III) to As(V).

The benefits of the microwave technique have also been described by Yehl et al. (2001) as being quicker and more versatile than the traditional shaking and sonication techniques. The heat is applied quicker and more directly to the sample because heating takes place internally rather than by convection. In addition, closed vessel systems can achieve higher temperatures, which accelerates desorption of the analytes from the matrix, and the increased pressure leads to improved interaction between the sample and the extraction solvent. The same authors listed a variety of

extraction techniques for arsenic species and compared a closed vessel microwave extraction with a sonication method for the determination of MMAA and DMA in spiked soil and sediment samples.

Microwave assisted extraction and sonication were performed by Yehl et al. (2001) on 0.5g of sample with 10 mL of different extraction solutions (50:50 acetone: methanol, isopropanol, and mixtures of 5% HCl with 50% respectively of acetone, methanol and isopropanol). The microwave extraction program used a maximum pressure of 1150 kPa, a power of 1000W and a maximum temperature of 160°C. The total duration was 21 minutes compared to a sonication duration of 2 hours. There was no significant difference in the extraction efficiency between both extraction methods when used with the same solvents. However, whilst the solvent mixtures containing 5% HCl, yielded extraction efficiencies around 100% for the spiked sediments, the isopropanol and the acetone: methanol mixtures showed maximum extraction efficiencies of 15% and 20% respectively. The authors proposed that HCl may enhance the extraction efficiency by protonating the solvents as well as producing hydronium ions for enhanced solubilisation of arsenic species by ion pairing. Additionally there was little oxidative potential to the extraction solutions so conversion of the arsenic species to As(V) was unlikely.

The more polar MeOH and acetone-HCl mixtures were shown to remove comparatively more arsenate then the isopropanol mixture. The polarity of the organic modifier also affects the ability of the extraction solution to permeate the organic components of the soil where neutral arsenic species reside. Therefore a less polar solvent would solubilise anions such as MMAA and arsenate to a lesser degree and hence be less efficient at removing them from the matrix. The dominant species in the soil samples studied was arsenate, and this was proposed to be a likely stable end product of degradation of other arsenicals in soils. It was also shown that sediment from anaerobic environments contained a comparatively higher proportion of MMAA than the soil from aerobic environments. This is thought to be due to the fact that methylation of arsenic species is favoured in anaerobic media. The forms of As found in anaerobic environments may also be better suited to methylation than those found in aerobic environments.

2.2.1 c) Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) has only been used for speciation studies in the recent past. One of the first publications using this technique for As speciation (McKiernan et al., 1999) showed a comparison of the extraction of arsenicals from fish samples using ASE and a sonication method based on that proposed by Alberti et al. (1995). The fish samples studied contained different proportions of fat and a de-fatting step was therefore included prior to the extraction of polar arsenicals using sonication and ASE with 50% MeOH. Speciation analysis was performed by anion exchange HPLC-ICP-MS. Total arsenic budgets were established between the total acid digest, and the sum of the fat extract, the polar extraction fraction and any residual material. The comparison showed that both methods are useful for the extraction of arsenicals from fish tissues. The percentage of As removed during the acetone extraction was ~ 5% of the total As measured, whilst the 50% MeOH extraction accounted for ~ 72% percent of the total. The authors concluded that the benefit of ASE lay in the automated approach of the technique, which could lead to significant improvements in time efficiency for the analytical procedure. It was also concluded that the elevated temperatures and pressures used during ASE may lead to more efficient and more reproducible extraction compared to sonication techniques.

Gallagher et al. (1999) applied ASE to the extraction of arsenosugars 328, 392 and 482 from ribbon kelp using a 30: 70 MeOH: H_2O mixture. The extracts were evaporated to dryness, re-dissolved in water and treated with a C-18 SPE clean up step prior to analysis by ion chromatography coupled to ICP-MS and electrospray MS and tandem MS. The main aim of the work was the identification of co-eluting species by IC-ICP-MS using ESI-MS and ESI-MS-MS in order to verify possible false positive signals. No comments were made about the suitability of the extraction except that ASE was capable of extracting these arsenosugars from the matrix.

In 2001 Vela et al. published a method for the extraction of arsenic species from carrots using ASE. As(III), As(V), MMAA, DMA, AsB and an unidentified arsenic compound were determined during the study. Different extraction programmes were tested, during which the number and duration of extraction cycles, the extraction temperature and different dispersion media were assessed using water as the extraction solvent. It was found that three separate cycles of 1-minute duration each were sufficient to extract 95% - 97% of total arsenic when 100°C was used

whereas only 81% - 87% were extracted at ambient temperature. Analysis of a spiked mixture of arsenicals indicated that Ottawa sand was a suitable dispersion medium due to the appropriate particle size, low arsenic background, and the fact that neither adsorption nor transformation (as monitored by the relative concentrations) of arsenicals took place. The importance of the dispersion medium was shown at sample weights above 1.25g of this material, which increased up to 6 times in volume during exposure to the extraction solvent and led to clogging of the cells without the use of dispersing agents. The extracts were subsequently filtered through a 0.45μm nylon/glass syringe filter and passed through a C-18 SPE cartridge. Filtration did not affect any of the arsenic species, but significant losses of DMA occurred during the SPE step in both spiked extracts and standard solutions and a loss of AsB was also noted from the spiked extracts. It was proposed that this may be due to de-protonation of DMA at higher pH values. The pH of the extract solutions was around 5.2. It was found that in the freeze-dried carrot materials analysed, As(III) and As(V) were the main species in samples with low total arsenic content, whereas MMAA was the major species in samples with a high total As content.

The most recent publication of an ASE method for arsenicals is a study by Gallagher et al. (2002) in which an ASE method using different MEOH: water mixtures was assessed using a certified reference material (DORM-2, Fish) and laboratory fortified blanks (LFB). Using the CRM, the retention of different arsenicals was verified on different dispersion media. When 'Filter Aid' was used for dispersing the sample, inorganic As, MMAA and DMA were bound, whereas AsB was not retained. Such losses of species may lead to a significant underestimation of the toxic species present in the sample and therefore the dispersion medium chosen must be inert. Analyte losses were not observed for Teflon beads and 'Q-beads', although Q-beads contained a considerable arsenic blank and thus needed pre-cleaning. Teflon beads were shown to be the best currently available dispersion medium for arsenic speciation studies using accelerated solvent extraction.

2.2.2 Analytical Techniques for the Separation of Arsenic Species and their Quantitative Detection

Due to the fact that most of the arsenic species are either cationic or anionic and non-volatile, high performance liquid chromatography (HPLC) has been used almost exclusively for separation of arsenicals prior to detection. Although ion exchange liquid chromatography has been most extensively reported in the literature (e.g. Thomas et al., 1997; Gallagher et al., 1999) the use of ion-pairing reversed phase chromatography has also been reported. Some separations by gas chromatography have also been reported for volatile arsine compounds and capillary electrophoresis (CE) is an active area of research within arsenic speciation studies.

ICP-MS offers good sensitivity for detection of trace level concentrations of arsenicals. When using quadrupole ICP-MS for the detection of arsenic species, the mass to charge ratio m/z 75 is monitored and this can be interfered with by formation of $^{40}Ar^{35}Cl$ in the plasma. The presence of this polyatomic species depends on plasma conditions as well as the presence of chlorine (Cl) in the sample matrix and whether it interferes with quantitation of As can be verified by injection of a solution containing Cl^- ions. Apart from ICP-MS, inductively coupled plasma optical emission spectrometry (ICP-OES) and various forms of atomic absorption spectrometry, such as graphite furnace (GFAA) and gaseous hydride (GHAA) can also yield suitable detection limits.

More recently, electrospray ionisation mass spectrometry (ESI-MS) has also been used in conjunction with HPLC separations as a complementary molecule-specific detection technique. It allows compound identification by molecular mass confirmation, particularly when used in tandem MS mode where the mass of fragments generated from the parent molecules can be measured (McSheehy and Szpunar, 1999). The confirmation of species identity is particularly important in cases where individual compounds co-elute. This could potentially lead to false positive signals and in such cases HPLC-ICP-MS cannot be used to quantify the species, unless the chromatography can be improved to resolve the species. ESI-MS-MS is not suitable for compounds that cannot be efficiently ionised or for mobile phases, which contain buffer salts that may interfere with the interface. In addition different compounds can have very different response factors by this technique

2.3 DISCUSSION

The literature reviewed highlights the fact that a significant number of analytical methods have been developed in recent years for the extraction, separation and detection of organotin and arsenic species in a variety of matrices. Most of the methodologies have been validated with regards to a single matrix (e.g. sediment, fish tissue, etc.) and at most several species of the same element. In addition, the performance of such methods may vary significantly for different species, which highlights the analytical challenges of speciation analysis. The fact that further methodologies continue to be developed and added to this array indicates that at present no single methodology is sufficient for determining all of the target species in any given matrix. In fact there are only a very limited number of extraction approaches, which have been reported for the simultaneous speciation of different elements in a variety of matrices.

The actual instrumentation used for both As and Sn speciation is often identical, particularly for the extraction step, and therefore there would be significant merit in developing a simultaneous extraction approach for species of these elements. Species of both elements are often of importance in the same sample type e.g. in contaminated sediments or seafood destined for human consumption, and a single extraction step could therefore have great benefits for streamlining the analytical procedure as well as reducing labour, time and resources required. The simultaneous separation of the different species of both elements is not as crucial in this regard, as the separation and detection methodologies available are less time and labour intensive than many of the currently used sample preparation procedures.

CHAPTER 3

INSTRUMENTATION

In this chapter, brief descriptions of the different analytical instruments that were used for the experimental work in this thesis are given. Most of the instrumentation was based in the Specialised Techniques department of LGC Limited (Teddington, UK).

3.1 EXTRACTION TECHNIQUES

In order to extract organometallic species from the sample matrix, a variety of extraction techniques can be employed. The extraction method used must extract the target analyte from the matrix in a quantitative manner without inducing changes in the chemical or physical form of the species. Depending on whether the target analyte is incorporated into the matrix and stored in the biological tissue (for example after ingestion by an organism), or loosely bound to the matrix by adsorption to particulate matter, determines how rigorous an approach is used. Arsenic species such as arsenobetaine (AsB) can be extracted from some fish tissues with a reasonably simple extraction by shaking with a methanol: water mixture (Section 2.2.1), whilst some organotin species such as monobutyltin (MBT) require much more rigorous conditions usually employing solvent-acid mixtures for the extraction from sediments (Section 2.1.1).

Generally, a variety of organic solvents of different polarity and mixtures of acids are used for extraction of organometallic species. The optimum extraction solvent is often specific to individual species, although simultaneous multi-species extractions can be performed for species with similar chemical characteristics. Specific instrumentation used for the different applications described in this work is given below.

3.1.1 Microwave Assisted Extractions

The extraction of certain analytes can be facilitated by heating the sample and extraction solvent in a microwave field. Microwaves are electromagnetic waves with frequencies from 300 MHz to 300 GHz. A common frequency used for microwave

assisted sample preparation is 2.45 GHz. Depending on a dielectric coefficient of a material, microwaves can be reflected (e.g. solid metals), pass through (certain plastics and glass) or be absorbed. Substances that absorb microwave energy are excited to perform molecular motions while free ions move through the electromagnetic field. Both of these effects cause heating of a substance. Heating by microwave radiation results in uniform and direct heating of the sample solution compared to the use of conventional heating techniques (e.g. heating blocks) where heat is transmitted by conduction. Generally, a known amount of sample is heated under microwave conditions in the presence of an extraction solvent. The behaviour of both sample and extraction solvent in a microwave field determine the extraction efficiency achievable. This approach can obviously not be applied to analytes that are degraded under microwave conditions.

Two types of microwave extraction systems are currently in use: open- and closed-vessel systems. Open vessel systems have no control over the pressure applied to the sample and extractant and therefore the main process facilitating extraction is heating in the microwave field. Closed-vessel systems make use of the pressure build-up in a sealed vessel during the heating process to increase the extraction or decomposition. This means that higher extraction temperatures can be achieved compared to open-vessel systems, which are limited by the boiling point of the sample and solvent mixture at atmospheric pressure. Both types of systems give the user a choice of maximum power applied, extraction duration and maximum temperature used. In addition, closed-vessel systems can also be set up to cut power when a set pressure in the extraction vessel has been reached. After the extraction, the sample and extraction solvent mixture are subjected to centrifugation or filtration in order to separate solid matter in the solution from the supernatant, which is collected for analysis.

A Paar Physica Multiwave (Anton Paar, Perkin Elmer, Beaconsfield, UK) closed vessel microwave system was used. This system can accommodate up to 6 high-pressure quartz or teflon vessels. The extraction conditions can be user defined according to maximum temperature and pressure and in terms of power applied and extraction duration. After the extraction, the extraction solvent had to be separated from the solid sample by either filtration or centrifugation. Actual conditions used are described in more detail in the relevant sections (Sections 4.4.1 and 5.2.3).

3.1.2 Accelerated Solvent Extractions (ASE)

Accelerated solvent extraction (ASE) also known as pressurised fluid extraction (PFE) involves the heating of a sample in the presence of an extraction solution at high pressures up to 2000 psi. Like the closed-vessel microwave approach, this technique utilises the fact that liquids at elevated pressures can be heated to temperatures above their respective boiling points without change to the gaseous phase. A schematic diagram of an accelerated solvent extraction system is shown in Figure 3.1. The sample is added manually into the stainless-steel extraction cell. The cell is automatically placed in the oven and connected to the solvent pump and the collection vial. The pump then fills the cell with extraction solvent. Once the cell is full, the heating cycle begins for a set-period of time. As the solvent heats up, the cell pressurised and once a set pressure is exceeded, solvent is allowed to vent into the collection vial, until the set pressure is obtained again. Fresh solvent is pumped into the cell to make up the displaced volume. After the extraction cycle(s) the cell is flushed with a pre-determined volume of fresh solvent and then purged with nitrogen gas (N_2) in order to recover all of the extraction solvent and analyte.

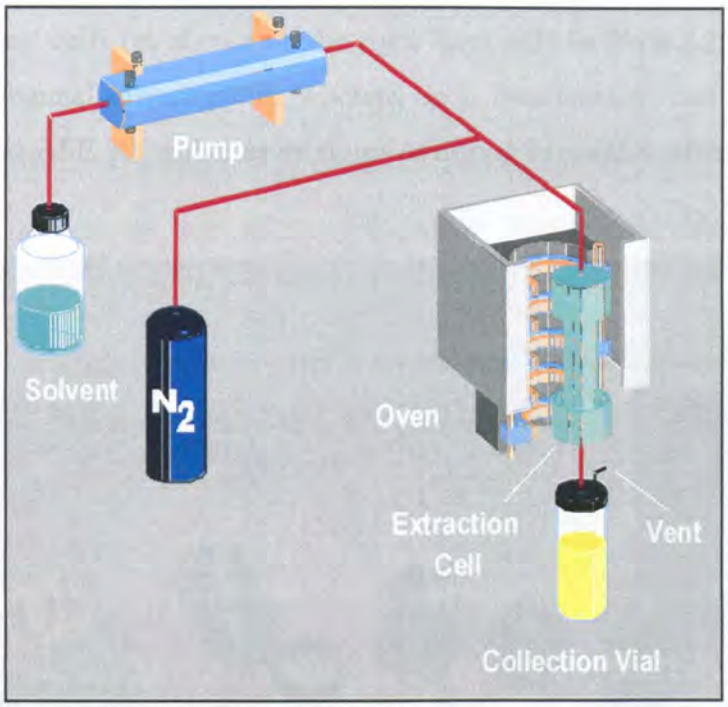

Figure 3.1 Schematic diagram of an accelerated solvent extraction system.

Plate 3.1 Photograph of a Dionex ASE 300 Accelerated Solvent Extractor.

Two different ASE instruments were used for the work described in this thesis: the Dionex ASE 200 and ASE 300 (Plate 3.1). All extractions were carried out using 11mL stainless steel cells (as shown on the right hand side in Plate 3.1). The ASE 200 has a single channel solvent pump, whereas up to four solvents can be used for extractions with the ASE 300 either as mixtures or during sequential extractions with different solvents.

Plate 3.2 Photograph of materials used during Accelerated Solvent Extraction.

A variety of different programs were used for the extractions carried out in this work and specific examples are given in the relevant sections of the thesis (Sections 4.4.2, 5.2.2 – 5.2.6 and Chapters 6 and 7).

3.2 CHROMATOGRAPHIC SEPARATION TECHNIQUES

After extraction, the different components in the extraction solvent must be separated in order to enable quantitation of individual species. Interfering components from the matrix material can be separated from the analytes during the sample clean-up as part of the preparation procedure. Techniques such as filtration, centrifugation, liquid-liquid extraction and solid-phase extraction (SPE) can be used for this purpose. The first two are used to remove particulate matter that may bind the analytes and thereby induce a bias, whilst the last two are chemical separation techniques which separate the analyte from the matrix on the basis of differences in the chemical properties such as polarity.

The clean extract may contain a number of different analytes of interest, which may be detected by a particular measurement technique, and these analytes therefore need to be separated from each other in order to allow separate quantitation. Two separation techniques that have been used extensively for this purpose in a wide range of application with a variety of detectors are gas chromatography and liquid chromatography. Both techniques utilise what is known as the elution technique. This is based on a column filled with a stationary phase and a mobile phase or eluent, which is continuously passed over the stationary phase by means of a pump or gas flow. The sample is introduced at the beginning of the column in a solvent that is miscible with the mobile phase. As the sample and the mobile phase are pushed along the stationary phase, the different components interact with the stationary phase according to their chemical or physical properties. These can vary from polarity to size, and a variety of stationary phases are available for both liquid and gas chromatography to achieve efficient separation for a great range of analytes.

3.2.1 Gas Chromatography (GC)

Gas chromatography is used for the separation of volatile compounds. An inert gas such as helium, argon, nitrogen or hydrogen is used as the mobile phase or carrier

gas. The chromatographic separation is achieved by a partition between the gaseous mobile phase and the liquid or solid stationary phase.

A schematic diagram of a conventional gas chromatograph is shown in Figure 3.2. The GC column is stored in a temperature programmable oven, and the sample is injected into the heated injection port by a syringe. From the injection port, the sample is pushed onto the column by the carrier gas and detection of the analytes is achieved at the column outlet by a suitable detector.

Figure 3.2 System diagram for a gas chromatograph (from Kellner et al., 1998).

Packed columns for gas chromatography are manufactured from stainless steel or glass, and a granular material supports the solid stationary phase inside. Packed column dimensions range from 3 - 8mm in internal diameter and from 1 - 3m in length. Nowadays, capillary GC columns are more commonly used because they provide more efficient separation due to the greater number of theoretical plates compared to packed columns. They are made from fused silica and contain no carrier material. Instead, the stationary phase is in the form of a liquid and adheres to the walls of the capillary. Capillary columns range in length from 15 - 100m and have internal diameters ranging from 0.15 - 1mm. Due to the narrow internal diameter of capillary columns, different injection systems are used in order to optimise transfer of the sample onto the column. In a split injector, the sample is injected into a hot evaporation chamber and a small proportion of the sample vapour goes onto the column while the remainder leaves the system via a split opening. In a split-less injector, the sample is injected while the splitter is open, and a large proportion of the

sample is therefore transferred directly onto the column. The splitter is then closed and the remainder of the sample vapour is purged away through the split opening. Split-less injection systems are therefore commonly used for trace analysis applications.

Gas chromatography can be coupled to a variety of detection systems. The most commonly used detector is the flame-ionisation detector (FID), which provides detection of all compounds containing C-C and C-H bonds. It has a linear range of 10^7 and a detection limit of 10^{-13} g/s. The electron capture detector (ECD) is specific to compounds containing electrophilic groupings such as halogens, peroxides, quinones, phthalates or nitro- groups. The linear range is smaller compared to the FID (10^4), but sensitivity is greater by an order of magnitude for the target analytes. Different types of mass-spectrometry (MS) can also be coupled to GC and provide structural information about fragment ions of the target analytes across a mass range from 50 - 500 m/z. For this work, gas chromatography was coupled to inductively coupled plasma mass spectrometry (Section 3.4.2).

The gas chromatograph used was an Agilent Technologies 6890, which was equipped with an autosampler and injector. The interface used to couple the GC to the ICP-MS was also provided by Agilent Technologies and consisted of a heated transfer line and injector (Agilent Technologies Technical Note 5988-3071EN).

3.2.2 Liquid Chromatography (LC)

A diagram of a liquid chromatography system is shown in Figure 3.3. Liquid chromatography is generally applied to compounds that are non-volatile and can therefore not be analysed by gas chromatography.

Organic solvents or aqueous buffer solutions are used as the mobile phase and delivery to the analytical column is achieved by means of either a single pump for isocratic runs or dual or quaternary pumps where the composition of the mobile phase can be changed throughout the run resulting in a gradient elution.

Figure 3.3 System diagram for liquid chromatography (from Kellner et al., 1998).

The mechanisms responsible for separation in high performance liquid chromatography (HPLC) are based on four principles: adsorption, distribution, ion exchange and exclusion. Normal, reversed phase, size exclusion and ion chromatography are distinguished and with any particular HPLC application a combination of the four separation principles is usually employed to achieve separation of the analytes. Columns dimension can vary from 50mm to 300mm in length and from less than 1mm (microbore columns) to 4.6mm in internal diameter. In order to reduce the volume of solvent used for HPLC separations, shorter microbore columns are being increasingly used for routine applications. HPLC separations can be coupled to a variety of detectors with ultra-violet (UV) absorption detection currently accounting for ~ 70% of applications. Other detectors such as mass spectrometers (e.g. MS, MS-MS or ICP-MS) are also increasingly being used, especially for trace analysis where high sensitivity is required or to gain structural information for identification purposes.

Initially a stand-alone single solvent channel Kontron HPLC pump was used for this research in conjunction with a Rheodyne injection manifold (Cotati, US). Injections were carried out manually using a syringe, and due to the single channel pump, only isocratic elutions could be performed. The HPLC column, injector and pump were connected by PEEK tubing. Subsequently, a fully automated Agilent Technologies 1100 HPLC system was used, which consisted of a quaternary pump,

allowing the use of gradient elutions, a temperature-controlled autosampler with positions for up to 100 vials, and a temperature-controlled column compartment, which could be used to maintain the column at a set temperature to ± 1°C. Chapters 4.1 and 5.1 contain more detail on the actual liquid chromatographic separations used including mobile phase composition and stationary phase materials.

3.3 DETECTION: INDUCTIVELY COUPLED PLASMA- MASS SPECTROMETRY (ICP-MS)

3.3.1 Inductively Coupled Plasma

The Inductively Coupled Plasma (ICP) has found widespread use in conjunction with atomic emission spectrometry (AES), mass spectrometry (MS) and time-of flight mass spectrometry (TOF-MS) detectors. ICPs are routinely used for ionisation of aqueous samples. Prior to introduction into the plasma, the solution is transformed into an aerosol by a nebuliser. Depending on the type of nebuliser used, the droplet size distribution of the aerosol is generally too large for efficient desolvation, volatilisation and atomisation in the plasma. Therefore, a spray chamber is used as a size-filter, which ensures that the average droplet size reaching the plasma is less than 10 μm.

Figure 3.4 shows a schematic diagram of an ICP. Argon gas is introduced as a tangential flow as the coolant and plasma gas, and the aerosol containing the sample is introduced along the injector tube. The coolant or main gas is the main supply of Ar to the plasma. At a flow rate of 12 - 15 L/min it also acts to protect the quartz walls of the torch from being melted. The auxiliary gas flows around the outside of the injector and apart from supplying gas to the plasma its main function is to push the plasma away from the injector tip. The ICP is generated by coupling energy from a radio frequency generator into a suitable gas (usually Ar) by means of a magnetic field, which is established through a water-cooled copper coil. The magnetic field also holds the plasma in place within the quartz torch. When a spark introduces electrons into the gas, they accelerate in the magnetic field and obtain sufficient energy to ionise gaseous atoms within the field. Further collisions with

other gaseous atoms induce a cascade of ionisation so that the plasma becomes self-sustaining.

Figure 3.4 Diagram of a) an Inductively Coupled Plasma (ICP) and b) the different zones of the ICP: PHZ- Pre-Heat Zone; BR- Boundary Region; IRZ Initial radiation Zone and NAZ- Normal Analytical Zone. (from Ebdon et al., 1998 a).

The plasma is divided into different regions according to the temperature distribution. In the pre-heat zone (PHZ), volatile components are burnt off and the initial dissociation of molecules in the sample matrix begins. The boundary region (BR) is defined as the area between the central channel and the plasma, because of the gas flow from the injector, this region is cooler than the adjacent regions of the plasma. The initial radiation zone (IRZ) contains excited species and free electrons. The normal analytical zone (NAZ) is used for ion sampling in ICP-MS applications.

In order to couple a mass-spectrometer to an ICP, a specialised interface has to be used in order to allow the quantitative transfer of ions from the plasma, which is at atmospheric pressure, to the mass analyser at reduced pressure in a vacuum chamber. This transfer is achieved in two stages as shown in Figure 3.5 below.

Figure 3.5 Interface design for coupling of ICP to mass-spectrometry detection. (from Ebdon et al., 1998 a).

The ICP-MS interface consists of two cones, which are usually made out of nickel or platinum. The sampler cone is water-cooled and in direct contact with the plasma. Ions are sampled from the NAZ through the cone orifice by the vacuum generated by a rotary pump connected to the interface. The ions are then transmitted into the analyser chamber, at a further reduced pressure through the skimmer cone. The ion transfer to the analyser chamber is usually also assisted by a number of extraction lenses behind the skimmer cone. Mass analysis coupled to ICP sources can be achieved by quadrupole, magnetic sector and time-of flight ion separation.

3.3.2 Quadrupole Mass Analysers

Mass analysis in ICP-MS is based on a mass to charge (m/z) ratio. The ions of interest in ICP-MS are almost exclusively singly charged, and mass to charge therefore usually equates to mass. Quadrupole mass analysers are made up of four hyperbolic rods, which are arranged in two pairs (Figure 3.6). An electric field is generated in-between the rods by a combination of radiofrequency and direct current voltages applied to each pair. The voltages applied can be controlled in such a way as

to only allow ions of certain m/z through to the detector, whilst the remainder strike the rods and are not detected.

Figure 3.6 Side and end view of a quadrupole mass-analyser (from Ebdon et al., 1998 a).

Quadrupole mass analysers are capable of resolving up to 0.8 m/z units. The entire mass range from m/z 2 to m/z 260 can be scanned in less than 100 ms and this allows for quasi-simultaneous isotope measurements. Generally, the instrument is set up to scan a number of masses of interest in sequence rather than scanning the whole mass range continuously, and this is referred to as peak hopping.

The advantages of quadrupole mass analysers are that they are compact, relatively cheap, robust and suitable for relatively interference free analysis of most trace elements. In addition, they can be used for quasi-simultaneous isotope ratio measurements. The disadvantages are that sensitivity is not as good as with magnetic sector instruments, interferences which are smaller than one m/z unit cannot be completely resolved, and isotope ratio measurements are not truly simultaneous because only one m/z ratio is measured at the same time.

A diagram of a quadrupole ICP-MS is shown in Figure 3.7. The components labelled in the diagram are:

A-	Sample solution	G-	Plasma
B-	Peristaltic pump	H-	Sampling cone
C-	Nebuliser	I-	Skimmer cone
D-	Aerosol	J-	Ion lenses
E-	Spraychamber	K-	Quadrupole mass analyser
F-	Torch	L-	Detector

Figure 3.7 Schematic of a quadrupole ICP-MS (from Evans et al., 1995).

Two different quadrupole ICP-MS instruments were used for the work described here. Initial experiments were carried out using an Elan 5000A (Perkin Elmer, Beaconsfield, UK), which provided a robust detector with good plasma stability for LC applications due to its free running RF generator. The majority of the measurements described in Chapters 4 - 7 were made using a newer Agilent Technologies 7500 ICP-MS. Due to the fact that the Agilent 7500 incorporated several years worth of improvements in technological innovation affecting parameters such as ion transmission and detection, the sensitivity provided by this instrument was superior to the Elan 5000A. The merits and drawbacks of both instruments when coupled to liquid chromatography are described in more detail in Section 3.4.

3.3.3 Magnetic Sector High-Resolution Instruments

Magnetic sector or High-resolution ICP-MS instruments are used for applications that require either superior mass resolution or sensitivity compared to quadrupole ICP-MS applications. The front-end parameters of such instruments are identical in principle to parts A – I shown in Figure 3.7.

Figure 3.8 Diagram of a double focussing magnetic sector instrument (from Ebdon et al., 1998 a).

However, the separation of ions with different mass to charge ratios is achieved differently. Ions are deflected in a magnetic field and the greater the mass of the ion, the greater the deflection. In normal high-resolution ICP-MS instruments an electrostatic analyser and a magnetic sector are arranged in sequence (Figure 3.8.). The electrostatic analyser acts as an energy filter for the ions sampled from the plasma. These ions are then deflected in the magnetic sector and mass spectra can be generated by scanning the magnetic field. The speed at which magnetic sector instruments can scan from m/z 2 to m/z 260 is about 1 second, i.e. 10 times slower than a quadrupole. For simultaneous detection after mass separation by magnetic sector, an array of detector cups can be assembled and instruments, which have this set-up, are referred to as multi-collector instruments. They allow for truly simultaneous isotope ratio measurements, and are therefore often used for applications where high-precision isotope ratio measurements are required. The isotope ratio measurement precision for this technique is also enhanced by the flat-

top of the spectral peaks when performing measurements at low resolution conditions (Becker and Dietze, 2000).

A Finnigan MAT Element 1 (Thermo, Bremen, Germany) was used for some of the work described here. The instrument was used in the low-resolution, high-sensitivity mode for the detection of minor organotin compounds in the liquid chromatography eluent. However, due to the poor plasma stability when eluents containing organic solvents were used and the cumbersome data transfer and peak area integration, this was not an approach suitable for moderate to high sample throughput in conjunction with some LC applications.

3.4 COUPLING OF CHROMATOGRAPHIC SEPARATION TECHNIQUES TO INDUCTIVELY COUPLED PLASMA MASS-SPECTROMETRY

3.4.1 Liquid Chromatography ICP-MS

As shown by the increasing number of publications employing LC-ICP-MS (Figure 3.9), this technique is becoming increasingly more important in a number of different applications (Figure 3.10).

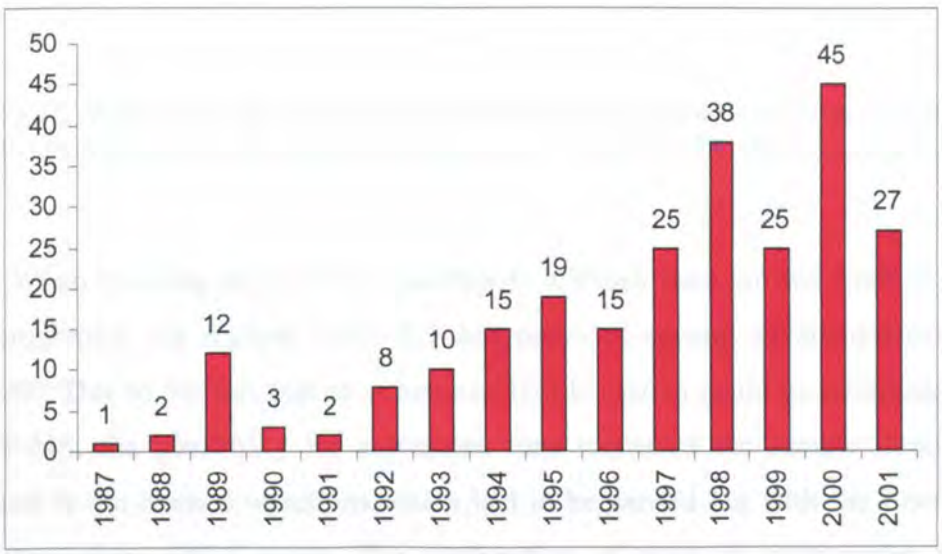

Figure 3.9 Trend in the number of research publications using LC-ICP-MS for analytical measurements (compiled from SciFinder 2002 database search).

ICP-MS was originally developed for the analysis of aqueous samples and therefore interfacing a liquid chromatography system to this detector is very practical

and cost-effective because no specialised interface is required. Some optimisation of the mobile phase for HPLC separations and the flow rate are required to ensure good compatibility of the liquid chromatographic method with the ICP-interface and plasma. This usually involves matching the ideal flow rate of the ICP-nebuliser to the column flow rate of the HPLC, and ensuring mobile phases containing organic solvents or buffer salts do not lead to clogging of the interface or carbon deposition and subsequent signal loss or plasma extinction.

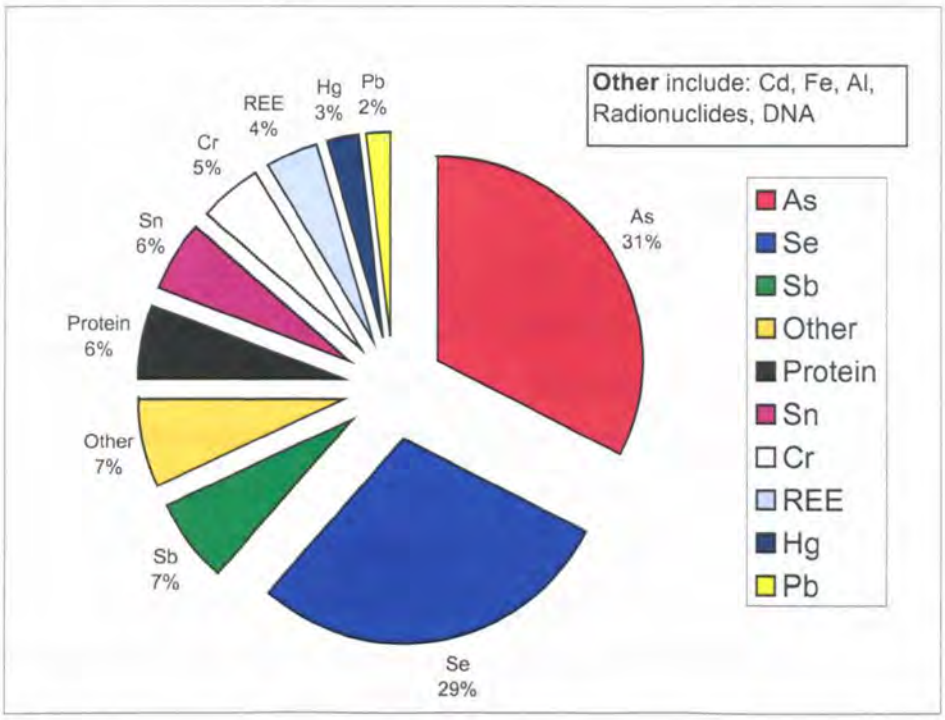

Figure 3.10 Diagram of the relative proportions of measurement applications using LC-ICP-MS (based on SciFinder 2002 search for 'LC-ICP-MS' publications. Date: 2002).

When coupling either of the quadrupole ICP-MS used for this work to liquid chromatography, the Agilent 7500i ICP-MS provided several advantages over the Elan 5000. Due to the fact that an automated HPLC system could be interfaced with the ICP-MS, the possibility for automated runs increased the sample throughput compared to the manual injections which had to be carried out with the Elan 5000 and a stand-alone HPLC pump. The combination of using an autosampler, which increased the precision of retention times, and the dedicated peak integration software provided with the Agilent system, significantly increased the precision in peak area measurements compared to the initial set-up, where the integration of the chromatographic peaks had to be carried out by exporting the data into Microsoft

Excel. The plasma stability was good for both quadrupole instruments when using organic solvents in the mobile phase. This was not the case with the High resolution ICP-MS, as described above.

3.4.2 Gas Chromatography ICP-MS

From a practical point of view the coupling of gas chromatography to ICP-MS is less straightforward than LC-ICP-MS for several reasons. The coupling of GC-ICP-MS usually relies on a more involved interface, and such interfaces vary in design (e.g. Montes-Bayon et al., 1999; Yang et al., 2002) and cost involved. The design of the interface can also have an impact on the analytical capabilities, because non-heated interfaces may be prone to adsorption of high-boiling point compounds such as TPhT.

In general, there are currently fewer publications using GC-ICP-MS compared to LC-ICP-MS. This reflects the fact that it is still considered a research tool rather than a routine methodology. However due to the potential for simultaneous multi-elemental speciation analysis of volatile compounds, such as species of Se (Gomez-Ariza et al. (2001), Pb (Leal-Granadillo et al., 2000), Hg (Snell et al., 2000) and Sn (Rajendran et al., 2000) and S, GC-ICP-MS will undoubtedly become more important as more robust and user-friendly interfaces are developed and provided commercially by instrument manufacturers.

A picture of the GC-ICP-MS system used for this work is shown in Plate 3.3, and actual working conditions are shown in Table 4.7 (Chapter 4).

Plate 3.3 GC-ICP-MS system consisting of an Agilent Technologies 6890 GC, a heated ICP transfer line and injector and the 7500i ICP-MS.

CHAPTER 4

ORGANOTIN SPECIATION

The toxic effects of organotin compounds in the environment have been well-documented (Nicklin and Robson, 1988), and have led to extensive research into analytical methodologies for their determination in a variety of matrices. The widespread use of organotin compounds in pesticides, anti-fouling paints and as heat- and light stabilisers in PVC products has resulted in their detection in most marine and fresh-water sediments as well as in open-ocean waters (Tao et al., 1999). In recent years, the focus of research in organotin analysis has begun to include matrices with human health implications, such as seafood (Keithly et al. 1999), artificial matrices such as PVC pipes used for drinking water distribution (Sadiki and Williams, 1996), and human blood (Kannan et al., 1999) and liver samples (Takahashi et al., 1999). Currently, a wide range of methods are being used for the extraction, separation and detection of organotin compounds, and there can be significant variation in the results obtained by different methodologies (Zhang et al., 1991; Pellegrino et al., 2000).

4.1 DEVELOPMENT OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD COUPLED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) DETECTION FOR ORGANOTIN SPECIATION

The aim of this work was to improve an existing HPLC-ICP-MS method for the separation and detection of organotin species in environmental and biological samples. The starting point was a methodology developed previously at LGC (White et al., 1998), which was capable of separating and detecting DPhT, DBT, TPhT and TBT. However, when coupling this LC separation to a more sensitive detector than that used in the original study by White and colleagues, it was shown that some minor organotin species in a mussel tissue extract interfered with the separation of DBT and TPhT. Therefore improvements in both the separation of the LC method, as well as the sensitivity of the proposed ICP-MS detection were required. Extracts of the mussel tissue CRM 477 (BCR, Brussels) were used for chromatographic method

development because it contains several organotin species in addition to those certified at μg/g level.

4.1.1 Experimentation with Liquid Chromatography coupled to Quadrupole and High-Resolution ICP-MS

Initial work was carried out according to the HPLC-ICP-MS method proposed by White et al. (1998). A Kontron Instruments 420 HPLC pump was coupled to a Perkin Elmer Elan 5000A quadrupole ICP-MS (Beaconsfield, UK) instrument using a 30-cm length of polyether ether ketone (PEEK) tubing. Manual injections were carried out using a syringe and a Rheodyne model 9725 injector (Cotati, California, U.S.A.) with a 50-μL PEEK sample loop. The LC column used was a Kromasil C-18 stationary phase (Hichrom, Reading), with a particle size of 5μm and column dimensions of 15cm × 2.1mm i.d. in conjunction with a mobile phase comprising 65: 25:10 % v/v of acetonitrile, water and acetic acid with 0.05% triethylamine (TEA).

Figure 4.1 shows a chromatogram of a CRM477 extract obtained by the HPLC-ICP-MS conditions described by White et al. (1998). The chromatogram shows separation of the main species DBT, TPhT and TBT, although DBT and TPhT are not base line resolved. MBT is not retained on the column and elutes in the void volume with this chromatography. The chromatographic peaks are ~50 - 60 seconds broad (especially for the butyl species) and the peak shapes are ragged due to signal noise, which is apparent on the baseline.

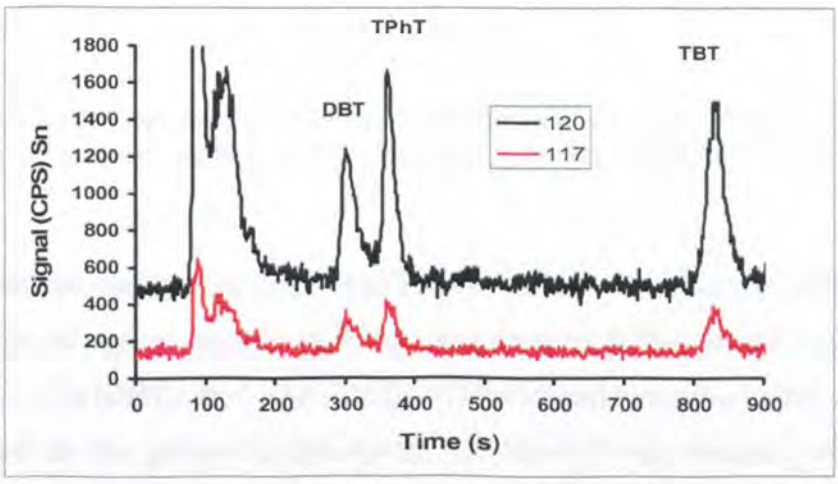

Figure 4.1 Chromatogram of a CRM477 extract using a Kromasil column (C-18 5μm 15cm x 2.1mm) with an Elan 5000A ICP-MS detector. Amount of TBT injected: 95pg as Sn.

In a second set of experiments, a High Resolution ICP-MS (HR-ICP-MS) was used at low-resolution, high-sensitivity setting with the same stand-alone pump and chromatographic conditions in order to determine interfering components. The isotopes monitored by both ICP-MS instruments were ^{120}Sn and ^{117}Sn. When the same sample extract is analysed using the same chromatographic set-up, but coupled to the HR-ICP-MS, it is apparent that parameters other than the chromatographic separation have an influence on the final signal output (Figure 4.2). As expected, the signals for all analytes in the sample are significantly greater when using the high-resolution ICP-MS as the detector. Also, due to the greater sensitivity of the instrument, minor peaks (UK, UK1 and UK2) are detected in the sample, which are masked by the background noise and insufficient sensitivity of the quadrupole ICP-MS. The peak labelled UK1 is clearly preventing baseline resolution between DBT and TPhT due to its retention time, which falls between both peaks.

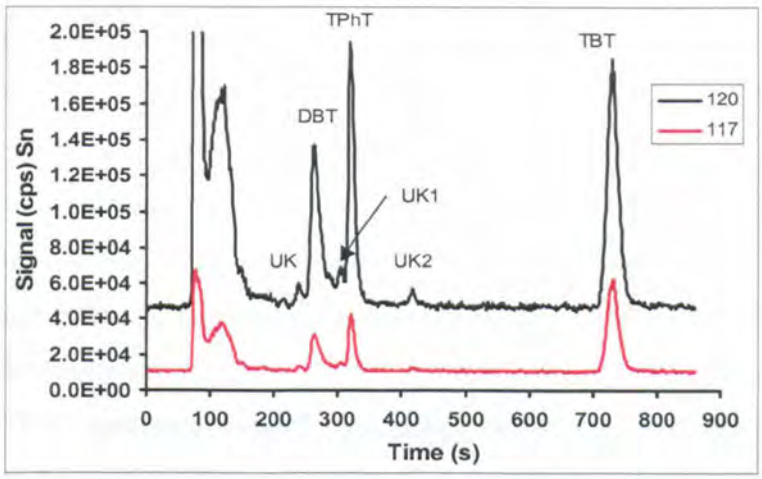

Figure 4.2 Chromatogram of a CRM477 extract using a Kromasil column (C-18 5μm 15cm x 2.1mm) with a MAT Finnigan Element High-Resolution ICP-MS detector.

When comparing Figure 4.2 with Figure 4.1 it is clear that the peak shape is improved for all major analytes and that the retention times for the analytes are reduced by ~ 50s (DBT) to ~ 100s (TBT). Whilst changes in the analyte signal can be attributed to the greater sensitivity of the HR-ICP-MS detector, changes in resolution and retention times are not a function of the detector, but rather of HPLC conditions and the front-end hardware of the ICP-MS, such as spray-chamber dimensions and nebuliser type, which together affect the aerosol transport into the

plasma. The detection of the interfering peak UK1 by HR-ICP-MS showed that the chromatographic conditions developed by White et al. (1998) needed further development in order to resolve DBT, UK1 and TPhT. Despite the great increase in sensitivity and the improvements in the peak shape of the target analytes, the HR-ICP-MS instrument suffered from significant plasma instability when the organic mobile phase was aspirated. The Elan 5000A did not suffer from these problems due to a free-running RF generator, but yielded insufficient sensitivity for the determination of the minor components for further chromatographic method development. For further improvements to the methodology, an ICP-MS was required that could provide the required sensitivity for detection of the minor species as well as offering sufficient plasma stability.

4.1.2 Futher Chromatographic Method Development with Quadrupole ICP-MS

A more modern quadrupole ICP-MS (Agilent 7500i) was used, which combined stable plasma conditions with sufficient sensitivity to detect the minor organotin species in the sample. When coupled to an HPLC system with an autosampler, this set-up also provided more reproducible HPLC conditions, combined with chromatographic software for accurate data interpretation. This set-up was used to determine the effects of changes in chromatographic parameters such as the stationary phase material, column dimensions or particle size, whilst maintaining the same ICP-MS conditions.

The HPLC system consisted of a quaternary pump, a vacuum degasser, an autosampler and a heated column compartment. All stainless steel parts of the HPLC system that come into contact with the sample were replaced by PEEK components. A 100-cm length piece of PEEK tubing (red) was used to connect the LC column to the nebuliser of the ICP-MS. Typical ICP-MS conditions are given in Table 4.1. Optimisation of the ICP-MS conditions was achieved prior to HPLC analysis by adjusting the torch position and tuning for reduced oxide and doubly charged ion formation with a standard tuning solution containing 10 ppb of ^{7}Li, ^{89}Y, ^{140}Ce and ^{205}Tl in 2% HNO_3. After this preliminary optimisation, the HPLC system was coupled to the nebuliser and a final optimisation was carried out using ~ 20 ppb ^{103}Rh added to the HPLC mobile phase. In order to reduce the solvent loading on the plasma, the double-pass spray-chamber was peltier cooled to -5°C. Oxygen (0.1

L/min) was mixed into the make-up gas by a mass-flow controller and added post-nebulisation in order to convert organic carbon to CO_2 in the plasma and avoid a carbon build-up on the cones.

Table 4.1 Typical ICP-MS parameters used with the Agilent 7500i.

Cones	Platinum
Plasma gas flow	14.9 L/min
Nebuliser gas flow	0.75 L/min
Make-up gas flow	0.25 L/min
RF power	1450 W
Sampling depth	5.5 mm
Integration time per mass	300 ms
Isotopes monitored	^{120}Sn ^{117}Sn ^{103}Rh
Other parameters	Injector diameter: 1.5mm Peltier-cooled spray-chamber at -5°C 0.1 L/min O_2 added post nebulisation Shield torch

Figure 4.3 shows a chromatogram for the HPLC conditions proposed by White et al. (1998), coupled to the Agilent 7500i ICP-MS. Peaks for DPhT, DBT, TPhT and TBT, as well as two minor unidentified peaks (labelled UK1 and UK2) are detected. Peak UK1 elutes on the tailing side of DBT and interferes with the quantitation of this compound as also shown by HR-ICP-MS (Figure 4.2). TPhT and TBT are sufficiently well resolved from UK1 and UK2 to allow accurate peak integration.

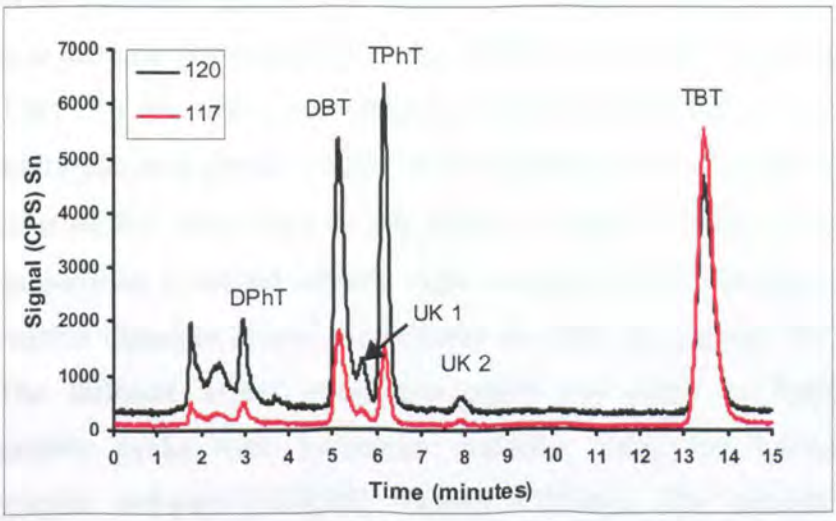

Figure 4.3 Chromatogram of a CRM477 extract using a Kromasil column (C-18 5μm 15cm x 2.1mm) with an Agilent 7500i ICP-MS detector. Amount of TBT injected: 38 pg as Sn.

When coupling HPLC to ICP-MS, the combination of spray chamber and nebuliser has a significant impact on the final chromatographic output. Rivas et al. (1996) described the impact of different spray chambers on the peak resolution, sensitivity and transport efficiency of organotin compounds by HPLC-ICP-MS. The front-end parameters for the ICP-MS instruments used in this study are compared in Table 4.2. The spray-chamber volumes of the Elan 5000A and the HR-ICP-MS are identical whilst the spraychamber of the modern quadrupole ICP-MS is ~18% smaller. Therefore the main difference in the chromatographic signal output obtained by the older quadrupole ICP-MS and HR-ICP-MS described above is most likely due to a combination of the nebuliser efficiency and the instrument sensitivity.

Table 4.2 Front-end parameters used with different ICP-MS instruments.

	Nebuliser (optimal flow rate)	**Spray chamber volume**
Quadrupole (old)	Cross Flow	85mL
Quadrupole (modern)	PFA Concentric (100μL/min)	70mL
HR-ICP-MS	Meinhard Concentric (200μL/min)	85mL

White et al. (1998) used a Kromasil C-18 stationary phase (Hichrom, Reading), with a particle size of 5μm and column dimensions of 15cm × 2.1mm i.d. (Conditions A in Table 4.3). In order to improve the resolution between DBT and TPhT, several stationary phases and column dimensions were selected. Attempts were made to increase the resolution between DBT and UK1 by sharpening the peak shape of DBT. To this end a new stationary phase (ACE) with a lower nominal silanol activity due to a greater degree of end-capping, and a reduced particle size was used. An ACE C-18 column (3 μm 15cm × 2.1mm i.d.) was selected for this purpose. In addition a second column with the same ACE stationary phase, but reduced internal diameter (1mm) was chosen in order to improve the compound signals. The different HPLC conditions tested are given in Table 4.3. All chromatographic peaks were integrated manually using the Agilent ICP-MS chromatographic software (G1824C Version C.01.00). The column efficiency (theoretical plates), resolution and retention times were calculated for each

chromatogram using the integration software of the Agilent Technologies LC/MSD ChemStation (Rev. A. 08.03 [847]).

Table 4.3. HPLC conditions for organotin separations.

Columns	All C-18 reversed-phase **Conditions A**: Kromasil 15cm × 2.1mm i.d., 5μm particle size **Conditions B**: ACE 15cm × 2.1mm i.d., 3μm particle size **Conditions C**: ACE 15cm × 1.0mm i.d., 3μm particle size
Mobile phase	**Conditions A**: 65:25:10 v/v/v Acetonitrile: Water: Acetic acid with 0.05% TEA pH 3.4 ± 0.1 **Conditions B and C**: 65:23:12 v/v/v Acetonitrile: Water: Acetic acid with 0.05% TEA pH 3.1 ± 0.1
Flow rate	**Conditions A and B**: 0.2 mL/min **Conditions C**: 0.06 mL/min
Other parameters	All stainless steel tubing and needle assembly replaced with PEEK (polyether ether ketone).

Initial experiments showed that using conditions B, i.e. a different C-18 stationary phase yielded improved resolution between DBT, UK1 and TPhT as well as decreasing their retention times significantly. The composition of the mobile phase was then altered by increasing the volume of acetic acid and decreasing the volume of water, so as to decrease the pH from 3.4 ± 0.1 to 3.1 ± 0.1, which resulted in a further reduction in retention times for all compounds. Figure 4.4 shows the result of the combined changes on the chromatography of the CRM477 extracts.

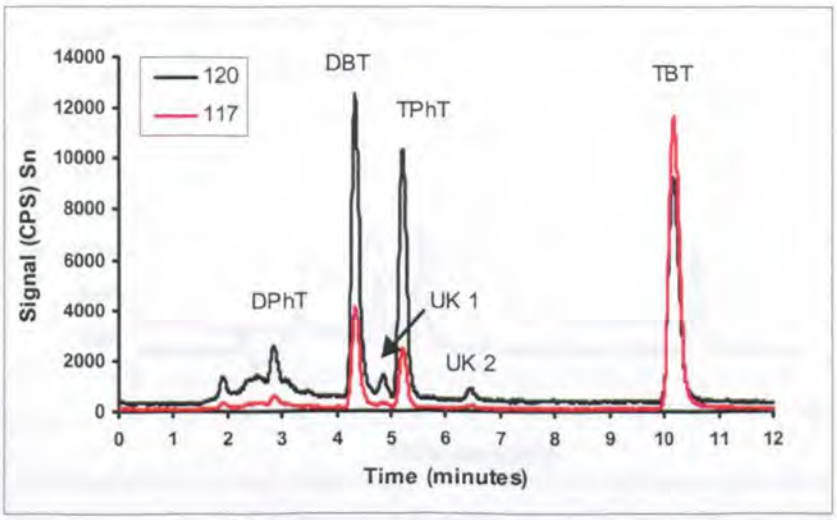

Figure 4.4 Chromatogram of CRM477 using ACE C-18 3μm 15cm x 2.1mm coupled to an Agilent 7500i ICP-MS. Amount of TBT injected: 32 pg as Sn.

The peak widths for the butyltin compounds were reduced significantly (~ 30s) compared to the conditions by White et al. (1998), and there was a reduction in the retention times of all compounds (Table 4.4).

Table 4.4 Changes in retention times (minutes) of different organotin species in mussel tissue CRM477 with different chromatographies.

All in minutes	**Kromasil C-18 (2.1 mm) Conditions A**	**ACE C-18 (2.1mm) Conditions B**
DBT	5.14	4.34
UK 1	5.68	4.86
TPhT	6.18	5.21
UK 2	7.94	6.46
TBT	13.48	10.17

Based on these improvements in resolution and retention, an attempt was made to increase the sensitivity by using the same ACE stationary phase with a reduced column diameter of 1.0 mm (Conditions C). Experiments using this column showed that after optimisation of the mobile phase flow rate to 0.06 mL/min, there was a significant increase in the signal for all analytes (Figure 4.5). However, the improvements in resolution between DBT and TPhT were reversed, and this approach was therefore not pursued for the separation of the analytes in this sample.

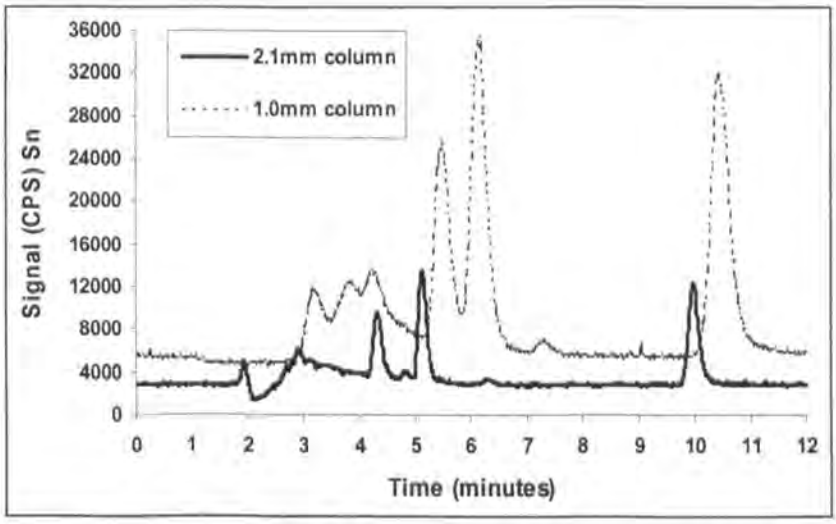

Figure 4.5 Chromatogram of a CRM 477 extract obtained with 15cm ACE columns of 2.1mm and 1.0mm internal diameters. (Amount of TBT injected: 46 pg as Sn). Isotope shown: ^{120}Sn.

In order to evaluate the differences between the HPLC conditions tested in quantitative terms, the column efficiency (theoretical plates), resolution and retention times were calculated for each chromatogram. These data are summarised in Tables 4.4 - 4.6.

The number of theoretical plates gives an indication of the column efficiency and was calculated by the half-width method. Table 4.5 shows a significant increase in the number of theoretical plates calculated for each of the compounds when the sample is analysed using HPLC condition B.

Table 4.5 Number of theoretical plates (half-width method) achieved with different chromatographic columns for organotin separations.

	Kromasil C-18 (2.1mm) Conditions A	**ACE C-18 (2.1mm) Conditions B**	**Factor increase (A – B)**
DBT	2257	4352	1.9
UK 1	2245	6118	2.7
TPhT	4973	6932	1.4
UK 2	5798	10615	1.8
TBT	6662	14134	2.1

The column efficiency is 1.4 – 2.1 times greater for the main analytes in CRM477 using the ACE 3C-18 2.1mm i.d. column, compared to the Kromasil C-18 stationary phase tested by White et al. (1998). This increase is probably due to a combination of the different C-18 material and the smaller particle size of the stationary phase.

The resolution between two adjacent peaks was calculated as:

$$Rs = 2\frac{(t_{R2} - t_{R1})}{(w_1 + w_2)} \qquad \textbf{Equation 1}$$

where t_{R1} and t_{R2} are retention times for peaks 1 and 2 respectively and w_1 and w_2 are peak width for peaks 1 and 2 respectively. When R_s is 0.5 the overlap between peaks is 16%, this is reduced to 2.3% at R_s equal to 1.0 and further to 0.1% when R_S equals 1.5 (Harris, 1991).

Table 4.6 Changes in resolution (R_S) from preceding peak with different chromatographies.

	Kromasil C-18 (2.1 mm) Conditions A	ACE C-18 (2.1mm) Conditions B	Factor increase (A – B)
DBT	-	-	-
UK 1	1.19	2.04	1.7
TPhT	1.20	1.39	1.2
UK 2	4.57	5.00	1.1
TBT	10.27	12.48	1.2

Table 4.6 shows that the resolution is also increased for all compounds using Conditions B compared to Conditions A. The most significant increase in resolution is seen between DBT and Peak UK1 from 1.19 using the HPLC method by White and co-workers (1998) to 2.04 using the ACE 3C-18 column.

Based on the experiments described above, the HPLC-ICP-MS conditions proposed here consist of an ACE C-18 LC column (15cm × 2.1mm, with 3μm particle size), a mobile phase of 65:23:12% v/v acetonitrile: water: acetic acid with 0.05 % TEA delivered at 0.2 mL/min coupled to a modern quadrupole ICP-MS with a small (70mL) cooled spray chamber and a concentric nebuliser. This method shows several improvements for the separation of DBT, TPhT, TBT and two unknown compounds detected in CRM477 when compared to the conditions proposed by White et al. (1998). These include increases in the resolution for all five peaks as well as significant improvements in the column efficiency by a factor of 1.4 - 2.7. The retention times for all compounds were also reduced and this lead to a decrease in the total run-time of ~ 25%.

4.2 GAS CHROMATOGRAPHY (GC) COUPLED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) DETECTION FOR ORGANOTIN SPECIATION

In order to have an independent methodology, which could be used to validate the accuracy of the developed LC-ICP-MS method for quantitative analysis a GC-ICP-MS method was used. In addition, a direct comparison between both methods has not been reported previously in the literature, and it was thought that this would provide useful data highlighting the relative benefits and drawbacks of both methods,

particularly with regards to the application of species-specific isotope dilution mass spectrometry (SS-IDMS).

4.2.1 Instrument Set-up

GC separations were performed on an Agilent 6890 GC. The GC-interface used to couple the GC to the ICP-MS was an Agilent G3158A, which consists of separately heated transfer line and injector. An Agilent 7500i ICP-MS was used for time resolved analysis of ^{120}Sn and ^{117}Sn. Table 4.7 shows typical conditions used for GC-ICP-MS analysis. The GC method used was according to Rajendran et al. (2000). The GC column was connected to a length of de-activated fused silica, which was inserted along the ICP transfer line and injector. The argon nebuliser gas for the ICP-MS was heated in a stainless-steel coil in the GC oven and transported along the transfer line and injector into the plasma. After installation of the interface, the ICP-MS torch position and the ion lenses were tuned using a 100 ppm xenon in oxygen mixture, which was added to the ICP-MS nebuliser gas at 0.1 L/min via a T-piece. ^{131}Xe was monitored for this adjustment.

Table 4.7 Typical conditions used for GC-ICP-MS analysis.

ICP-MS	
Cones	Platinum
Plasma gas flow	14.5 L/min
Carrier gas flow	0.80 L/min
Make-up gas flow	Not used
RF power	1100 W
Sampling depth	7 mm
Integration time per mass	100 ms
Isotopes monitored	^{120}Sn, ^{117}Sn, ^{131}Xe
Other parameters	0.1 L/min N_2 or O_2 added Shield torch used 2 roughing pumps
GC	
Column	HP5 (30m ×0.32mm i.d. × 0.25μm)
Carrier gas	He at 2 mL/min (controlled by electronic pressure control)
Inlet temperature (mode)	250°C (pulsed splitless)
Injection volume	1 μL
Transfer line temperature	280°C
Injector temperature	240°C

4.2.2 Effect of optional gas addition on the sensitivity of GC-ICP-MS measurements

During initial tuning experiments, it was found that the addition of 0.1 L/min oxygen as the optional gas enhanced the organotin signals of the GC-ICP-MS measurements significantly. Mixed gas ICP discharges have been shown to improve detection limits for elements with high ionisation energies. This has been attributed to the fact that mixed-gas plasmas offer a greater level of interaction between the plasma and the sample, because of their enhanced thermal conductivity and smaller axial channel compared to an argon plasma (Montaser and Zhang, 1997). An experiment was set up to quantify the sensitivity increase with a) no added gas, b) 0.1 L/min O_2 added and c) 0.1 L/min N_2 added, using repeat injections of a 20 ng/mL as Sn mixed organotin standard. Oxygen or nitrogen was added to the nebuliser argon stream via a T-piece.

The increase in peak area measurements for MBT, DBT, TBT, TeBT, MPhT, DPhT and TPhT as a result of addition of 0.1 L/min O_2 and 0.1 L/min N_2 is illustrated in Figure 4.6 and results are shown in Table 4.8. Adding 0.1 L/min O_2 results in increases in the measured peak area ranging from 9 (DBT and MPhT) to 12-fold (MBT). The addition of N_2 results in a further increase in the response factor compared to analysis without addition of an optional gas. Response factor increases range from 105 (DBT and TPhT) to 136 for MBT and 150 for TeBT. When comparing the effects of adding N_2 to O_2 there is a further increase in peak areas ranging from 11-fold (TPhT) to 16-fold (TeBT) for analysis of the same 20 ng/mL (as Sn) mixed standard.

Snell et al. (2000) showed that adding O_2 to the auxiliary gas during mercury speciation by GC-ICP-MS increased sensitivity by a factor of two. Montes-Bayón and co-workers (2002) reported increased response factors for the analysis of volatile Se species when adding O_2 and N_2 during GC-ICP-MS analysis. Although Sn is efficiently ionised under normal plasma conditions (first ionisation potential of 7.30 volts) (Harris, 1991), the mixed gas conditions used for this study probably facilitate the efficient breakdown and ionisation of the ethylated Sn species that are delivered from the GC.

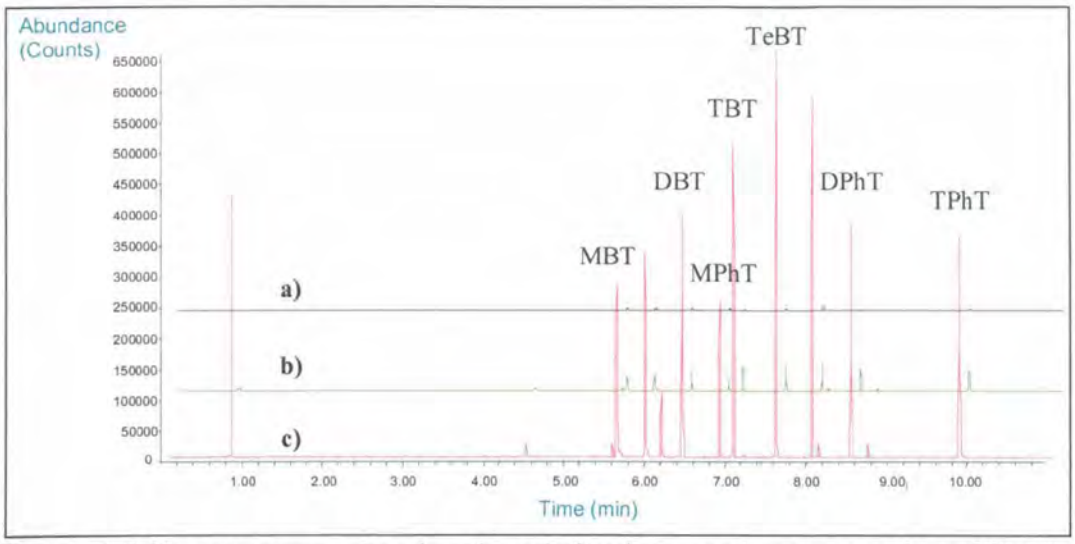

Figure 4.6 Chromatogram showing the relative increase in response for a 20 ng/mL (as Sn) mixed organotin standard analysed using a) no additional gas, b) 0.1 L/min O_2 and c) 0.1 L/min N_2.

Table 4.8 Comparison of GC-ICP-MS peak area measurements using a 20 ng/mL as Sn mixed organotin standard analysed with a) no additional gas, b) 0.1 L/min O_2 and c) 0.1 L/min N_2.

Compound	Retention Time (min)	a) No gas added	b) 0.1 L/min O_2 added	Response factor compared to a)	c) 0.1 L/min N_2 added	Response factor compared to a)	Response factor compared to b)
MBT	5.57	2274	27029	12	309702	136	12
DBT	6.38	3247	29238	9	340436	105	12
MPhT	6.84	2026	18173	9	215182	106	12
TBT	7.02	3490	33132	10	399868	115	12
TeBT	7.54	3717	34225	9	558916	150	16
DPhT	8.46	3181	29665	9	338057	106	11
TPhT	9.81	4287	41119	10	450803	105	11

During HPLC-ICP-MS analysis (Wahlen and Catterick, 2003), the addition of oxygen was mandatory to avoid blocking of the ICP-MS interface by carbon build-up and therefore it could not be assessed whether this gas addition resulted in increased sensitivity with this method. However, adding an oxygen-nitrogen mixture to the HPLC set-up did not result in increased signal intensity when an aqueous sample was introduced into the plasma. With HPLC-ICP-MS systems it has been shown that the ionisation of elements such as As is enhanced by the addition of organic solvents such as MeOH (Larsen and Stürup, 1994). It is likely that any

possible signal enhancing effect of additional gases added to the LC-ICP-MS system described here is masked by the high percentage of organic solvent (65% acetonitrile) that is introduced into the plasma.

4.3 COMPARISON OF HPLC AND GC SEPARATIONS COUPLED TO ICP-MS FOR ORGANOTIN DETERMINATIONS IN SEDIMENT EXTRACTS

The aim of this study was to establish a direct comparison between the respective performance characteristics such as detection limits, analyte capabilities and isotope ratio measurement precision for SS-IDMS between HPLC and GC separations for organotin compounds. This was achieved by coupling both chromatographic separations to a quadrupole ICP-MS and analysing the same sediment extracts for tributyltin using species specific IDMS. This approach ensured that any differences in the final results would be closely linked to the separation technique used rather than being due to factors affecting the extraction procedure or sample preparation. Uncertainty estimates, according to the EURACHEM (2000) are provided for the results obtained by both methodologies.

4.3.1 General Comparison of the Chromatographic ICP-MS Techniques

It is useful to compare both chromatographic ICP-MS couplings in terms of time-efficiency of the analysis, analyte capabilities, ease-of-use and cost. From a practical point of view the coupling of liquid-chromatography to ICP-MS is more straightforward than GC-ICP-MS for several reasons. Firstly, ICP-MS was designed for the analysis of liquid samples and therefore the coupling to a liquid chromatography column is readily and cheaply achieved by means of PEEK tubing. In contrast, the coupling of GC-ICP-MS usually relies on a more involved interface, and such interfaces vary in design (e.g. Montes-Bayon et al., 1999; Yang et al., 2002) and cost involved. The design of the interface can also have an impact on the analytical capabilities, because non-heated interfaces may be prone to adsorption of high-boiling point compounds such as TPhT.

The analysis of organotin species by gas-chromatography involves a derivatisation step, which makes the sample preparation more time-consuming compared to the simple dilution necessary for the LC-ICP-MS method described here; this is despite recent improvements in derivatisation provided by new reagents

such as $NaBEt_4$ in comparison to alkylation with Grignard reagents. The use of derivatisation agents can also be a cost-factor for routine field laboratories. One of the benefits of GC-couplings with ICP-MS lies in the greater separation power, which enables the analysis of more species within the same chromatographic run time. For the methods described here, 5 species (DBT, TBT, DPhT, TPhT and TPrT) can be separated by LC compared to up to 14 species by GC-ICP-MS. Although the addition of tropolone and slight alteration to the LC mobile phase (Chiron et al., 2000) can enable the separation of MPhT and MBT in addition to the species listed above, the separation and peak resolution is still inferior to the GC-ICP-MS method described here.

4.3.2 Comparative Figures of Merit

4.3.2 a) Detection Limits

The signal-to-noise ratio for GC-ICP-MS for TBT is ~ 450:1 for 0.014 ng TBT (as Sn) injected, whilst HPLC-ICP-MS has a signal-to-noise ratio of ~ 11:1 for 0.035 ng TBT (as Sn) injected. Estimation of the detection limits is based on the relation between the height of the analyte peak and the amplitude of the baseline noise (3σ), so the reasons for the differences in the signal to noise ratios of both approaches need to be emphasised. A comparison of the chromatograms in Figure 4.7 and Figure 4.8 shows that the level of the background noise is ~ 3000 counts for the major isotope ^{120}Sn when measured by LC-ICP-MS. The background level for the same isotope measured by GC-ICP-MS is usually below 50 counts.

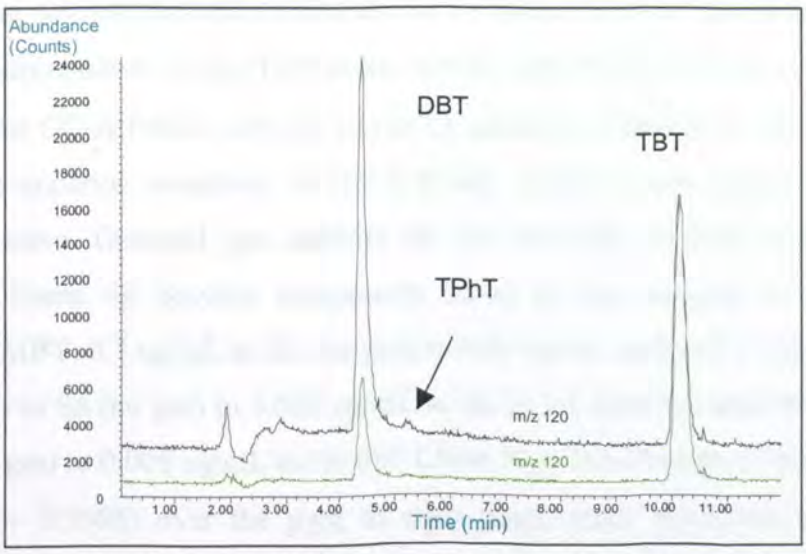

Figure 4.7 HPLC-ICP-MS chromatogram of the sample sediment.

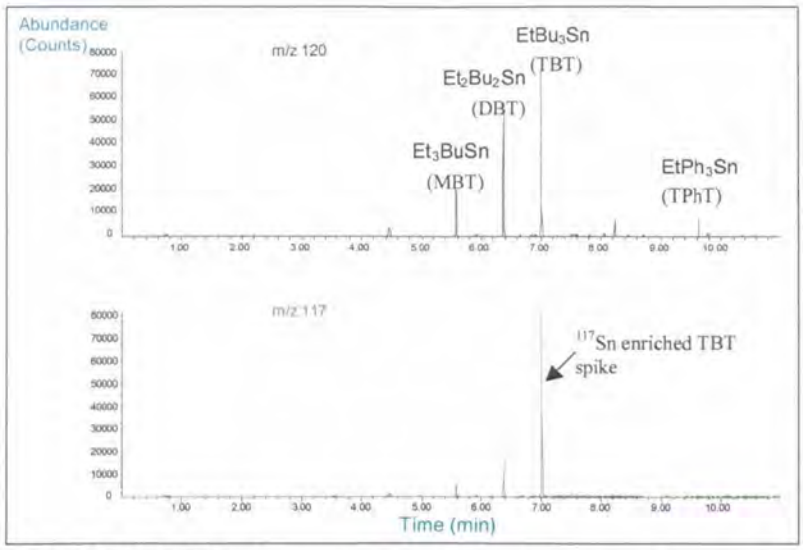

Figure 4.8 GC-ICP-MS chromatogram of the sediment extract after ethylation.

The reasons for this significant difference lie in the different chromatographic systems used and the mobile phases introduced into the plasma from them. Gases for GC-analysis are not usually prone to contamination by Sn, and contact with metallic surfaces inside the GC that could give rise to metal contamination is minimal. For liquid chromatography on the other hand, it is much more difficult to eliminate this background noise. It can be reduced by using ultra-pure reagents and replacing metal components of the LC-system with inert materials such as PEEK, but unless the liquid chromatograph is exclusively used for ultra-trace analysis, contamination from reagents used with other applications usually result in a rise in background levels with time.

The absolute detection limits for the sediment extracts was estimated by peak height measurements as 3 pg TBT as Sn (3σ) for HPLC-ICP-MS and 0.03 pg TBT as Sn (3σ) for GC-ICP-MS with 0.1 L/min O_2 addition (Table 4.9). This comparison shows the superior sensitivity of GC-ICP-MS, which is two orders of magnitude more sensitive. Optional gas addition for GC-ICP-MS resulted in the following detection limits for butyltin compounds based on the analysis of a calibration standard. MBT: 0.7 ng/mL as Sn (no gas) to 0.01 ng/mL as Sn (0.1 L/min N_2), DBT: 0.5 ng/mL as Sn (no gas) to 0.008 ng/mL as Sn (0.1 L/min N_2) and TBT: 0.4 ng/mL as Sn (no gas) to 0.006 ng/mL as Sn (0.1 L/min N_2). The linearity of both methods is good ($r^2 > 0.9996$) over the pg/g to ng/g range used. Injections of μg/g level

standards were not carried out to avoid contamination of the system due to carry-over.

Table 4.9 Comparative figures of merit for the analysis of TBT in sediment.

			Isotope ratio measurement precision [d]	
	L.O.D. (as Sn) [a]	Linearity [b]	Sample solution (R'_B)	Calibration solution (R'_{Bc})
LC-ICP-IDMS	3 pg	>0.9998	0.3 – 1.4%	0.7 – 1.3%
GC-ICP-IDMS	0.03 pg [c]	>0.9996	0.5 – 1.0%	0.4 – 0.7%

[a] Absolute detection limit in sediment for ^{120}Sn
[b] Typical correlation coefficient measured over pg/g to ng/g calibration range
[c] Obtained with addition of 0.1 L/min of O_2
[d] Expressed as percent relative standard deviation of multiple (n = 4) measurements.

4.3.2 b) Isotope Ratio Measurement Precision

The precision of the isotope amount ratio measurements for both methods can be compared for repeat injections of the mass-bias calibration blend solutions and the sample blends. As shown in Table 4.9 the typical percent relative standard deviations achieved by HPLC-ICP-IDMS range from 0.3% to 1.4% for R'_B and from 0.7% to 1.3% for R'_{Bc}. For GC-ICP-IDMS the respective precisions achieved range from 0.5% to 1.0% for R'_B and 0.4% to 0.7% for R'_{Bc}. The isotope measurement precision is influenced by a combination of the chromatographic parameters of the peaks obtained and by the ICP-MS measurement conditions used with each method. The most important parameters that affect the reproducible integration of a peak are chromatographic parameters such as peak width, resolution, tailing and signal-to-noise ratio and instrumental parameters such as the number of isotopes monitored and the integration time per isotope used, which influences the sensitivity and hence the peak area obtained. The combination of the peak width, the number of isotopes monitored and the integration time determine how many individual measurement points define each peak.

For the LC-ICP-MS chromatogram shown in Figure 4.7, the number of measurement points for the ^{120}TBT peak is calculated as follows: The peak width (~ 48 seconds) is divided by the product of the isotopes monitored (2) and the integration time per isotope (0.3 seconds). Therefore ~ 80 points define this peak. For the ^{120}TBT peak in the GC-ICP-MS chromatogram (Figure 4.8) the equivalent

number of points is 15 (~ 4.5 second peak width/ 3 isotopes per 0.1 seconds). By decreasing the integration time of the GC-ICP-MS method to 0.075 seconds and reducing the isotopes monitored to 2, the number of points measured for GC peaks was increased by 50%. This resulted in a significant improvement in the isotope ratio measurement precision by ~ 0.5%. Despite the greater number of measurement points for the LC-ICP-MS peaks, the peak area integration by GC-ICP-MS is more reproducible. This is because the GC peaks are narrower, there is no significant background noise for the isotopes monitored, and the peaks do not suffer from tailing as much as the HPLC peaks. The tailing of the LC peaks in combination with the baseline noise has the biggest influence on the isotope ratio measurement precision by this method.

The differences in peak area integration are reflected by the respective isotope amount ratio precision data for R'_B and R'_{Bc}, which shows GC-ICP-MS to have lower maximum RSDs (1.0% and 0.7% respectively) compared to HPLC-ICP-MS (1.4% and 1.3% respectively). The chromatogram in Figure 4.7 also highlights the fact that the background noise for the less abundant isotope (^{117}Sn) is smaller and with the 'approximate matching' IDMS spiking used improves the signal-to-noise ratio for the ^{117}TBT peak in comparison to the ^{120}TBT peak.

4.3.3 Species-specific IDMS Calculations

The same approach for isotope dilution analysis was used for TBT in sediment as that described for non-speciation IDMS measurements described by Catterick et al. (1998). This IDMS methodology relies on the 'approximate matching' of the isotope ratios in both the sample (R'_B) and the calibration solution (R'_{Bc}). As described by Catterick et al. (1998), this matching approach negates errors associated with mass-bias effects, detector dead-time, and the characterisation of the spike. The concentration of the spike is eliminated from the calculation of the mass fraction in the sample (Equation 2), and therefore the time consuming reverse isotope dilution (RIDMS) used to characterise this value is not required.

Alternating injections of the sample (SB) and calibration solutions (MB) were carried out. In order to obtain sufficient data for the calculation of full uncertainty budgets, each sample extract was injected four times and the calibration solution was injected before and after each sample injection. A typical injection sequence used to

calculate the mass fraction for a single sample extract was therefore: MB Inj. 1, SB Inj. 1, MB Inj. 2, SB Inj. 2,....., MB Inj. 4, SB Inj. 4, MB Inj. 5., The calibration blend isotope ratio (R'_{Bc}) was calculated for each injection from the ratio of the peak areas of ^{120}TBT and ^{117}TBT. The chromatographic peaks were integrated manually using the ICP-MS chromatographic software. The sample isotope ratios (R'_B) were calculated in the same way for each injection. A sample mass fraction was calculated for each sample blend ratio and the average of the bracketing mass-bias calibration blend ratios according to Equation 2.

$$w'_X = w_Z \cdot \frac{m_Y}{m_X} \cdot \frac{m_{Zc}}{m_{Yc}} \cdot \frac{R_Y - R'_B \cdot \frac{R_{Bc}}{R'_{Bc}}}{R'_B \cdot \frac{R_{Bc}}{R'_{Bc}} - R_Z} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}} \qquad \textbf{Equation 2}$$

(Where X = Sample, Y = Spike and Z = Primary Standard. Other notation used in Equation 2 is given in Table 4.10)

The isotope ratios of the primary or natural TBT standard were calculated on the basis of the representative isotopic composition of Sn according to IUPAC. For the spike TBT, the isotopic composition was obtained from the certificate supplied with the ^{117}Sn enriched material from AEA Technology plc (UK). The mean of the four sample mass fractions was then calculated as the mass fraction obtained for that sample extract. The final mass fraction was recalculated back to the original sample, and corrected for moisture content.

Table 4.10 Notations used in Equation 2.

R'_B	measured isotope amount ratio of sample blend (X+Y)
R'_{Bc}	measured isotope amount ratio of calibration blend (Bc=Z+Y)
R_{Bc}	gravimetric value of the isotope amount ratio of calibration blend (Bc=Z+Y)
R_Z	isotope amount ratio of Primary standard Z (IUPAC value)
R_Y	isotope amount ratio of spike Y (value from certificate)
w'_X	mass fraction of Sn in sample X obtained from the measurement of one aliquot
w_Z	mass fraction of Sn in primary standard Z
m_Y	mass of spike Y added to the sample X to prepare the blend B (=X+Y)
m_X	mass of sample X added to the spike Y to prepare the blend B (=X+Y)
m_{Zc}	mass of primary standard solution Z added to the spike Y to make calibration blend Bc (=Y+ Z)
m_{Yc}	mass of spike Y added to the spike Y primary standard solution Z to make calibration blend Bc (=Y+ Z)

4.3.4 Comparison of HPLC-ICP-MS and GC-ICP-MS Data for the Species-specific Isotope Dilution Measurement of TBT in Sediment Extracts

The main aim of this work was to make a direct comparison of HPLC and GC separations for organotin analysis and therefore the uncertainties, which are discussed with respect to this data refer solely to the uncertainty associated with the measurement by hyphenated ICP-MS and not the sample extraction and preparation procedure.

All of the variables present in Table 4.10 have associated standard uncertainties that contribute to the combined standard uncertainty of the mass fraction of analyte of concern, w_X. A complete description of the estimation of the standard uncertainties of the different variables has been described elsewhere (Wolff-Briche et al., 2001). Figure 4.9 shows the major components for a representative uncertainty budget for the analysis of TBT in the sediment extracts obtained.

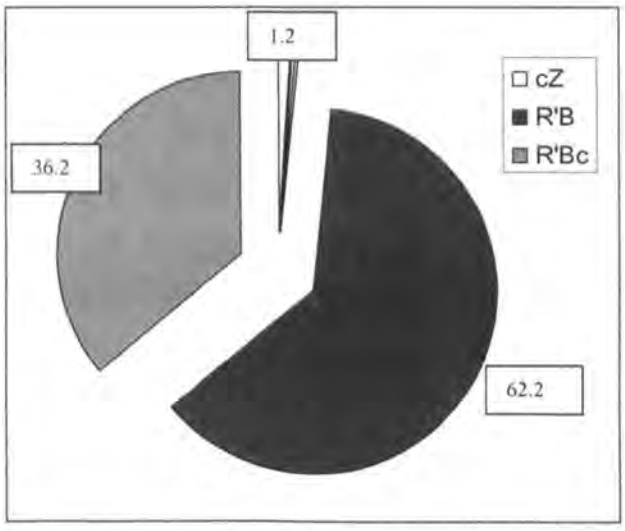

Figure 4.9 Representative uncertainty budget for the hyphenated ICP-MS methods described, (components with a contribution of >1% are listed in the legend).

All weighings were carried out on a 4-figure balance and the standard uncertainty for each mass was obtained by differential weighing and calculated as 0.00006 g for the range of masses used. The dilution factors were based on weight to weight and their standard uncertainties were calculated by combining the standard uncertainties of the individual weighings.

The mass fraction of the primary standard, w_Z and its associated standard uncertainty was calculated from the data of its gravimetric preparation. The mass

fraction of the spike was determined by performing reverse isotope dilution mass spectrometry analysis.

The combined standard uncertainty of the isotope ratio R_{Bc} was calculated by applying the uncertainty propagation law as described in the EURACHEM (2000) guide. The standard uncertainties of the measured isotope ratios R'_B and B'_{Bc} were calculated as the standard deviation of the repeat measurements respectively of the sample blends (n = 4) and the mass-bias calibration blends (n = 5).

The same sample extracts were analysed by both separation methodologies, and therefore variations in extraction efficiency should not influence the comparative data obtained. The use of species-specific IDMS as the calibration method should overcome differences in the results obtained by both methodologies due to sample losses, dilution errors or the derivatisation procedure, all of which may influence results obtained by external calibration techniques, assuming spike equilibration.

Table 4.11 shows the TBT results obtained by both methods for the sediment extracts. A comparison of the mean mass fractions and the associated uncertainties by both methods shows that there is no significant difference in the results. Neither of the mean mass fractions for PACS-2 lies within the certified range for TBT in PACS-2 (980 ± 130 ng/g TBT as Sn). The deviation from the certified value is not relevant here because the aim was a comparison of the methodologies and not an evaluation of the extraction efficiency. In addition, the PACS-2 sediment used for this comparative study was from a bottle that was more than a year old and had been frequently opened.

Analysis by HPLC-ICP-IDMS yielded a mean mass fraction of 828 ± 87 ng/g TBT as Sn, whilst GC-ICP-IDMS analysis resulted in a mean mass fraction of 848 ± 39 ng/g TBT as Sn. For the lower level sediment P-18/HIPA-1 there is also good agreement between the data obtained by both separation methodologies, which in turn agree well with the consensus mean value of 0.680 ± 0.015 nmol/g TBT^+ (80.1 ± 1.8 ng/g as Sn) obtained during the CCQM P-18 study (Sturgeon et al., 2003). The precision (calculated as the percent relative standard deviation from the mean and expanded uncertainties) of the LC-ICP-IDMS measurements in the extracts from both sediment (PACS-2: 10.5%, P-18/HIPA-1: 12.8%) is slightly worse than that achieved by GC-ICP-IDMS (PACS-2: 4.6%, P-18/HIPA-1: 5.1%.). This difference is

a direct result of the differences in the peak area integration characteristics as discussed above.

The comparison of the data obtained by each method for the two different sediment types highlights a decrease in the measurement precision with decreasing TBT content. With a decrease of the TBT content in the P-18/HIPA-1 sediment by more than 10-fold compared to the PACS-2 sample, the measurement precision by LC-ICP-IDMS decreases by 2.3% compared to an equivalent change in the GC-ICP-IDMS measurement precision of only 0.5%. The reason for this difference lies in the greater sensitivity of the GC-ICP-MS approach, which results in the fact that even the TBT peaks for the P-18/HIPA-1 sediment extracts can be integrated with a high degree of reproducibility.

The major contributions to the standard measurement uncertainty associated with the individual results after sample preparation are the ^{120}Sn TBT/ ^{117}Sn TBT isotope amount ratios in the sample blend (R'_B) and in the mass-bias calibration blend (R'_{Bc}). The poorer reproducibility of the peak area integration by HPLC-ICP-MS, resulted in the standard uncertainty estimates obtained being greater than those obtained by GC-ICP-MS.

Table 4.11 TBT data for the analysis of two different sediment samples (PACS-2 and P-18/HIPA-1) by both methods.

All as ng/g Sn[a]	PACS-2[b]	P-18/ HIPA-1
LC-ICP-IDMS	828 ± 87 (10.5)[c]	78.0 ± 9.7 (12.8)[c]
GC-ICP-IDMS	848 ± 39 (4.6)[c]	79.2 ± 3.8 (5.1)[c]

[a]The values represent the mean result and their associated expanded uncertainty with a coverage factor of k = 2.
[b]Old bottle used for comparison
[c]Values in brackets represent relative standard deviations of multiple measurements

Table 4.12 shows data for DBT and TBT determined in a newly opened bottle of PACS-2 by GC-ICP-MS analysis of the ASE extracts obtained using extraction A. The values are contained within the 95% confidence intervals of the certified values and correspond well with other IDMS data published (Encinar et al., 2001/ 2002 b). An independent verification of the accuracy of the ASE approach and the HPLC-ID-ICP-MS method shown was obtained during the P-18 study of the CCQM for the determination of TBT in a blind trial sediment (Metrologia, 2002).

The value obtained by this method 0.657 ± 0.082 nmol/g TBT^+ (expanded uncertainty with a coverage factor of 2), was in very good agreement with the mean result of 0.680 ± 0.015 (one standard deviation) nmol/g TBT^+ obtained by 14 international expert laboratories.

Table 4.12 TBT and DBT determined in PACS-2 by ASE and GC-ICP-IDMS analysis.

Sample	**DBT [a] (ng/g Sn)**	**TBT [b] (ng/g Sn)**
1	1084	885
2	1081	879
3	1086	872
4	1097	869
Mean	1087	876
Expanded uncertainty (k = 2)	77	51

[a] Certified value: 1090 ± 150 ng/g Sn (95% confidence interval)
[b] Certified value: 980 ± 130 ng/g Sn (95% confidence interval)

The comparative data provided for analysis of the same sediment extracts by HPLC-ICP-IDMS and GC-ICP-IDMS shows that the type of chromatographic separation used has no significant influence on the mass fractions determined. Therefore, the GC and HPLC methods can be used as an independent check on one another.

4.4 BEHAVIOUR OF ORGANOTIN SPECIES DURING EXTRACTION AND SAMPLE PREPARATION

The procedure used to extract organotin compounds from the matrix can have a significant impact on the final analytical result for individual species (Pellegrino et al., 2000). Sample preparation is often also the major step of the analytical procedure in terms of time and effort required, particularly when considering conventional non-automated extraction methodologies such as liquid-liquid extractions or soxhlet extractions that can require extraction times from 1 - 24 hours for organotin species (Pellegrino et al., 2000; EDANA 2000).

In order to develop a fast and reliable analytical method for organotin speciation, two semi-automated extraction techniques were used: closed vessel microwave extraction, and accelerated solvent extraction. The instrument used for microwave extractions was capable of extracting up to six samples simultaneously according to user-defined conditions (temperature, time, power, pressure). This makes it a practical technique for the assessment of different extraction solutions, because their performance can be assessed in a single experiment. Compared to microwave extraction, accelerated solvent extraction has the advantage that a higher sample throughput can be achieved due to the automated extraction of up to 24 cells. The extraction solution is collected in glass vials without the need to transfer the solution manually and separate the solid sample from the extraction solvent by centrifugation. This can also result in significant timesaving during the sample preparation and thus simplifies the analytical procedure as a whole. With this instrument, it is possible to extract each sample with a number of extraction steps of varying duration and thus establish an 'extraction profile' of a particular analyte from a matrix sample.

Several certified reference materials (CRMs) and candidate reference materials (Table 4.13) were used to test the robustness of the developed extraction procedures. These were chosen to reflect a variety of environmental conditions such as organotin concentrations, and include sediments and biological materials.

Table 4.13 Materials used for method development experimentation.

Reference number	Matrix	Organotin species	Concentration range	Comments	Source
CRM 477	Mussel tissue	MBT*, DBT*, TBT*, MPhT, DPhT, TPhT,	ng/g (ppb) to µg/g (ppm)	Certified reference material	BCR, Brussels, Belgium
CRM 710	Oyster tissue	DBT, TBT	ng/g (ppb)	Candidate material for 'MULSPOT' certification study	BCR, Brussels, Belgium
PACS-2	Sediment	MBT*, DBT*, TBT*, TPhT	ng/g (ppb) to µg/g (ppm	Certified reference sediment from polluted harbour	National Research Council Canada, Ottawa, Canada
P-18/ HIPA-1	Sediment	DBT, TBT	ng/g (ppb)	Prepared as candidate material by 10 – 15-fold dilution of PACS-2 with clean sediment.	National Research Council Canada, Ottawa, Canada

* Species concentration certified

4.4.1 Closed Vessel Microwave Extraction

4.4.1 a) Stability and Species Integrity of TBT

Initial extraction experiments were based on a published procedure (Arnold et al., 1998), but employed microwave extraction using quartz digestion vessels rather than accelerated solvent extraction. An Anton Paar Physica Multiwave (Perkin Elmer, Beaconsfield, UK) closed vessel microwave system was used for the work described. Extractions were carried out using 10 mL of 0.5M sodium acetate/1.0M acetic acid in methanol with the conditions shown in Table 4.14. Aliquots of the supernatant were diluted five-fold in ultra-pure water for analysis.

Table 4.14 Conditions used with the PAAR Physica Multiwave microwave.

Power (W)	Time (min)	Power (W)	Fan
30	5.0	150	25%
150	35.0	200	25%
0	15.0	0	100%
	Total time: 55.0		

In order to assess the integrity of an analyte during sample preparation, it is common practice to expose aliquots of single compounds to the sample preparation conditions and monitor changes compared to aliquots that have not been exposed to those conditions. These are generally referred to as 'spike recovery experiments' and

can be carried out in the presence or absence of matrix material. An aliquot of a ^{117}Sn enriched TBT standard solution was subjected to extraction in a closed-vessel microwave system whilst another was not. Both were then analysed by HPLC-ICP-MS. The reason an isotopically labelled standard was chosen instead of a commonly available natural abundance TBT standard was that the experiment was less likely to be influenced by contamination when using an enriched standard. Figure 4.10 shows the chromatograms for both aliquots. It is noticeable that this enriched spike contained a small quantity of dibutyltin (retention time: 275s) which is an impurity in the standard.

The ratios between the peak areas of TBT and DBT were measured in the digested and undigested standard TBT solutions in order to determine whether any degradation of TBT had occurred during the microwave extraction process. If significant breakdown of TBT to DBT had taken place during microwave digestion, this ratio would be smaller in the digested spike compared to the undigested one. However, the TBT/DBT ratio was fractionally (1.7%) greater in the digested spike solution which indicates that no significant degradation of TBT to DBT had taken place.

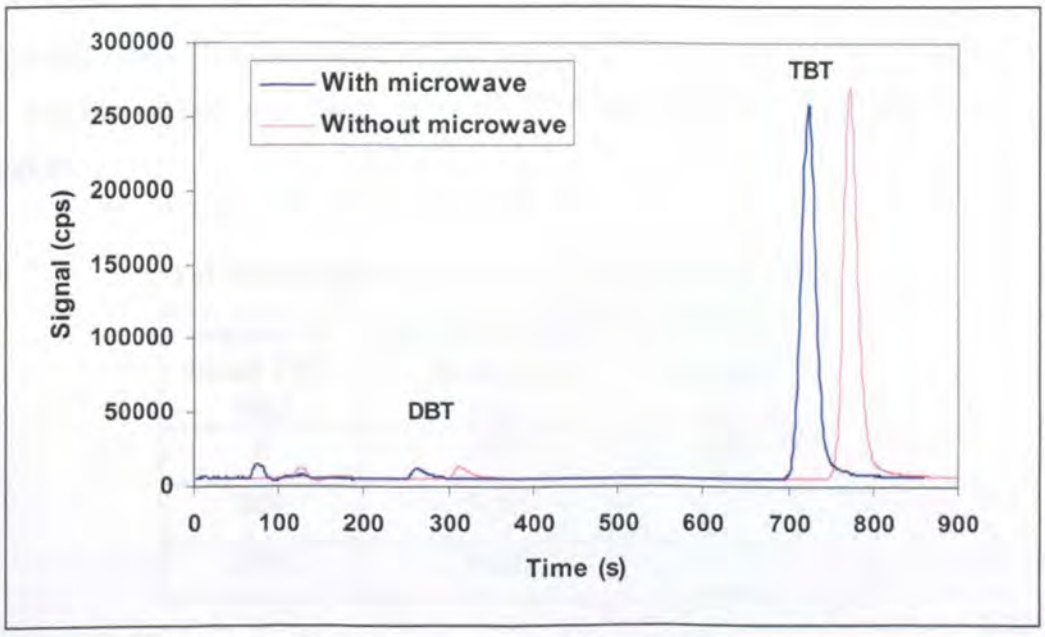

Figure 4.10 HPLC-ICP-MS chromatogram of ^{117}Sn TBT standard after extraction by closed vessel microwave (blue line) and without microwave extraction (red line). The red trace is offset by 50s to show both chromatograms clearly. The DBT peak is due to an impurity in the standard.

There is a slight variation in the peak height of TBT in Figure 4.10 which is probably due to variations in the instrument sensitivity, but the fact that no breakdown of TBT has occurred is demonstrated by the fact that the ratio between TBT and one of its potential breakdown products (DBT) has remained the same. The DBT peak constitutes ~3.1% of the TBT peak in terms of peak area in both standards. If breakdown of TBT had occurred during the extraction then this percentage would be different. Isotopically labelled compounds can also be used to identify species transformations in the presence of other indigenous analytes, which would otherwise make the results of spiking experiments difficult to interpret.

4.4.1 b) Spike Recovery of TBT from Oyster Tissue

The extraction efficiency of the microwave method was determined by spiking two sub-samples of an oyster tissue to one- and two-fold of the sample concentration with a natural isotopic abundance TBT standard in methanol. The mixture was homogenised using an ultrasonic bath and left overnight to equilibrate and evaporate the methanol fraction. After the microwave extraction step, the samples were transferred to plastic 15mL centrifuge tubes, and the digestion vessels and stoppers were washed with two 1mL aliquots of the extractant which were also added to the centrifuge tubes. The samples were then centrifuged for 5 min at 3900 - 4000 rpm and the supernatant was transferred into 40mL pre-cleaned amber glass vials and weighed.

Table 4.15 Spike recovery of TBT from oyster tissue.

Amount of added TBT^+ (ng)	Amount of TBT^+ measured (ng)	Recovery (mean ± 3σ as %)
0	554	104 ± 6
508	1110	
1009	1610	

This spike recovery experiment indicates that 104 ± 6% of the added TBT standard is recovered by this method (Table 4.15). However, the recovery of a superficial spike is a potentially inaccurate reflection of the extraction efficiency because recoveries based on extraction of spiked compounds from a matrix material may not be

representative for extraction of the inherent analytes, which may be bound much more strongly to the matrix.. These data are valuable because they indicate that the added TBT has not been degraded under these conditions.

4.4.1 c) Extraction of TBT from Mussel Tissue

The extraction efficiency was determined using a certified reference material mussel tissue CRM 477 from the EC Reference Bureau. Several aliquots were spiked with a ^{117}Sn TBT enriched standard for isotope dilution analysis (IDMS). After addition of the extraction solution, the samples were ultra-sonicated for 1 min. Following this, microwave extraction was used as described above to extract the TBT from the mussel tissue. Table 4.16 summarises the results obtained. The mean TBT^+ concentration is 1.93 ± 0.14 mg/kg (± one σ). This equates to a mean recovery of 88% ± 6%. Although this value is lower than the certification value (BCR, 1997), it falls within the range of values obtained by laboratories involved in the certification process for CRM 477 (Figure 4.11). The fact that the mean value for TBT obtained here falls outside of the uncertainty limits for the mean certified value, but is contained within the values used for certification of the material for TBT is probably due to the way the uncertainty limits (95% confidence interval) were calculated from the data set.

Table 4.16 TBT^+ recoveries from CRM 477(*).

Extract	TBT^+ (mg/kg)	Recovery (%)	Average recovery (%)
1	2.10	95	
2	1.83	83	
3	1.97	90	
4	1.81	82	**88**

(*) CRM 477 is certified as 2.20 mg/kg TBT^+, uncertainty ± 0.19 mg/kg

Considering that the data were obtained by species-specific IDMS both the accuracy and the precision (7%) of the measurements are relatively poor. This is most likely due to an insufficient period for spike equilibration, as well as variable extraction of TBT from the matrix. The latter is a result of the fact that the mussel tissue settled in

the bottom of the extraction vessels, which led to a reduction in the surface area for interaction between the matrix and extractant.

The difference between the recovery from CRM analysis and the spike recoveries shown in Table 4.15 demonstrate the fact that extraction efficiencies obtained by spiking must be treated with caution because they can vary to a great extent depending on the spiking regime used and the affinity of the analyte for the matrix.

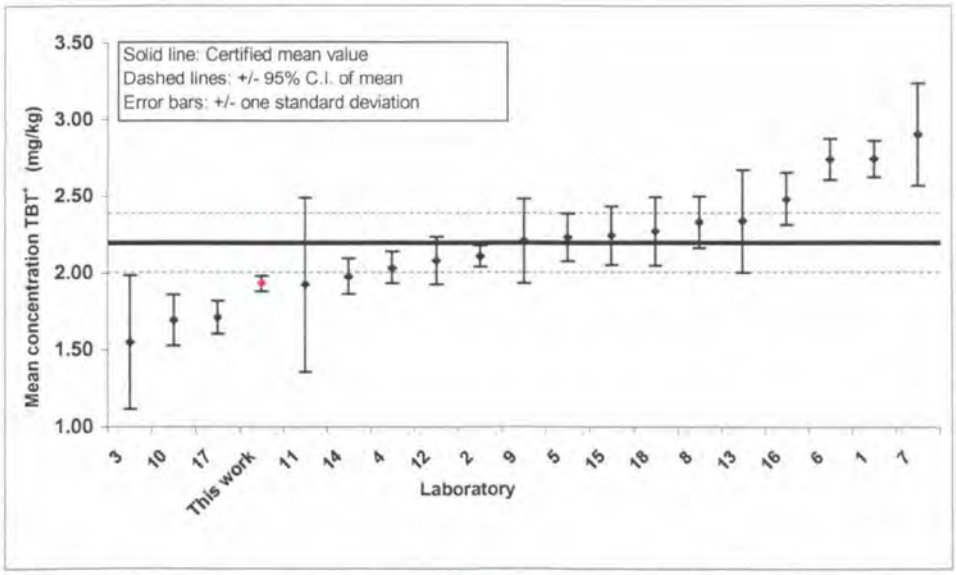

Figure 4.11 Graph showing data from this work (red data point) in relation to the data used for certification of TBT in mussel tissue CRM 477.

In order to determine whether significant amounts of the analyte remained in the matrix after the first extraction, a second extraction was carried out. The residual mussel material from the first extraction was washed out of the centrifuge tubes with 10 mL of the extractant and this solution was transferred to the digestion vessels for a second extraction. After the second extraction, the slurry was again centrifuged. The supernatant was transferred into amber, pre-cleaned 40mL glass vials and weighed. Chromatograms of the first and second extraction steps of CRM 477 are shown in Figure 4.12 a) and b), respectively. The amount of TBT determined in the second extract was ~ 9 % of TBT measured in the first extract. Small peaks were also visible for DBT and TPhT (monitoring the ^{120}Sn isotope), but these were contained within the limit of detection, which was taken as three times the amplitude of base-line noise. The second digestion step should therefore always be included in the method if TBT is the analyte of interest. It is also interesting to note that the ratio between

the enriched ^{117}TBT spike and the inherent analyte change from the first extraction step, where significantly more ^{117}TBT is detected to the second extract, which contains roughly equal amounts of ^{117}TBT and natural TBT. This is a further indication that the spike compound is preferentially removed during the first extraction.

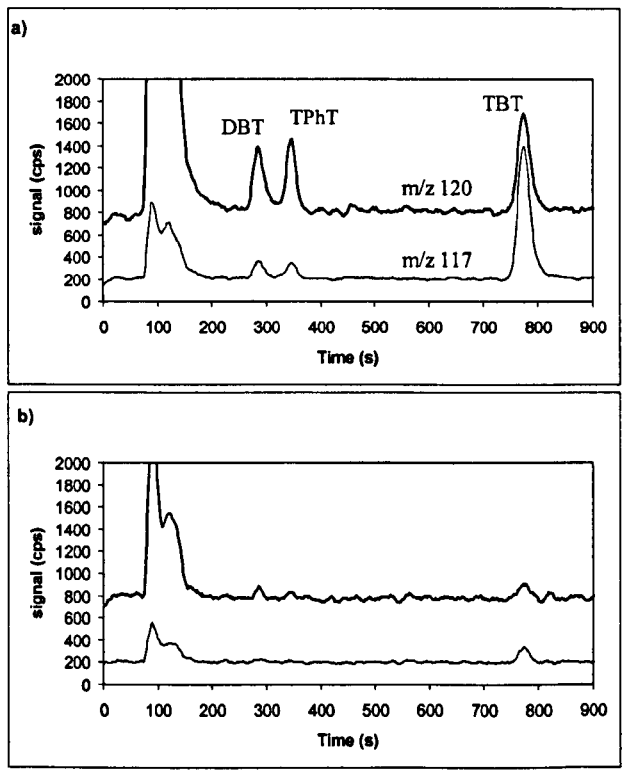

Figure 4.12 HPLC-ICP-MS chromatogram of a) the first extraction of CRM 477 spiked with ^{117}Sn TBT and b) the second extraction of the same sample. Dibutyltin (DBT) at 297s, Triphenyltin (TPhT) at 348s and Tributyltin (TBT) at 776s (^{120}Sn is shown as bold line, ^{117}Sn is shown as regular line width.)

4.4.1 d) Multi-compound Extraction of Organotin Compounds by Microwave Extraction

The initial method development of microwave extraction focussed on the development of an extraction procedure for TBT because this is the organotin compound of the greatest toxicological concern in the environment. However, it is obvious from the analyte peaks in chromatograms such as shown in Figure 4.12 a) that other compounds such as DBT and TPhT were extracted simultaneously. In order to develop a suitable multi-species microwave extraction method, six reagent mixtures reflecting a range of those currently used were chosen from the literature

(Pellegrino et al., 2000) for the assessment of a simultaneous extraction of organotin species:

(a) methanol

(b) 0.5M acetic acid in methanol

(c) 0.5M hydrochloric acid in methanol

(d) 0.5M hydrochloric acid in methanol with 0.03% tropolone

(e) 0.5M sodium acetate 1.0M acetic acid in methanol and

(f) glacial acetic acid

Aliquots (0.2 g) of mussel tissue CRM477 were extracted using a previously published procedure (Yang and Lam, 2001) by closed vessel microwave extraction with 10 mL of each solution for 6 minutes at 450W. This method had been shown to quantitatively extract butyltin compounds from sediments. After the extraction, the samples were transferred to centrifuge tubes and centrifuged for 10 minutes at 4000 rpm. The supernatant was then diluted two-fold in de-ionised water and analysed by HPLC-ICP-MS.

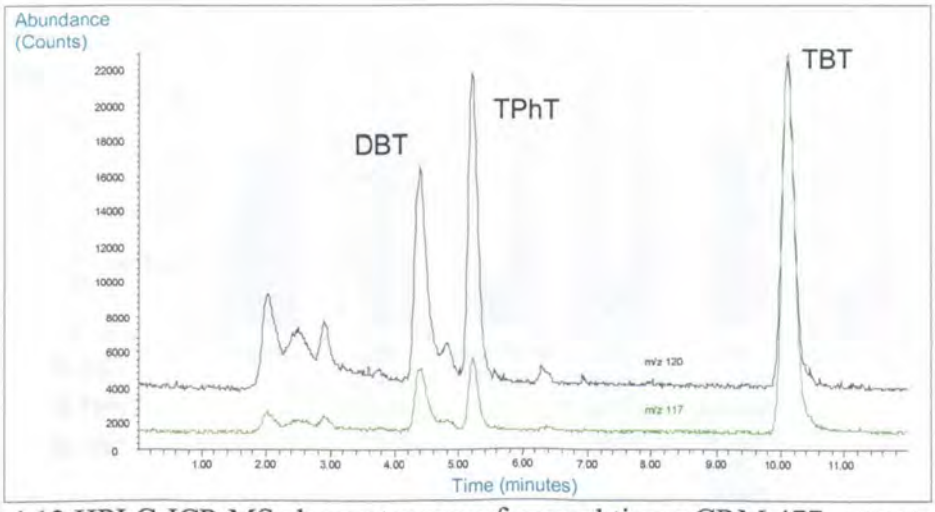

Figure 4.13 HPLC-ICP-MS chromatogram of mussel tissue CRM 477 extracted with 0.5M acetic acid in methanol [solvent (b)].

The chromatography for solvents (b) and (e) is shown in Figures 4.13 and 4.14 and the changing proportions of the major species (DBT, TPhT and TBT) are obvious. Figure 4.15 shows a diagrammatic summary of the data obtained. The bar chart and both chromatograms indicate that there is significant variation in the extraction of the three main analytes depending on the extraction solution used. TPhT recoveries are much lower when hydrochloric or concentrated acetic acid is used, whilst the DBT signal is significantly higher in hydrochloric or highly molar acetic acid extracts than

in the other solutions. The extraction of TBT is subject to much smaller variations than DBT and TPhT.

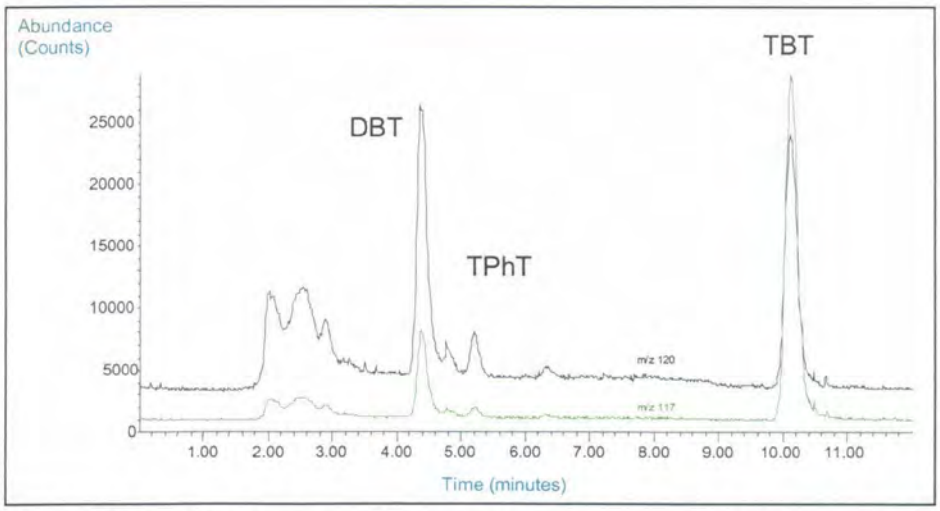

Figure 4.14 HPLC-ICP-MS chromatogram of mussel tissue CRM 477 extracted with glacial acetic acid [solvent (e)].

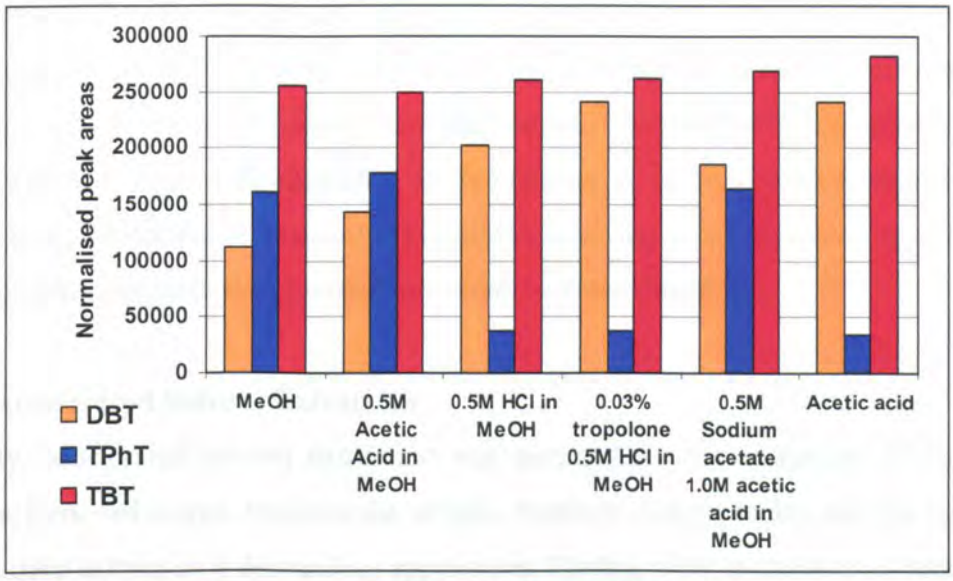

Figure 4.15 Relative responses of DBT, TPhT and TBT after extraction of mussel tissue CRM 477 in a closed vessel microwave (450W, 6 min.) using different extraction solutions.

Increasing the amount of acetate in solution seems to enhance the extraction of the butyltin species from the mussel tissue. When comparing 0.5M sodium acetate/ 1.0M acetic acid in methanol to 0.5M acetic acid in methanol the signal for both DBT and TBT is increased. This has also been described in the literature (Arnold et al., 1998) for sediment extraction by ASE using sodium acetate in a methanolic solution of

acetic acid. The reduced TPhT signals when strong acid mixtures are used are most likely due to breakdown of this species. The breakdown of TPhT in a microwave field has been highlighted in the literature (Donard et al., 1995) and the results presented here suggest that this breakdown predominantly occurs when solvent mixtures containing strong acids are used. This was verified by microwave extraction of a TPhT standard in the presence of glacial acetic acid, which indicated that DPhT, which is one of the possible breakdown products, was present in greater proportions after microwave exposure. The other breakdown products (MPhT and Sn) could not be monitored by the HPLC-ICP-MS method used.

The best compromise for the extraction of the organotin species from this mussel tissue was 0.5M sodium acetate/1.0M acetic acid in methanol for the microwave experiments described above. Although the microwave approach was shown to be capable of extracting several organotin species simultaneously it was not further investigated as a multi-species extraction approach due to several factors. The decanting of the supernatant and sample for centrifugation after the extraction was both time consuming and, more importantly, prone to sample losses by spillages and solvent residues in extraction and centrifuge tubes. The potential for degradation of TPhT was also a great disadvantage as this species is of toxicological significance due to its common use in pesticides. Therefore, attention was focused on accelerated solvent extraction with the potential to overcome these shortfalls.

4.4.2 Accelerated Solvent Extraction

Initially, accelerated solvent extraction was assessed for the extraction of butyltin species from sediments, because the affinity between clay particles and the species makes their extraction a demanding application. Further work on biological materials is described in Sections 4.4.2 b) and 4.4.2 c).

4.4.2 a) Optimisation of Extraction Conditions for Butyltin Species in Sediments

In order to establish optimal conditions for accelerated solvent extraction of organotin compounds from sediments, samples of PACS-2 were extracted under different extraction times, pressures and temperatures. Experimentation with sediment extraction at pressures ranging from 1000psi to 2000psi and with varying

extraction durations (from 5 minutes to 40 minutes) showed that neither of these parameters influenced the extraction of DBT and TBT significantly.

Extraction temperature

In order to determine the effect of the extraction temperature on the recovery of DBT and TBT from sediment, seven separate aliquots of sediment were extracted in duplicate using 1500psi pressure and five separate 5-minute cycles. The recovery of DBT and TBT increased with temperature from 40°C to 100°C (Figure 4.16) and fluctuates at temperatures above 100°C, especially for DBT. It is possible that this was the result of species transformations at high temperatures, namely the de-butylation of TBT to DBT. If this is indeed the case, then the breakdown rates for DBT and TBT appear to be similar, as both analytes displayed similar patterns at temperatures above 100°C. The other breakdown products (MBT and inorganic Sn) could not be monitored using HPLC-ICP-MS and therefore no information was available to confirm whether breakdown of DBT has indeed taken place.

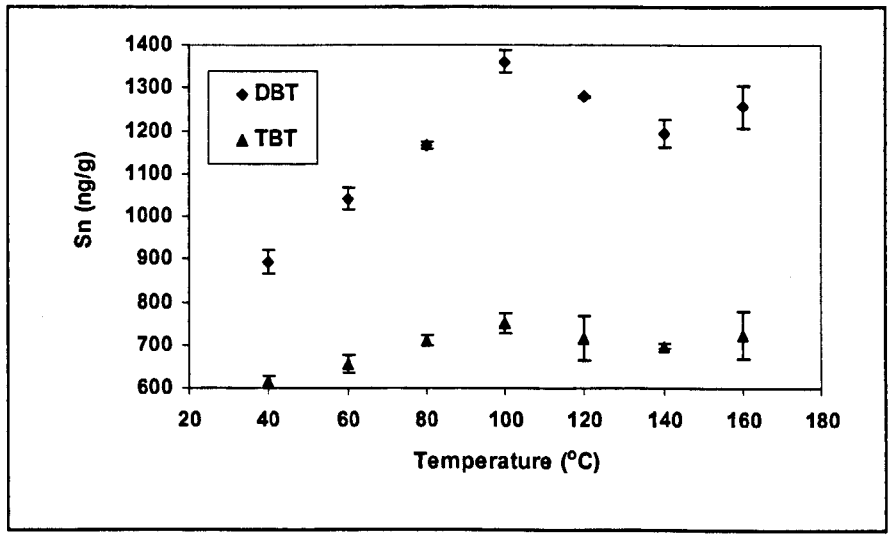

Figure 4.16 Effect of temperature on the accelerated solvent extraction of DBT and TBT from sediment (error bars represent one standard deviation).

During the extraction at and above 100°C, it was noted that the recovery of DBT was ~ 115%, whereas the recovery of TBT was ~ 85% of the certified concentrations. The most likely process for this apparent increase of DBT would be breakdown of TBT to DBT by the loss of a butyl-group during extraction. In order to assess whether this was the case, the sediment was spiked with a known quantity of

^{117}Sn enriched DBT prior to extraction. After extraction, the ratio of ^{120}DBT to ^{117}DBT was measured (Figures 4.17 and 4.18) and was found to be about 10 - 15% greater than the theoretical ratio based on the amount of sample and spike material added.

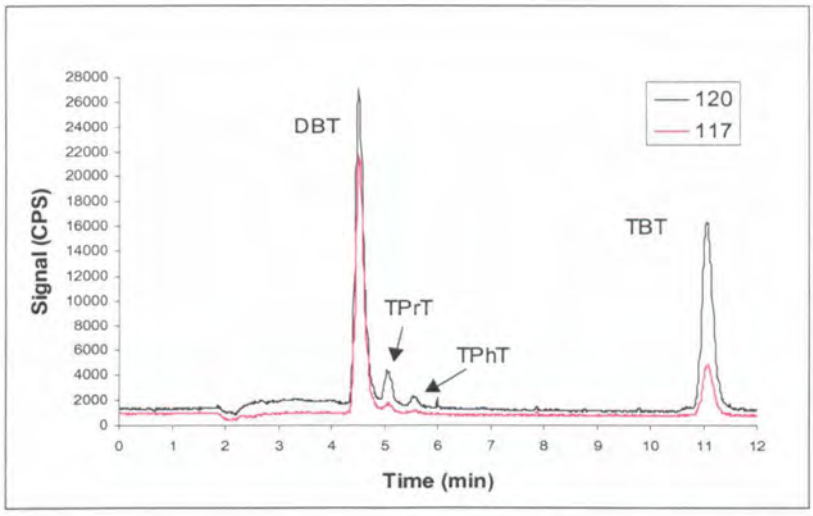

Figure 4.17 Chromatogram of PACS-2 sediment spiked with ^{117}Sn enriched DBT prior to ASE extraction. (The ratio of ^{120}Sn to ^{117}Sn for TBT is the natural, unspiked ratio of 4.2:1, whereas the ^{120}Sn to ^{117}Sn for DBT is close to 1:1.)

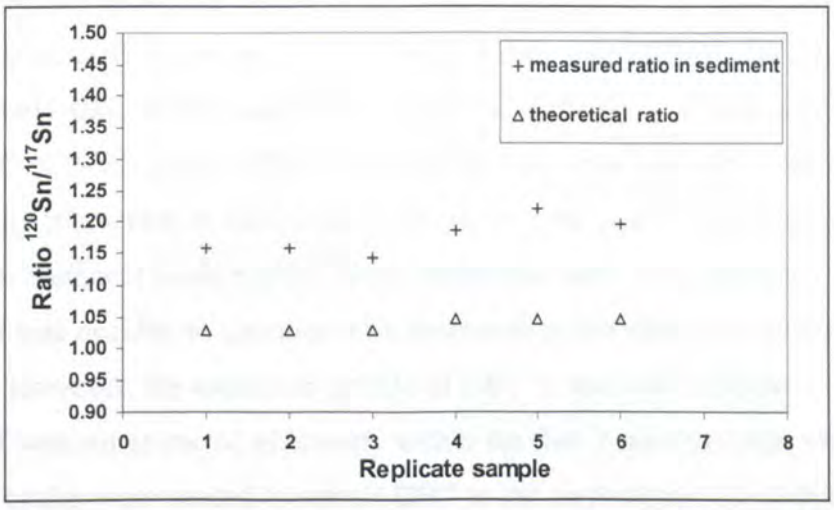

Figure 4.18 Diagram of theoretical and measured ratios of ^{120}Sn to ^{117}Sn of DBT in PACS-2 sediment after ASE extraction. The theoretical ratio is based on the amount of ^{117}Sn DBT spike added to a known amount of sediment containing inherent ^{120}Sn DBT. The measured ratio is that determined by peak area measurements of the extracted sample.

Further experiments indicated that the degradation was most likely due to contact of TBT with the hot cell walls of the stainless-steel ASE extraction cells. The use of PTFE liners inserted into the cells prevented this degradation and this was verified by

measurements of the ratio between DBT and TBT in sediment extractions with and without PTFE liners.

4.4.2 b) Experiments with Different Extraction Solvents for the Extraction of Organotin Species from Sediments and Biological Materials

The solvent mixture containing 0.5M sodium acetate/ 1.0M acetic acid in methanol used previously with microwave extraction caused problems with the ASE instrument. In particular there were leaks on the extraction cell valves due to precipitation of acetate. Species-specific IDMS could be used to correct for losses but the practical drawbacks of using this solvent mixture with ASE meant that it was not suitable for automated high-throughput extractions.

A comparison of hexane and methanol was carried out using ASE. The solvents were chosen because of their different polarities and good compatibility with the ASE instrumentation. The performance was tested by extraction of organotin species from a sample with high lipid and organic carbon content (oyster tissue CRM 710), and a clean sediment containing organotin compounds at environmental background levels (CCQM P-18/ HIPA-1). The analysis of the extracts was carried out by GC-ICP-MS, which allowed for the separation and detection of MBT, MPhT and DPhT in addition to the species separated and detected by HPLC-ICP-MS (DBT, TPhT and TBT). The data indicated that hexane was suitable for extraction of MBT, DBT and TBT in the oyster tissue, but not from the sediment. Methanol could extract these compounds from both matrices.

It was decided to continue with methanol as the main solvent for extractions by ASE. However, the extraction profile of DBT in sediment (Figure 4.19) indicated that DBT was not extracted efficiently within the first 2-minute cycle. More than five separate cycles were needed to extract DBT to the equivalent of the blank level. This was attributed to the greater affinity of this compound to the clay fraction of the sediment compared to TBT. The use of acetic acid has been proposed in the literature (Arnold et al., 1998, Yang and Lam, 2001) to enhance extraction of such compounds from sediments, because acetate can act as a counter ion and enhance the removal of the species. In order to facilitate the extraction of the more polar species such as DBT and MBT, acetate was added to the methanolic solvent in the form of acetic acid for further experiments.

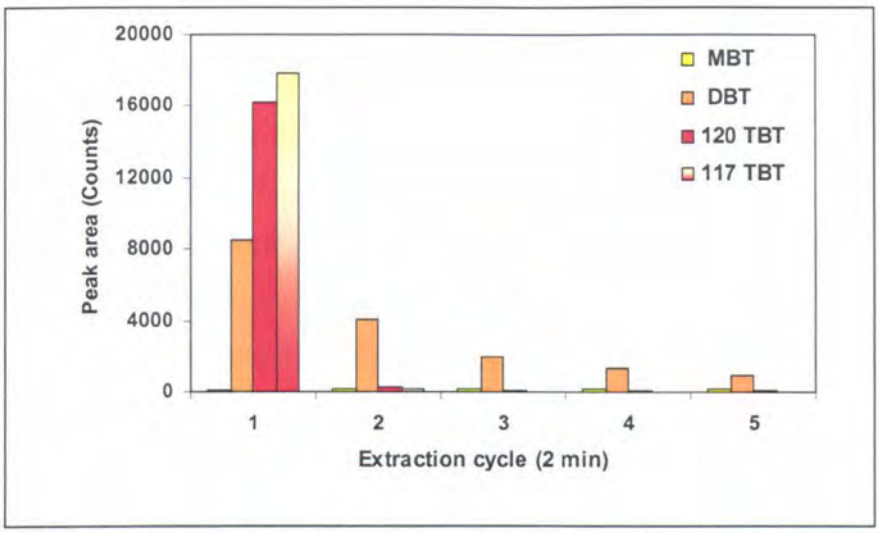

Figure 4.19 Extraction profile of DBT and TBT obtained by accelerated solvent extraction of sediment P-18 with methanol.

Another significant finding of this study was that the spiked ^{117}Sn TBT is extracted with the same efficiency for both matrices as the inherent TBT. This condition is vital for the applicability of IDMS quantitation. The efficiency of this acetic acid-methanol mixture is highlighted in Figure 4.20. This sequential extraction showed that ~ 99% of DBT and TBT were extracted in the first two 1-minute cycles. This explains why there is no further increase in TBT recovery at extraction durations longer than 10 minutes.

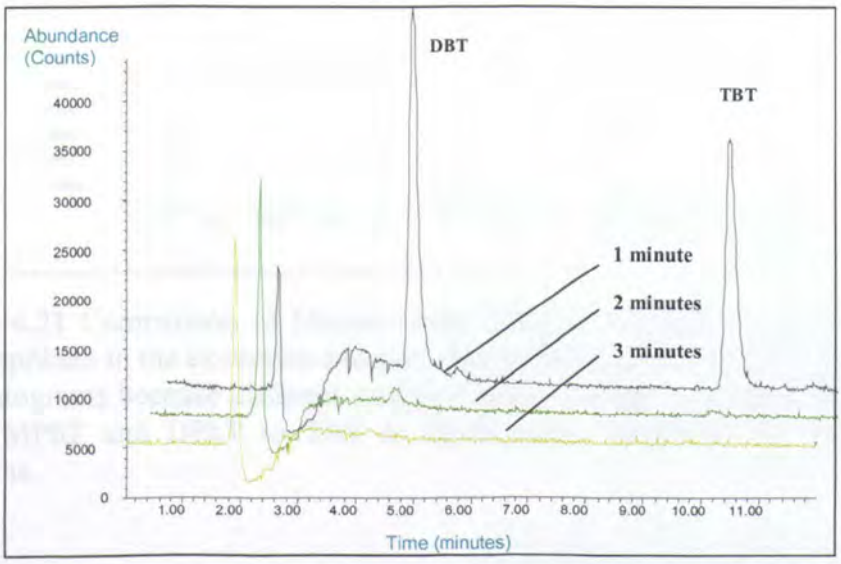

Figure 4.20 HPLC-ICP-MS chromatogram showing the sequential extraction of butyltin compounds from PACS-2 during the first three 1-minute cycles.

4.4.2 c) Extraction of More Polar Mono- and Di-Substituted Species

The analysis of mono-substituted species such as MBT and MPhT has proved to be very challenging and some discrepancy between results by different methodologies has been reported (Figure 2.1; Pellegrino et al., 2000). This is partly due to the fact that the extraction behaviour of the mono-substituted species is different to those of the di- and trisubstituted species, but all of these analytes are usually extracted and analysed by the same experimental procedure. For the extraction of the mono-substituted species the use of tropolone (2-hydroxycyclohepta-2,4,6-trienone), which is a strong chelating agent for Sn and its organometallic molecules has been shown to increase the extraction efficiency significantly (Chiron et al., 2000) and was investigated here. Solutions of 0.5M acetic acid in methanol were prepared with and without addition of 0.1% tropolone for the ASE extraction of six organotin species from mussel tissue CRM 477. The results obtained for this experiment are shown in Figures 4.21 and 4.22 and Table 4.17.

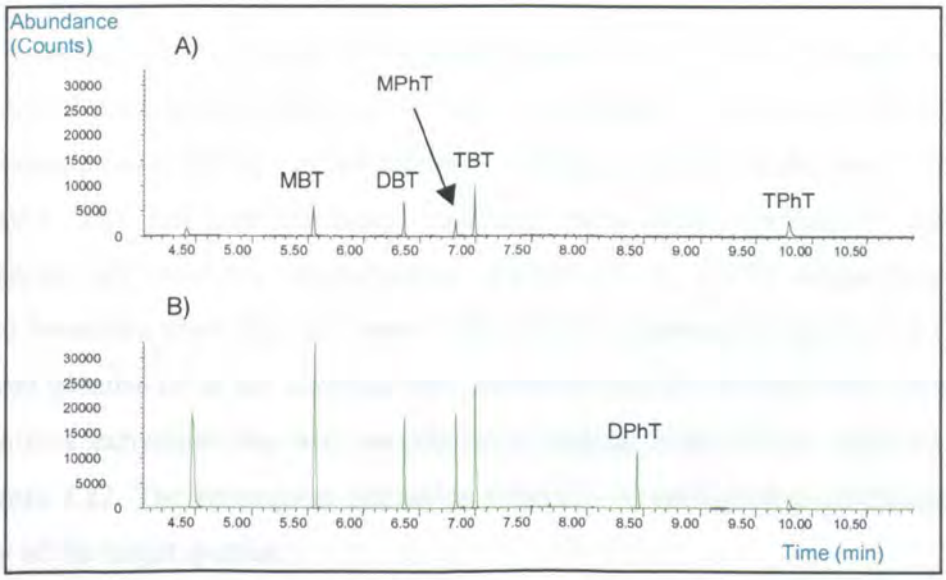

Figure 4.21 Comparison of Mussel tissue CRM 477 extracted A) without and B) with tropolone in the extraction solution. The signal intensity is different between the chromatograms because different sample dilution factors were used, but the ratio of MBT, MPhT and DPhT to TBT is significantly influenced by the addition of tropolone.

The GC-ICP-MS chromatograms shown in Figure 4.21 and the data in Table 4.17 clearly indicate the significant increase in the recovery of MBT, MPhT and DPhT. Although the effect of tropolone on mono-substituted species has been reported, the

increase in the recovery of DPhT was not anticipated. The significant increases in the recovery of some species (MPhT, DPhT and MBT) ranging from factors of 2.1 to 2.8 indicate that the presence of tropolone or a similar chelating agent during the extraction is necessary for optimal recovery of these compounds.

Table 4.17 Data for organotin species extracted from CRM477 with and without the use of tropolone in the extraction solution.

Organotin Compounds (ng/g as Sn)			
	Extraction without tropolone	Extraction with tropolone	Factor change
MPhT	760	1910	2.5
DPhT	40	110	2.8
TPhT	310	290	0.9
MBT	1350	2820	2.1
DBT	460	450	1.0
TBT	680	650	1.0

Further work is needed to elucidate whether the influence of tropolone is due to an actual increase in the recovery of these compounds or whether an improvement in the ethylation efficiency in the presence of tropolone leads to the observed signal increases. The fact that increased recoveries have been reported by analytical techniques not involving derivatisation (Chiron et al., 2000) suggest extraction related benefits rather than the latter. This solvent combination proved to be very efficient because all of the analytes were extracted from the mussel tissue in the first two-minute extraction step with an efficiency ranging from 97% to 100% as shown in Figure 4.22. The subsequent extraction steps (2 - 4) contained a maximum of 3% of any of the target species.

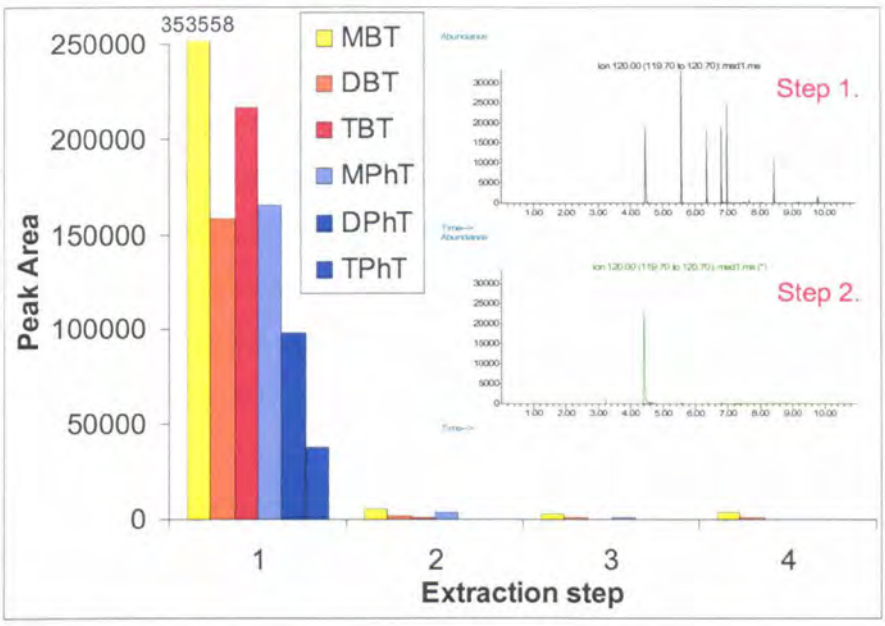

Figure 4.22 Extraction of organotin compounds from Mussel tissue CRM477 with 0.5M acetic acid in methanol containing 0.1% tropolone. The insert shows the chromatograms for the first and second 2-minute extraction steps, highlighting the efficiency of the extraction procedure.

Although the efficiency of extraction of the compounds using the tropolone mixture studied here in combination with accelerated solvent extraction showed significant improvements for the extraction of MBT and DBT, this approach had to be discontinued because the use of tropolone resulted in frequent blocking of the static valve on the ASE instrument. This was probably the result of the chelation of other residual materials by tropolone in the solvent lines of the instrument, and the subsequent accumulation of this material in the valve parts. Even thorough cleaning of the instrument lines did not overcome this problem, and further experimentation with tropolone was therefore abandoned for cost reasons.

4.4.2 d) Recommended Conditions for Accelerated Solvent Extraction of Organotin Species

Based on the experiments described above, the optimal solvent for the extraction of DBT and TBT by ASE was 50% acetic acid in MeOH. The increased proportion of acetic acid compared to the 0.5M concentration previously used negated the need for sodium acetate as a counter ion and overcame problems with leaking of the valves on the extraction cells due to precipitation of the salt.

The extraction conditions proposed are: 5 consecutive cycles of 2 - 3 minute duration, a temperature of 100°C and an extraction pressure of 1500psi. The use of PTFE liners is recommended to avoid thermal degradation of TBT to DBT or MBT. The suitability of this approach was confirmed by the analysis of the certified reference sediment PACS-2. The results obtained by GC-ICP-IDMS were 1087 ± 77 ng/g Sn for DBT and 876 ± 51 ng/g Sn for TBT (expanded uncertainties with a coverage factor of 2), which are in good agreement with the certified values of 1090 ± 150 ng/g Sn and 980 ± 130 ng/g Sn respectively.

4.4.3 Derivatisation of Organotin Compounds

Most organotin species are not volatile in their molecular form, and therefore cannot be analysed directly by gas chromatographic techniques. Different derivatisation techniques including pentylation with Grignard reagents (Gui-bin and Qun-fang, 2000) and ethylation (Schubert et al., 2000) or propylation (Bergmann and Neidhart, 2001) in aqueous media have been described in the literature to convert organotin species into a volatile form. A derivatisation technique, which is increasingly being used in analytical laboratories for many organometallic compounds, is ethylation with sodium tetraethylborate ($NaBEt_4$, STEB). This is usually performed at pH values between 4 - 5 in aqueous media. The derivatisation step forms an integral part of the sample preparation, and the efficiency of derivatisation for the individual species has to be assessed to establish a quantitative procedure.

4.4.3 a) Derivatisation Behaviour in Matrix-free Multi-Species Standard Solutions

In order to assess the derivatisation behaviour of MPhT, DPhT, TPhT, MBT, DBT and TBT with sodium tetraethylborate under conditions where other matrix components could not interfere with the ethylation, an experiment was set-up using a mixture of these compounds in methanol. A stock standard was made up as a methanolic solution containing the species in the proportions shown in Figure 4.23.

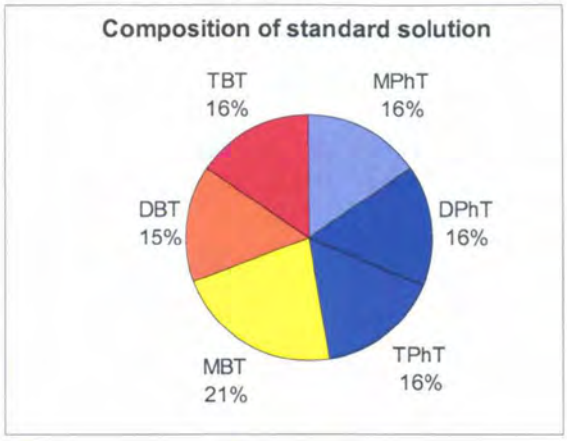

Figure 4.23 Composition of the matrix free stock standard by weight of Sn.

Different amounts of this stock standard were then weighed into separate vials to yield total amounts of the organotin compounds ranging from 100 ng to 1700 ng (Table 4.18). Each of these solutions was derivatised using 1 mL of 2% w/w $NaBEt_4$ and shaken with 2 mL of hexane for 10 minutes. The hexane fractions were transferred to GC vials for analysis by GC-ICP-MS.

Table 4.18 Weights of stock standard, sodiumtetraethylborate and hexane used.

Total weight of organotin compounds for derivatisation (ng)	Weight of $NaBEt_4$ (g)	Weight of hexane (g)
100	0.9947	1.3144
200	0.9998	1.3287
500	1.0076	1.3200
1000	0.9976	1.3120
1700	0.9872	1.3075
Mean	**0.9974**	**1.3165**
RSD (%)	**0.7**	**0.6**

The results for the analysis of the different derivatised standards are shown in Figures 4.24 and 4.25.

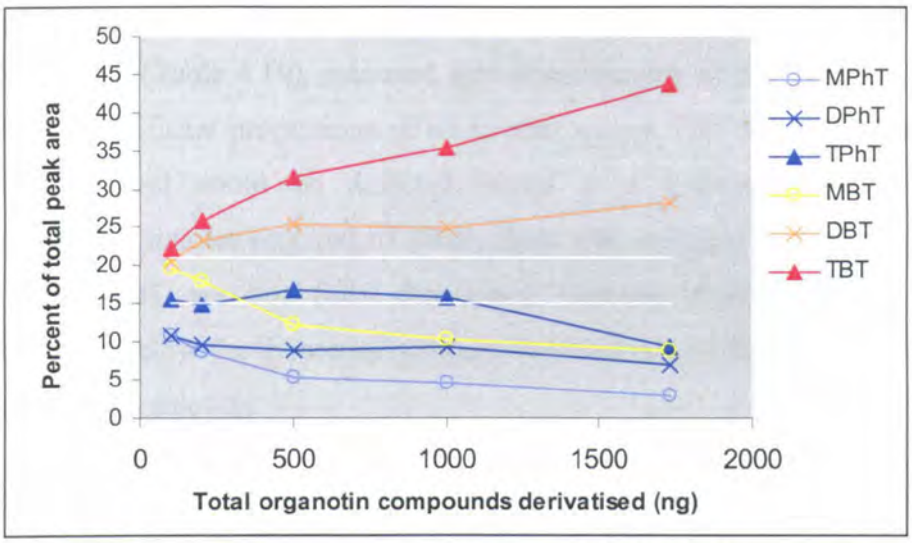

Figure 4.24 Peak area percentage contribution of individual organotin species in derivatised standard solutions. The white lines indicate the maximum and minimum gravimetric contributions in solution.

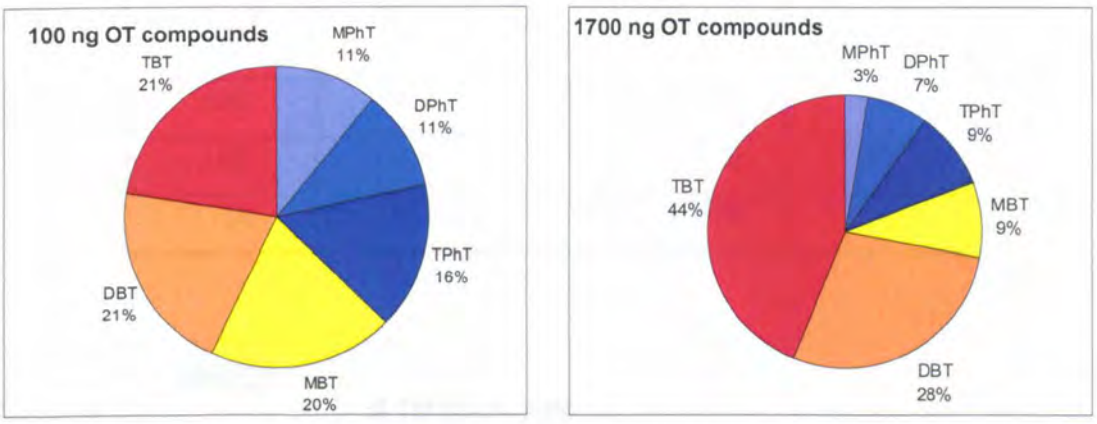

Figure 4.25 Peak area percent contribution of individual species determined in the standard solution containing 100 ng and 1700 ng of organotin species.

The patterns shown in Figures 4.24 and 4.25 are based on the percentage of the peak area of individual compounds compared to the total peak area for each chromatogram. The figures show that the relative proportions of the ethylated species are changing as the amount of organotin compounds increases from 100 ng to 1700 ng. The relative proportions of TBT and DBT are increasing, whereas the proportions of MBT and the phenyltin species are decreasing below their respective theoretical contributions. It is most likely that this is either due to a) a preferential saturation of the hexane layer with derivatised organotin species of TBT and DBT; or b) a preferential ethylation of TBT and DBT over the other species present in solution.

Although the amount of $NaBEt_4$ used was always in excess of the theoretically required amount (Table 4.19), a second derivatisation step of the 1700 ng standard showed that significant proportions of all species except TBT were still derivatised (Figure 4.26) and could be detected. Even at a theoretical ratio between nmoles supplied/ nmoles required of 300:1, there was evidence that species such as MPhT and DPhT are not fully derivatised, because their measured relative proportions are below the theoretical proportion based on the gravimetric amount of species present in solution.

Table 4.19 Amount of organotin compounds (nmoles) in each standard solution and nmoles of ethylgroups required for derivatisation.

Amount organotin (ng)	Amount organotin (nmoles)	nmoles required	nmoles supplied	Ratio supplied/ required
100	0.8	1.7	520	300
200	1.7	3.5	520	150
500	4.2	8.7	520	60
100	8.4	17	520	30
1700	14.6	30	520	20

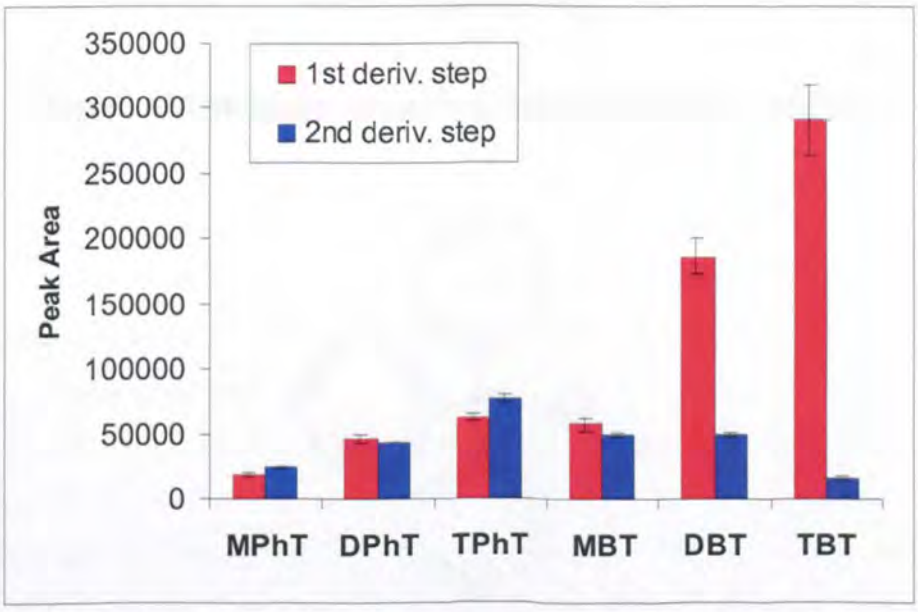

Figure 4.26 Derivatisation of organotin species present in 1700 ng standard in two consecutive reaction steps with 1 mL of 2 % w/w $NaBEt_4$ each.

The pattern of ethylation efficiency that is indicated in Figure 4.26 is TBT> DBT> MBT> TPhT> DPhT> MPhT. This is perhaps surprising as it might be expected that the order would be based on the number of ethylgroups needed for full derivatisation, i.e. tri-substituted compounds > di-substituted compounds > mono-substituted compounds. The fact that this appears not to be the case could be an indication that the molecular structure may have an influence on the derivatisation chemistry due to stearic hindrance.

The structures for TBT and TPhT are shown as their chlorides in Figure 4.27 and 4.28 respectively. During derivatisation of these compounds the molecular ion (e.g. TBT^+) reacts with an ethylgroup (CH_2CH_3) from the sodium tetraethylborate derivatising agent $NaBEt_4$ (Figure 4.30). The ethylgroups provided can probably react more easily with a Sn atom that has straight alkyl chains such as butylgroups ($CH_2CH_2CH_2CH_3$) attached rather than phenyl-rings, which may shield the central Sn-atom by their spatial arrangement around it.

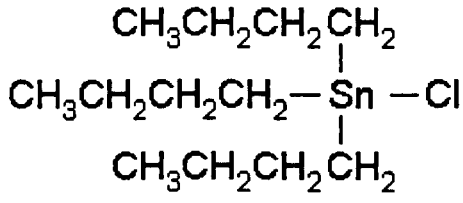

Figure 4.27 Molecular structure of Tributyltinchloride (TBTCl).

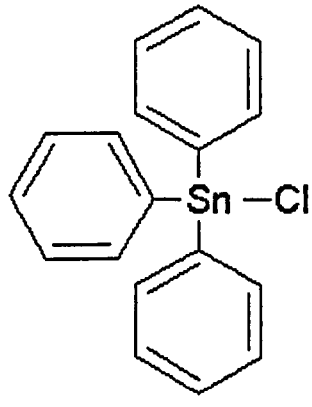

Figure 4.28 Molecular structure of Triphenyltin (TPhTCl).

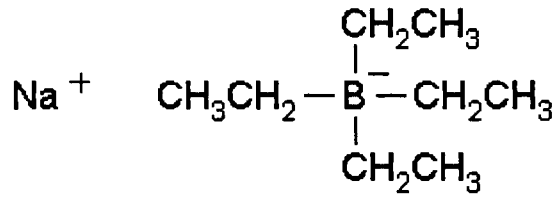

Figure 4.29 Molecular structure of sodium tetraethylborate $NaBEt_4$.

4.4.3 b) Verification of the Derivatisation Efficiency in Matrix Samples using IDMS

An assessment of the derivatisation efficiency in environmental matrix samples that contain components, which may also react with the derivatising agent can be carried out in several ways. One is to quantitate the analytes of interest by a method that does not require derivatisation using conventional calibration curves. Alternatively, species-specific isotope dilution can be used directly to assess the derivatisation of a GC based method. The data generated by IDMS is compared to data obtained by external calibration for the same sample. If the derivatisation efficiency is sufficient, both approaches will give comparable results within the precision of the measurement. However, if the derivatisation efficiency for an analyte in a particular matrix is low, there will be a significant discrepancy in the results, because the data obtained by conventional calibration would show a low bias compared to the IDMS data. This is the case if the analytes in the calibration standard are fully derivatised because no matrix components interfere with the process, whereas reactions other than derivatisation of the target analyte can occur in the matrix samples. The IDMS data will not be affected by an inefficient derivatisation, because the spike material and the inherent analyte are both derivatised with the same yield and the ratio therefore remains the same.

Data for such a comparative derivatisation experiment using IDMS for DBT, TPhT and TBT in mussel tissue CRM477 are shown in Table 4.20. The data indicate that TPhT and TBT are effectively derivatised at the levels present in the mussel tissue CRM, because there is no significant difference in the results obtained by external calibration and IDMS for these two species. This indicates that the derivatisation step does not alter the species or affect the quantitation in any way.

The data for DBT on the other hand shows a significant difference between the result obtained by external calibration 460 ± 30 ng/g Sn compared to 560 ± 30 ng/g Sn by IDMS. The fact that the result obtained by external calibration is low compared to the IDMS reference value gives an indication that the derivatisation of this compound may not have been complete in the sample. The total amount of organotin compounds in this mussel tissue is about 4900 ng/g Sn. Inorganic Sn as well as other organometallic species and matrix components may be responsible for the insufficient derivatisation of DBT.

Table 4.20 Comparison of results obtained by external calibration and isotope dilution analysis (IDMS) for organotin species in mussel tissue CRM477 (n = 3).

Species	External calibration (ng/g Sn ± Std Dev.)	IDMS (ng/g Sn ± Std Dev.)
TPhT	300 ± 15	310 ± 5
DBT	460 ± 30	560 ± 30
TBT	660 ± 40	700 ± 5

4.5 SUMMARY

An existing LC-ICP-MS method was assessed and improved after the instrumental impact of non-chromatographic parameters on the separation performance of up to six organotin species were investigated. Significant improvements were made to the resolution, column efficiency and time-efficiency of the LC-ICP-MS approach after a different stationary phase material was used.

The performance of this methodology was compared to a GC-ICP-MS approach, which was adapted from an existing methodology for the use of a newly available commercial ICP-MS interface. The detection limits for up to 12 organotin species were lowered significantly by addition of optional gases such as oxygen and nitrogen. This development allowed the subsequent detection of species at environmental background concentrations at the sub- part per billion level. A comparison of GC and LC separations coupled to ICP-MS indicated that LC-ICP-MS was the more time-efficient and practical approach, but suffered from lower resolution, inferior sensitivity and more limited analyte capabilities.

GC-ICP-MS provided superior isotope ratio measurement precision by species-specific isotope dilution analysis. This was due to the greater precision in the

integration of peak areas which is related to both the peak shape and signal to noise ratio by this method. Because of the greater reproducibility of the peak area integration with GC-ICP-MS, the typical isotope amount ratio precisions achieved by GC-ICP-IDMS were superior by 1.5 to 2-fold compared to HPLC-ICP-IDMS. This difference was reflected in the expanded uncertainty associated with the final results, which was greater by HPLC-ICP-MS (10.5 - 12.8%) compared to GC-ICP-IDMS (4.6 - 5.1%) for analysis of the same extracts. The accuracy of both methods was in good agreement with each other and certified reference materials for the determination of TBT in sediment. GC-ICP-MS analysis yielded greater sensitivity with method detection limits based on sediment analysis (0.03 pg TBT as Sn) being two orders of magnitude better than achieved by HPLC-ICP-MS.

In an attempt to develop a time-efficient extraction methodology for organotin species, microwave extraction and accelerated solvent extraction were investigated due to their potential for fast and semi-automated extraction. A closed vessel microwave extraction method was developed which was capable of recovering close to 100% of TBT spiked to an oyster tissue and ~ 88% of TBT in a mussel tissue CRM. However, it was shown that TPhT was degraded under microwave conditions when solutions containing concentrated acetic and hydrochloric acid were used. Accelerated solvent extraction was further investigated in order to overcome the shortfalls of the microwave approach. During these studies, it was found that TBT degraded above temperatures of 100 - 110°C and this was verified by monitoring the isotope ratios of DBT in a sediment CRM spiked with a ^{117}Sn labelled DBT standard. This degradation could be avoided by the use of PTFE liners inserted into the ASE extraction cells, which minimised contact between the analytes and the heated stainless steel walls of the cells.

Experiments were carried out with a variety of solvent mixtures in order to establish the best compromise between simultaneous and efficient extraction of multiple organotin species and the practical applicability for high sample throughput extractions. The use of sodium acetate and tropolone in conjunction with acetic acid and methanol had been previously investigated in the literature, but it was found that sodium acetate lead to leaking of the extraction cells due to precipitation of the salt on the cell valves. This usually occurred within extraction of 4 - 6 samples and led to abortion of the instrument run, which meant that high sample throughput was

unreliable. Tropolone was shown to significantly increase the extraction of MBT, MPhT and DPhT, but also led to reactions with residual materials in the solvent lines of the ASE resulting in blockages of internal valves, which had to be replaced. This was both impractical and costly and the use of tropolone was discontinued. The best compromise for practical and efficient extractions was achieved using 50% acetic acid in methanol.

The derivatisation of six organotin species with a commonly used derivatising agent (sodium tetraethylborate) was examined using matrix-free calibration standards and matrix samples. This research showed that the procedure was more suitable for butyltin species than for compounds containing phenyl rings in their structure. This may be due to stearic hindrance. The derivatisation efficiency was tested for DBT, TBT and TPhT by a comparison between quantitation of these analytes in mussel tissue by external calibration and species-specific isotope dilution analysis. This showed that only DBT was not fully derivatised in the mussel tissue extracts.

CHAPTER 5

ARSENIC SPECIATION

5.1 DEVELOPMENT OF LIQUID CHROMATOGRAPHIC CONDITIONS FOR THE SEPARATION OF AS-SPECIES WITH ICP-MS DETECTION

A variety of different liquid chromatography conditions were selected from the literature for the evaluation of their separation capabilities of As-species with ICP-MS. Quadrupole ICP-MS was chosen as the detector for this work due to its low limits of detection and good compatibility with ion-exchange liquid chromatography. The evaluation was carried out using matrix-free calibration standards containing Arsenobetaine (AsB), Dimethylarsinic Acid (DMA), Monomethylarsonic Acid (MMAA) and Arsenite (As(III)). Several of the separation procedures proposed in the literature, which made use of gradient elutions (Thomas et al., 1997; Lindemann et al., 1999; Jackson and Bertsch, 2001) could not be replicated successfully for this work, as described below. Although some As-species can be present at ppm level in marine tissues, the detection of many of the minor species requires instrumentation capable of detection limits at or below the part-per-billion level.

5.1.1 Preliminary Chromatographic Development

The benefit of gradient elution profiles generally lies in enhanced separation of arsenicals in different matrices, and the subsequently smaller risk of co-elution and mis-identification and erroneous quantitation. The chromatography for a deionised water blank by one gradient elution approach is shown in Figure 5.1. Significant variations in the baseline signal and a marked signal increase were observed at the point where the greater concentration of HNO_3 elutes from the column. The signal variation and the 'peak' point are probably due to solubilisation of contaminants from metal components of the HPLC system with increasing acidity of the mobile phase used. The separation of As-species in an oyster tissue extract using this chromatography (Figure 5.2.) was not sufficient in terms of resolution and peak shape, and this approach was not investigated further. Instead, a combination of two different isocratic separation methods was chosen in order to minimise possible analytical errors due to co-elution of compounds.

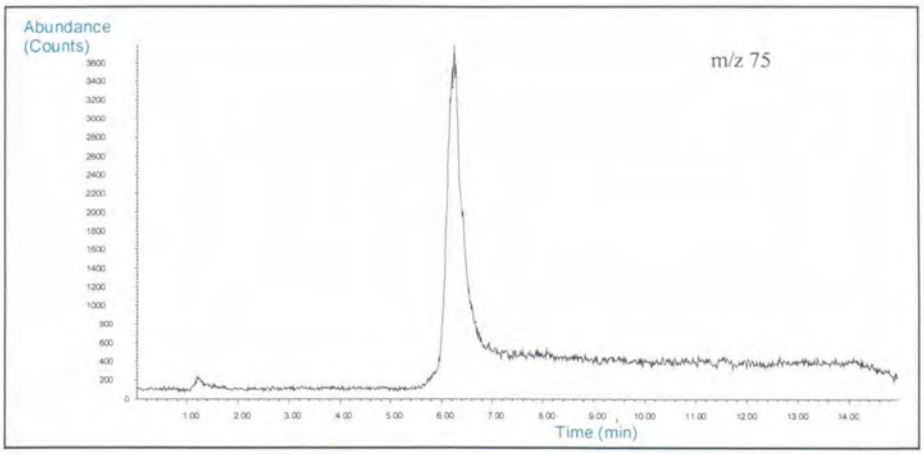

Figure 5.1 HPLC-ICP-MS chromatogram obtained for m/z 75 after injection of a deionised water blank on a Dionex AS-7 column with a 0.5mM – 50mM HNO_3 gradient elution.

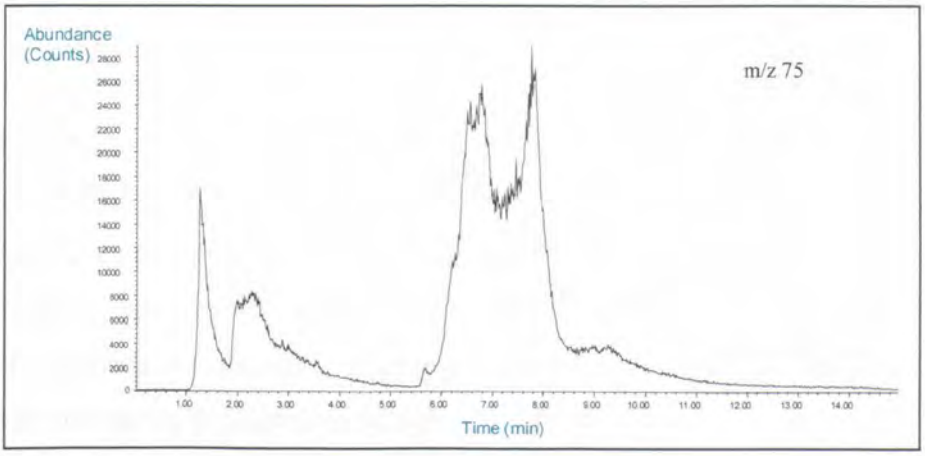

Figure 5.2 HPLC-ICP-MS chromatogram obtained for m/z 75 after injection of an extract of oyster tissue BCR 710. Dionex AS-7 column with a 0.5mM – 50mM HNO_3 gradient elution.

The first method was developed on the basis of the approach used by Lindemann et al. (1999), except that a Hamilton PRP-X100 ion exchange column was used instead of Dionex IonPak14. The mobile phase consisted of 2.2mM NH_4HCO_3/2.5mM Tartaric acid with 1% MeOH at pH 8.2, and was delivered at a flow-rate of 1 mL/min isocratically. The second approach was used as described by Zheng and Kosmus (1998), except that two PRP-X100 columns were installed in sequence to enhance resolution between AsB and DMA at higher concentrations. The chromatograms for both separations are shown below for calibration standards (Figures 5.3 and 5.4).

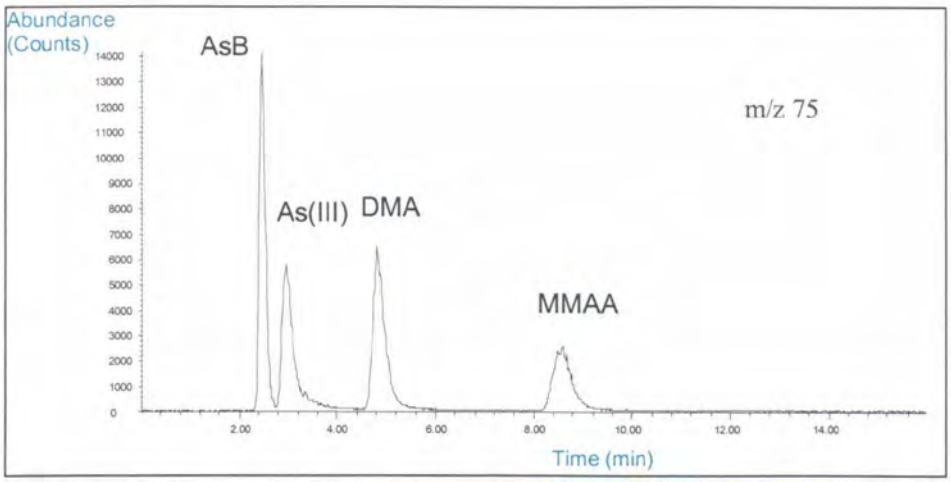

Figure 5.3 Chromatogram of an As standard mixture (~ 5ng/g as As) using Chromatography A: 2.2mM NH_4HCO_3 2.5mM Tartaric acid, 1% MeOH, pH 8.2, Hamilton PRP X-100 column.

The difference between the two separations is obvious from the fact that the order of elution has changed from AsB, As(III), DMA, MMAA using chromatography A to AsB, DMA, MMAA, As(III) using chromatography B. These chromatographies could therefore be used in a combined approach to overcome problems with interferences or insufficient resolution of particular analytes encountered with any one of the methods. An example of an application where the use of complementary methods is mandatory is illustrated in Section 5.1.4.

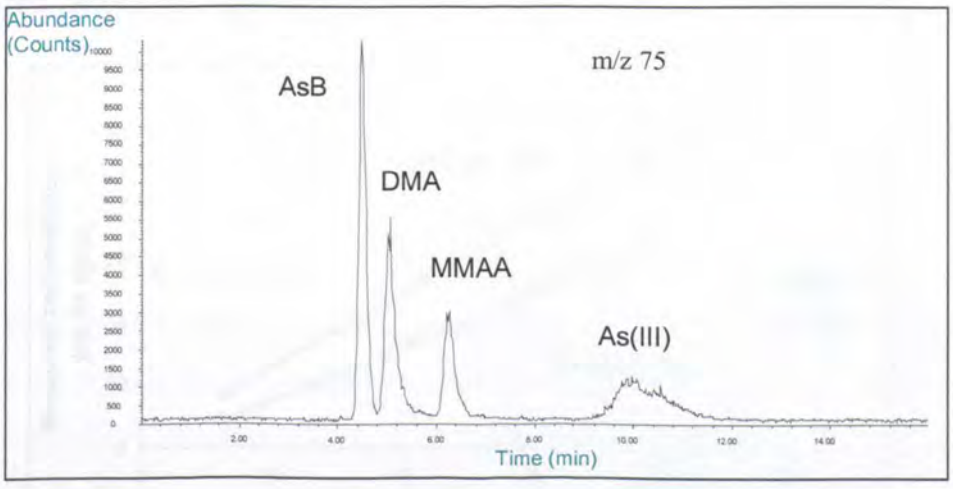

Figure 5.4 Chromatogram of an As standard mixture (~ 5ng/g as As) using Chromatography B: 15mM tartaric acid, 1% MeOH, pH 2.9, two Hamilton PRP X-100 columns in sequence.

For further work Chromatography A was predominantly used, because the peak shapes were better for all analytes and because of the better resolution between AsB and DMA. This was particularly important in samples of marine origin containing part-per million levels of AsB as well DMA, where the AsB peak may potentially mask the DMA peak or prevent its accurate integration.

5.1.2 Evaluation of Commercially Available Calibration Standards for Arsenic Speciation

Di-Sodium hydrogen arsenate heptahydrate ($AsHNa_2O_4.7H_2O$) ≥ 98.5%, Sodium (meta)arsenite ($AsNaO_2$) ≥ 99.0% and Cacodylic Acid (dimethylarsinic acid, DMA) $C_2H_7AsO_2$ ≥ 99.0% were obtained from Fluka (Sigma-Aldrich Co., Gillingham, UK). Arsenobetaine (AsB, $C_5H_{11}AsO_2$) 97% and Monomethylarsonic acid di-sodium salt (MMAA, $CH_3AsNa_2O_3$) > 98% were obtained from Argus Chemicals (Vernio, Italy). A second AsB standard was obtained from BCR (Brussels, Belgium) as a solution of AsB in water at 1031 ± 6 (95% C.I.) mg/kg.

The AsB standards from different suppliers were compared. The AsB from Argus Chemicals was in crystalline form and the BCR AsB was diluted in ultra-pure water. Aliquots of both were dissolved gravimetrically to ng/g level in 1% v/v HNO_3 and measured against an inorganic As-standard in 1% v/v HNO_3 by ICP-MS. The total As signal obtained by both standards showed a significant difference in response as shown in Figure 5.5.

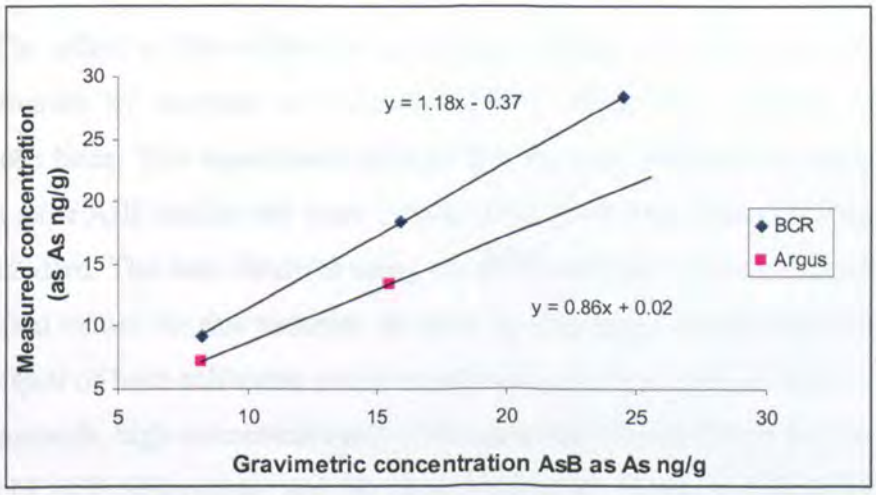

Figure 5.5 Difference in the total As measured in AsB standard solutions from two separate suppliers diluted to similar gravimetric concentrations as As.

This indicates that the total As response obtained by the BCR standard is ~ 13% - 17% higher than expected according to the gravimetric dilution, whereas the response obtained by the diluted Argus Chemicals standard is ~ 14% below the expected concentration based on the gravimetric dilution. A similar effect was observed when both standards were used for quantitation of samples by the developed LC-ICP-MS methodologies. Calibration curves based on gravimetrically prepared standards of AsB as As from both sources are shown in Figure 5.6 and the slopes of both calibrations lines indicate that the average response of the BCR standard is up to 40% greater than that of the Argus calibrant.

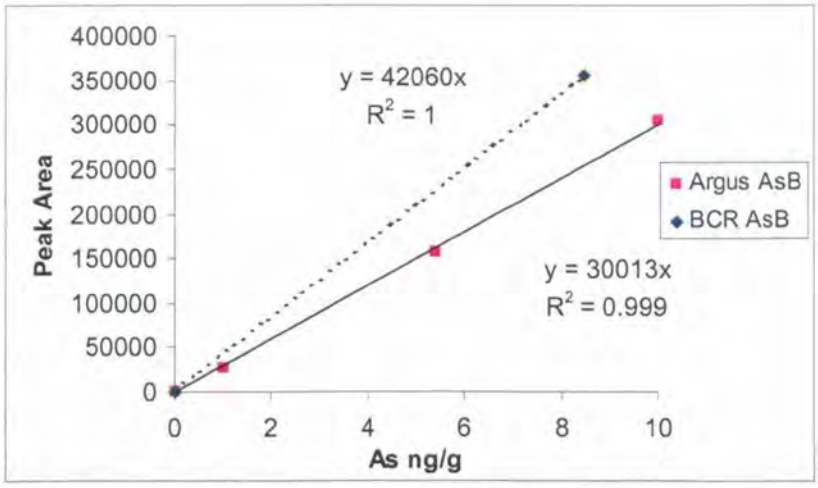

Figure 5.6 Calibration lines obtained by measuring the LC-ICP-MS peak areas for gravimetrically prepared calibration standards of AsB from Argus Chemicals and the BCR.

The effect of this difference on the quantitation of AsB in sample extracts was evaluated by analysis of Tuna-fish CRM (BCR 627) extracts using both calibrations lines. This experiment showed that the data obtained by using the BCR calibrant gave AsB results that were 20% to 36% lower than those obtained with the Argus standard. The data obtained using the BCR calibrant were within the range of the certified values for this material. In order to determine whether the difference in the As signal of both calibrants could be attributed to impurities of other As species in the standards, high concentrations (> 500 ng/g As) of both standards were injected onto the LC-ICP-MS system, and the chromatographic traces monitored for other As species. This showed that the difference was not due to impurities of As species, such as AsIII, DMA or MMA that the methods could separate and detect. Another

possibility could be non-As containing impurities or the absorption of moisture by the AsB granules of the Argus standard during storage, delivery and preparation. The BCR solution may have been subject to evaporation losses during storage or the preparation of the calibration solutions although this would be considered to be minimal, because the solution was stored in a fridge at +4°C when not in use. Further investigation and comparison with standards from other sources is needed as such a difference in response has an obvious influence on the accuracy of the analytical result. The BCR AsB was used for all further work because it was considered the more reliable material due to the accuracy of the analysis of the Tuna fish CRM (BCR627).

5.1.3 Comparison of Different Calibration Techniques for Quantitation of Arsenic Species

Due to the fact that arsenic is mono-isotopic, isotope dilution analysis cannot be used for the high-accuracy quantitation of this compound by LC-ICP-MS. In such circumstances, calibration by standard addition is often used in order to achieve matrix matching of standards and samples to the highest degree possible, and in order to avoid differences in signal intensities of standards and analytes caused by matrix components. In addition, it is also a useful technique for chromatographic applications where the possibility of retention time shifts of analytes in the sample due to matrix components exists. This could result in misidentification and thus erroneous results. However, standard addition calibration can be very time-consuming, because several aliquots of the sample have to be spiked with different levels of a calibration standard, and at least three levels of standard addition are needed for accurate quantitation of the same sample. External calibration by non-matrix matched standards can be used for applications where the difference in the matrix between samples and standards does not influence the accuracy of the result to a significant extent. This technique is useful for analysis of large batches of samples bracketed by injections of standards making up the calibration curves.

Standard addition calibration and non-matrix matched external calibration were compared using Chromatography A for AsB and DMA in methanolic ASE extracts of two certified reference materials (DORM-2, dogfish muscle and BCR627, tuna fish) in order to assess whether the calibration technique used significantly

influenced the accuracy or precision of the analytical result. Tables 5.1 and 5.2 summarise the data obtained by both calibration techniques for AsB and DMA in the certified reference materials DORM-2 and BCR627 respectively.

Table 5.1 Comparison of standard addition calibration and non-matrix matched external calibration in DORM-2.

All as ng/g As	**AsB**			**DMA**		
DORM-2 (dogfish muscle)	Mean	Standard Deviation	RSD (%)	Mean	Standard Deviation	RSD (%)
Standard addition	15240	210	1.4	300	30	10.0
External calibration	15440	520	3.4	320	10	3.0
Certificate value	16400	1100*		n.a.		

* (95% C.I.)

The data in both tables indicate that there is no conclusive evidence that AsB or DMA are influenced by matrix components in either of the CRMs with the developed methodology. Generally, quantitation by external calibration yields higher results for both analytes in both matrices, but this difference is not significant as the mean results for both techniques are within the standard deviations for both calibration techniques. The accuracy of the results obtained by both calibration approaches is not significantly different, and there is no indication that either of the approaches results in consistently better measurement precision. As these data were acquired as part of a measurement sequence, the precision data could be improved for both approaches by increasing the number of injections for the samples and standards. This may show a difference that is not apparent with these data.

Table 5.2 Comparison of standard addition calibration and non-matrix matched external calibration in BCR 627.

All as ng/g As	**AsB**			**DMA**		
BCR 627 (Thuna fish)	Mean	Standard Deviation	RSD (%)	Mean	Standard Deviation	RSD (%)
Standard addition	3560	120	3.4	175	12	6.9
External calibration	3720	105	2.8	191	21	11.0
Certificate value	3900	220*		150	23*	

* (95% C.I.)

5.1.4 Effects of Plasma Disturbance of the ICP due to Elution of Organic Solvent Fractions from the Liquid Chromatograph

In order to avoid losses of volatile arsenic species prior to analysis, the ASE methanol fractions were not evaporated to complete dryness, but instead were diluted in deionised water. This meant that for quantitation by external calibration, the calibration standards were prepared in deionised water, whilst the sample extracts still contained a fraction of methanol. When the samples were injected, a signal response was observed when the organic solvent eluted from the column. It has been shown in the literature that the organic carbon content in the plasma of the ICP-MS can have a significant influence on the signal intensity of both inorganic As and the individual arsenic species (Larsen and Stürup, 1994). In order to overcome this effect, 1% MeOH was added to the mobile phase, but the addition of higher concentrations decreased the efficiency of the chromatographic separation. This addition did not overcome the plasma disturbance effects, as shown in Figure 5.7 unless the samples were diluted at least 10-fold and therefore it was attempted to add additional MeOH post-column.

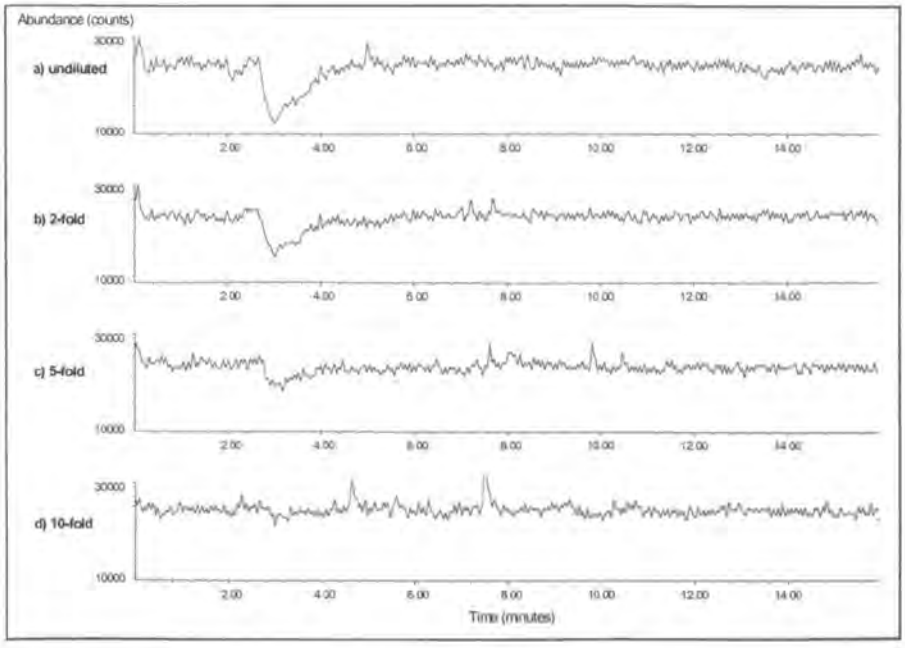

Figure 5.7 Signal of the ^{103}Rh internal standard, added post-column for fish sample extracts a) undiluted and diluted b) 2-fold, c) 5-fold and d) 10-fold in deionised water.

MeOH was added post-column via a T-piece prior to nebulisation in order to negate any difference in signal intensity that may result from the MeOH content of the sample extract compared to the standard solutions. Although the respective volume of MeOH from this source was more than 5 times greater than the MeOH from undiluted sample extracts, there was still an obvious plasma disturbance when sample extracts were diluted less than 5-fold. This is shown in Figure 5.7 for the signal of the internal standard ^{103}Rh added to the MeOH post column for fish extracts diluted from 0- to 10-fold.

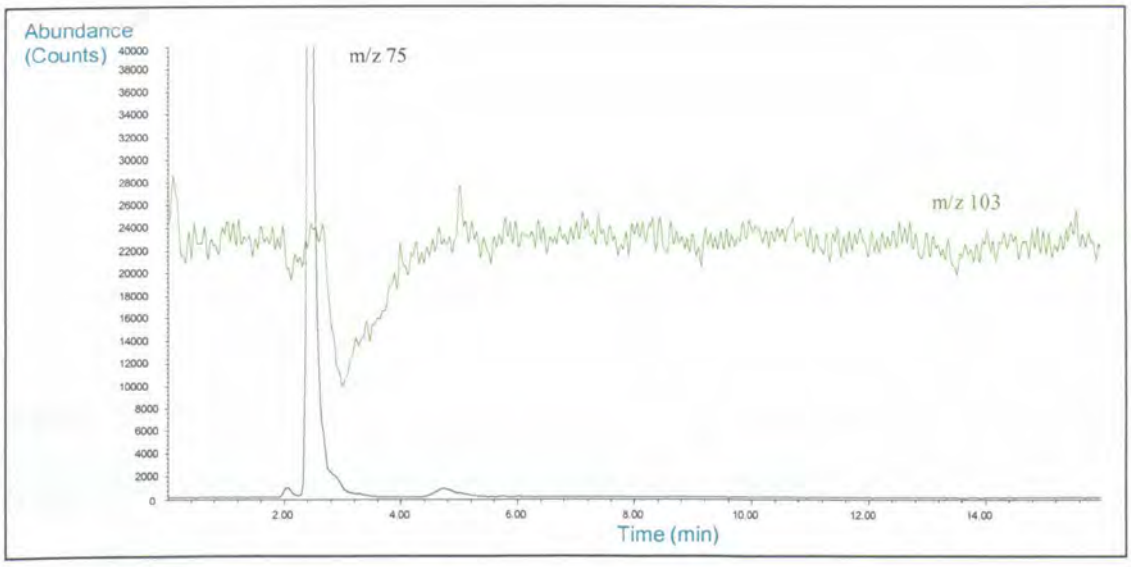

Figure 5.8 ^{103}Rh and ^{75}As signals for an undiluted fish extract. Notice the increase in the ^{75}As signal at the tailing side of the major peak (AsB) coinciding with the decrease in the ^{103}Rh signal.

Figure 5.8 shows the impact of the solvent elution on both the internal standard and As signal. Whilst the signal intensity for Rh is reduced between 2.8 and 4.8 minutes, the signal for ^{75}As increases on the tailing side of the AsB peak. This increase was also apparent in other isotopes monitored such as m/z 77, 78 and 51. Although this sample did not contain As(III) above the detection limit, this signal increase in m/z 75 coincides with the elution of this species, and an accurate quantitation by external non-matrix matched calibration would be affected for As(III) using this approach. In this case, a standard addition calibration represents a more suitable approach for quantitation, unless the arsenite concentrations in the extracts are high enough to allow greater than 10-fold dilution to reduce the effect described.

For samples where neither of these approaches is possible because of low arsenite concentrations a different chromatographic separation must be used which is capable of resolving such interferences chromatographically. This approach is demonstrated in Figures 5.9 and 5.10. Figure 5.9 shows a chromatogram for a soil extract containing As(III), DMA and MMAA separated using Chromatography A. Figure 5.10 shows the same sample analysed using Chromatography B.

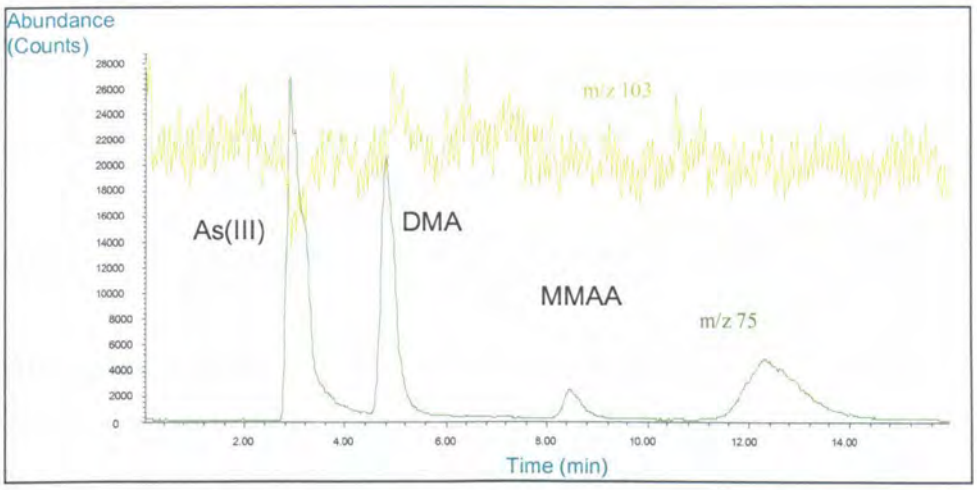

Figure 5.9 Soil sample extract analysed using Chromatography A. The matrix interference elutes with the same retention time as As(III) making the accurate quantitation of this compound by external calibration difficult.

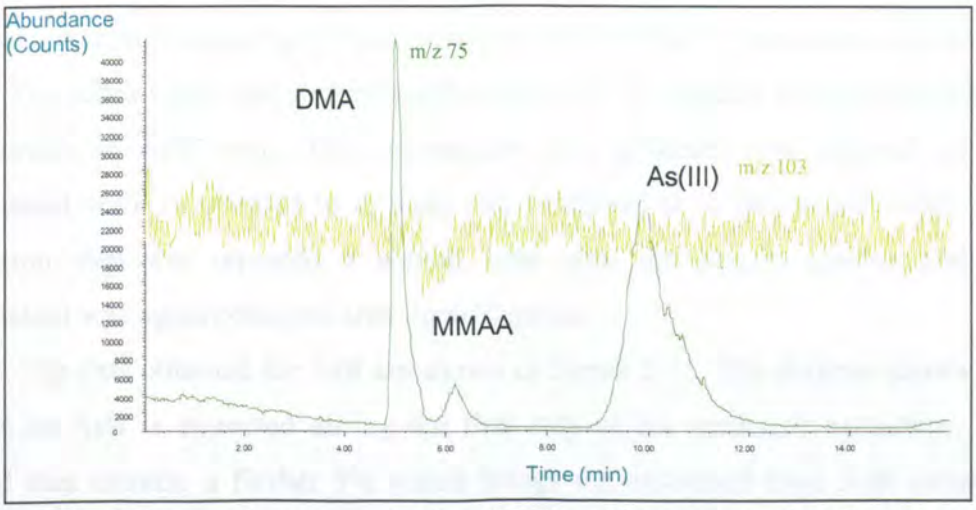

Figure 5.10 Soil sample extract analysed using Chromatography B. Using this chromatography, As(III) is not interfered with by the elution of the organic solvent fraction and can be quantified by non-matrix matched external calibration.

Both sets of chromatographic conditions resolve the three arsenic species present in the sample, but the organic solvent interference affects different species in each case.

With Chromatography A, the As(III) signal is affected whereas using Chromatography B, it is the DMA peak that is interfered with. By using both sets of chromatographic conditions on the samples, the interference can be resolved and each species can be quantified free from interference with at least one method. The analytical data for this sample shows that the influence of the interference on DMA is almost negligible (A: 3400 ± 300 ng/g compared to B: 3700 ± 170 ng/g) whereas the arsenite data is affected significantly (A: 6400 ± 140 ng/g compared to B: 7700 ± 220 ng/g). The difference in the arsenite data for the soil sample is probably due to an enhanced peak area measurement of the As(III) in the sample compared to the aqueous standard using Chromatography A.

5.2 DEVELOPMENT OF EXTRACTION TECHNIQUES FOR ARSENIC SPECIATION

5.2.1 Mechanical Shaking

Experiments were carried out with a Tuna fish CRM (BCR 627) certified for AsB and DMA in order to assess extraction of these species by mechanical shaking with methanol and acetone. The extraction with acetone was carried out to determine whether a significant fraction of arsenicals was present in the material in a non-water soluble form or associated with lipids. Aliquots of ~ 0.2g of the material were placed in 50mL centrifuge tubes and 10mL of acetone and methanol were added to different tubes. The tubes were then shaken mechanically for 15 minutes and centrifuged for 10 minutes at 4200 rpm. The supernatant was collected and aliquots of the supernatant were evaporated to dryness and re-dissolved in de-ionised water. The extraction step was repeated a second time with the sample residue and the supernatant was again collected after centrifugation.

The data obtained for AsB are shown in Figure 5.11. The diagram shows that ~ 95% of AsB is extracted during the first step of the methanol extraction. The second step extracts a further 5% which brings the combined total AsB extracted with methanol to the certified value for this compound. In contrast, the extraction with acetone only extracts a total of 2% of the certified value, of which about 72% is extracted during the first step. These data indicates that the amount of AsB, which is associated with lipids or associated with non water-soluble components in this

sample is negligible, and that two extraction steps with methanol are required for full recovery of the AsB in this CRM.

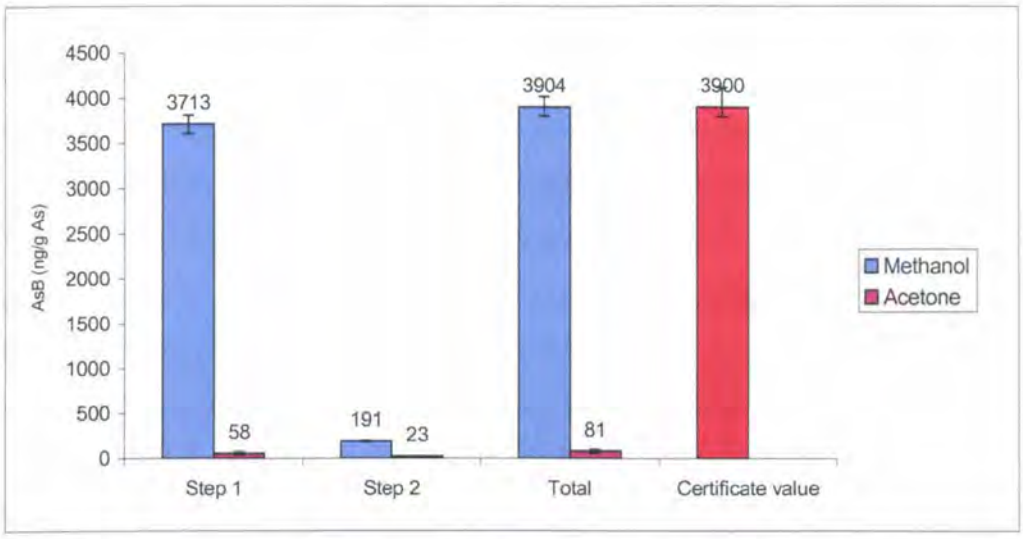

Figure 5.11 Extraction of AsB from BCR 627 Tuna fish by mechanical shaking with acetone and methanol.

Mechanical shaking can therefore be a suitable and inexpensive extraction approach for AsB from this matrix, and this has been confirmed by other studies (e.g. Jackson and Bertsch, 2001). However, the practicalities of this approach are not appropriate for high sample throughput, and therefore no further experiments were carried out with the extraction of other As-species by this method.

5.2.2 Extraction of Arsenicals by Accelerated Solvent Extraction (ASE)

Although accelerated solvent extraction has been used previously for the extraction of arsenicals (Gallagher et al., 1999; McKiernan et al., 1999; Vela et al., 2001) this technique is not very widespread at present and its suitability for the samples used in this study had to be validated. For this purpose the candidate CRM oyster tissue BCR710 was used because it contains the greatest number of arsenicals of currently available CRMs (McSheehy et al., 2001; Kohlmeyer et al., 2002) and consensus mean data were available to assess the extraction efficiency.

One of the advantages of ASE is that the instrument allows the sequential extraction of the same material in several extraction steps. This feature can be used to assess the extraction profile of analytes from a particular matrix. In order to establish an extraction profile of arsenicals, the oyster tissue was extracted using 100% MeOH with 5 consecutive cycles of 2 min duration. The extracts were collected in separate

vials and analysed by HPLC-ICP-MS. Figure 5.12 shows that all of the species except AsB are extracted within the first 2-minute extraction step. Figure 5.13 shows a diagrammatic representation of the results obtained. Of the seven arsenicals that are separated and detected in the extracts, only AsB is present in the second extract in a detectable quantity. The AsB concentration measured in the second extract represent a minor proportion (0.4 %) of the AsB extracted in the first step. All other species including DMA, MMA and three unidentified arsenicals are fully extracted during a single 2-minute extraction step. This pattern is probably partly due to the difference in the concentrations of the different arsenicals present in this sample, where AsB constitutes the major fraction of the total As in the sample, whilst the other species represent minor constituents.

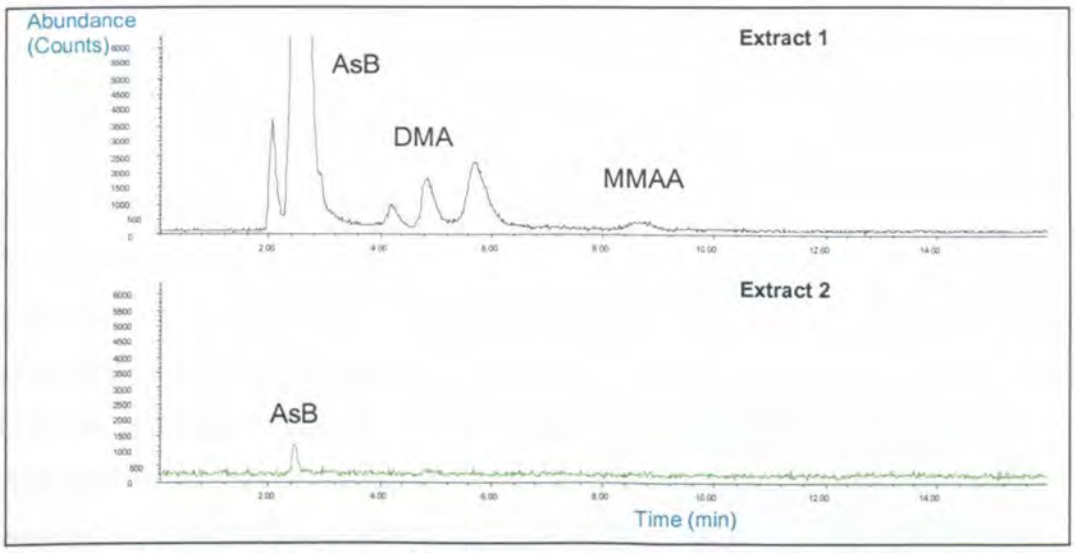

Figure 5.12 As-species detected in Oyster tissue BCR710 during the first two sequential extraction steps by accelerated solvent extraction. The chromatogram at the top (black trace) represents the first extraction cycle and the chromatogram at the bottom (green trace) represents the second extraction cycle.

The accuracy of the ASE method was proven by the good agreement between the AsB and DMA data obtained during the experiment with the consensus mean values from the 'Mulspot' intercomparison exercise (Data courtesy of the BCR, Brussels) obtained by eight and six international expert laboratories respectively (Figures 5.14 and 5.15).

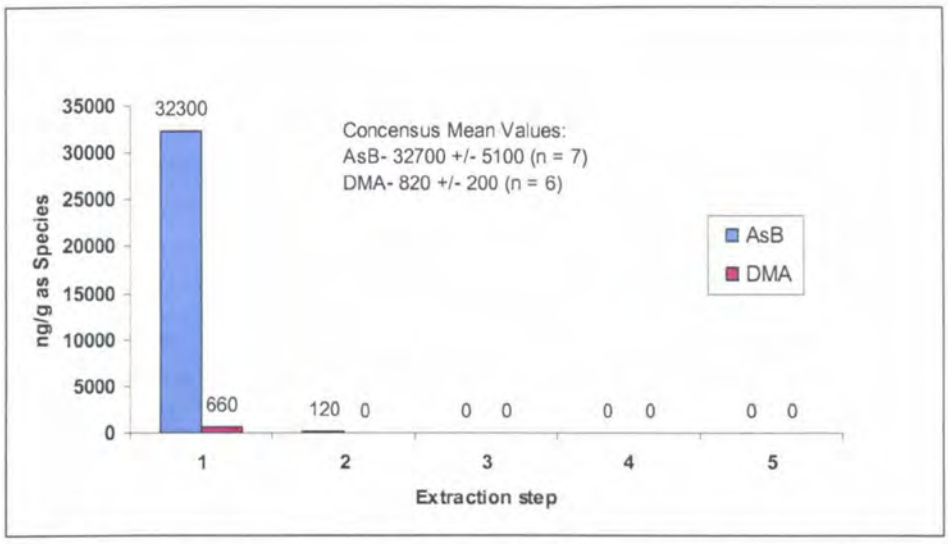

Figure 5.13 Extraction profile of AsB and DMA from BCR 710 (candidate material) using accelerated solvent extraction with methanol (sequence of 5 separate 2 minute extraction steps).

5.2.3 Comparison of Microwave Extraction and Accelerated Solvent Extraction for Arsenic Speciation

Experiments carried out by other laboratories indicated that the use of microwave extraction could also be applied successfully for the extraction of As-species from different matrices (Yehl et al., 2001). In order to obtain a direct comparison between ASE and closed vessel microwave extraction, the same oyster material (BCR 710) was extracted by both methods using methanol. No lipid pre-extraction by ASE was used for this experiment. The extraction conditions used for both methods are summarised in Table 5.3. The major As-species in the oyster material are water-soluble and can be extracted relatively easily, as shown by the mechanical shaking experiments above, so a low power (100W) microwave program was chosen with a short duration (10 minutes) and a moderate maximum temperature of 60°C.

Table 5.3 Extraction conditions used for ASE and closed-vessel microwave extraction.

ASE (Dionex ASE 300)		**Lipid pre-extraction**	**Compound extraction**
	Pre-Heat	2 minutes	2 minutes
	Heat	5 minutes	5 minutes
	Extraction steps	3 x 5 minutes	5 x 2 minutes
	Temperature	60°C	100°C
	Pressure	1500 psi	1500 psi
	Solvent	Hexane	Methanol
Microwave (Paar Physica Multiwave)	(Closed Vessel)		
	Power		100 W
	Duration		10 minutes
	Temperature		60°C
	Solvent		Methanol

The results for this experiment are summarised in Table 5.4. Data for both AsB and DMA by ASE were within the standard deviations of the consensus mean for BCR710 (Figures 5.14 and 5.15) during the certification campaign. The AsB recovery by closed-vessel microwave extraction was also within 5% of the consensus mean, whereas DMA recovery by this method was 32% lower than the consensus mean and on average 23% lower than the value obtained by ASE. The mild extraction conditions used for microwave extraction could be responsible for this and this recovery may be improved by altering the extraction conditions used, for example by increasing the duration and temperature, or by adding a second extraction step with a fresh aliquot of methanol. These factors were not tested further, but the reproducibility of three microwave extracts for AsB suggests this technique warrants further investigation.

Table 5.4 Arsenobetaine and DMA extracted from BCR710 by closed-vessel microwave extraction and ASE (n = 3).

All in ng/g	**AsB (± 1σ)**	**% of consensus mean**	**DMA (± 1σ)**	**% of consensus mean**
Microwave	31000 ± 200	95	560 ± 20	68
ASE	32600 ± 1000	100	750 ± 30	91
Consensus Mean (C.M.) MULSPOT study	32700 ± 5100		820 ± 200	

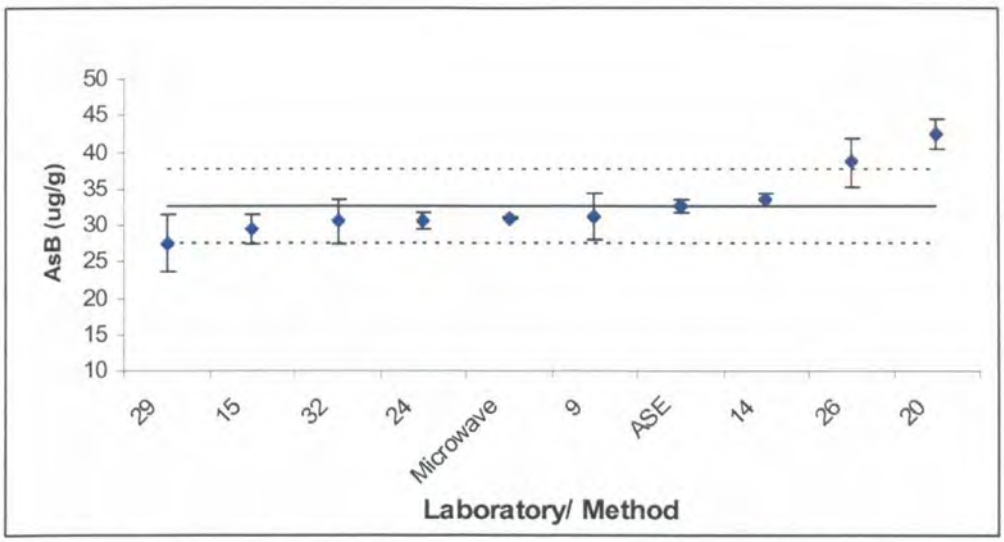

Figure 5.14 Diagram showing the performance of the tested extraction methods compared to the AsB data accepted for certification for the oyster tissue BCR 710 under the BCR MULSPOT study. The solid line is the mean of all submitted results and the dashed lines show the limits of one standard deviation about the mean.

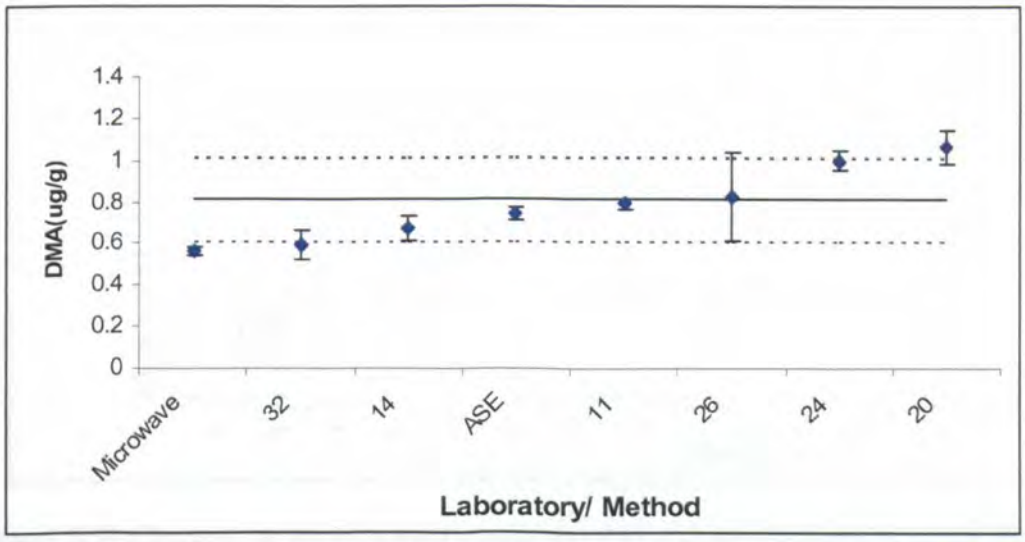

Figure 5.15 Diagram showing the performance of the tested extraction methods compared to the DMA data accepted for certification for the oyster tissue BCR 710 under the BCR MULSPOT study. The solid line is the mean of all submitted results and the dashed lines show the limits of one standard deviation about the mean.

If both methods are compared in terms of time-efficiency and practicality, the ASE approach has several advantages. First, the sample extract is collected in a vial after extraction and therefore there is no need for transferring both sample and solid residue to a centrifuge vial, centrifuging it in order to separate out solid material and collecting the supernatant for analysis, which are the steps needed for sample

preparation by microwave digestion. On the other hand, the microwave system does not provide as many possibilities for contamination or species transformation as described in an earlier report (Wahlen, 2002), and this may be reflected in the smaller standard deviations shown for this method in Table 5.4.

An additional advantage of the ASE approach is that it offers the possibility of extracting the same material with a series of solvents in sequence. This can be of benefit if a sample is analysed that contains matrix components, which can interfere with the subsequent analytical steps. Such effects were observed when a chicken tissue was extracted and analysed by LC-ICP-MS conditions A. Figure 5.16 shows that the DMA peak is uncharacteristically broad when the methanolic chicken extract was injected. Other samples and standards that were injected during the same analysis did not show the same peak broadening and column deterioration was therefore ruled out as the cause.

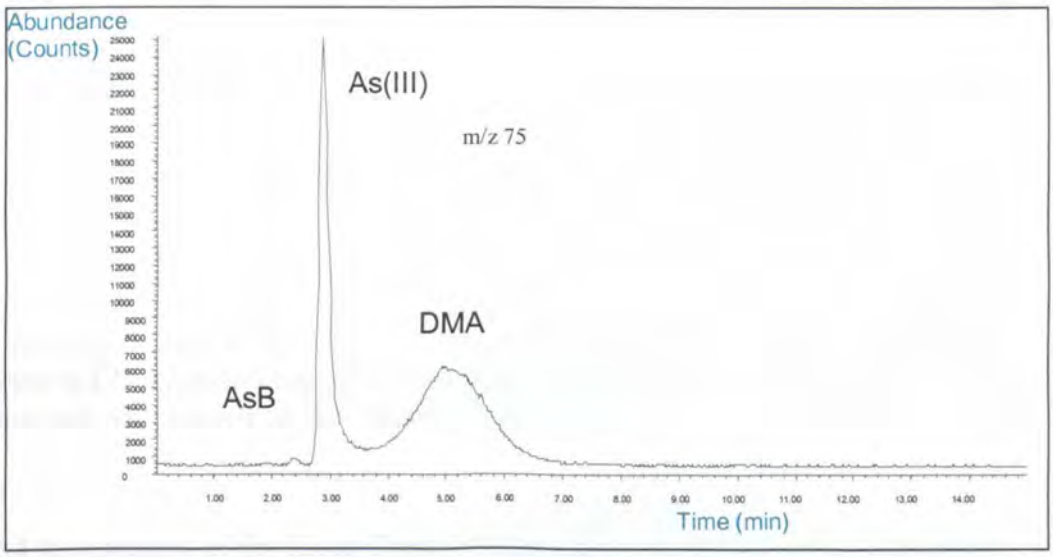

Figure 5.16 Chromatogram of a methanolic chicken extract analysed by LC-ICP-MS (conditions A).

A clean-up of the lipid matrix components in the chicken material was attempted with a sequential extraction, by a de-fatting extraction using hexane and 3 sequential ASE cycles of 5 minute duration at 60°C followed by extraction of the arsenicals using the proposed ASE conditions comprising 5 separate 2 minute cycles at 100°C (See Table 5.4). The effect of this pre-extraction on the chromatographic separation of the arsenicals is shown in Figure 5.17. The difference in signal intensity and signal to noise ratio for the chromatograms in Figures 5.16 and 5.17 are due to

the fact that they were acquired on different days with different instrument sensitivities. The hexane fractions were also monitored for arsenic species by HPLC-ICP-MS after evaporating the hexane to dryness and dissolving the residue in de-ionised water. No species were detected at concentrations above the limits of detection (AsB 0.03 ng/g, DMA 0.07 ng/g, AsIII 0.16 ng/g). Such a sequential extraction of the same sample with different solvents would be considerably more laborious with a microwave approach because the sample in the extraction vessels would have to be dried until all of the previous solvent is removed. Using the ASE approach, this is achieved by purging the extraction cell with nitrogen gas, which also ensures optimal collection of the extraction solvent.

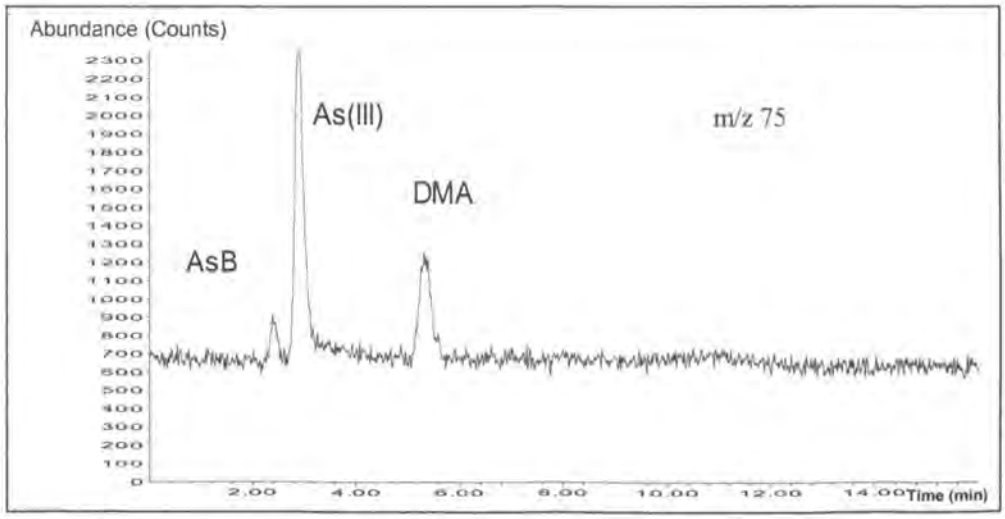

Figure 5.17 Chromatogram of the methanol extract of the chicken material after pre-extraction with hexane as described in Table 5.3.

5.2.4 Assessment of the Long-Term Performance of the Developed Methods

The suitability of the combined ASE extraction and the developed chromatographic separations was verified for AsB and DMA over an 8-week period during which three materials were repeatedly extracted and analysed. DORM-2 and BCR-627 are certified reference materials containing these species, and BCR710 is a candidate CRM for which consensus mean and standard deviations data were available as the result of an comparison exercise of European expert laboratories.

Table 5.5 shows the data obtained for these three materials in comparison to their certified or consensus mean values. The mean recoveries for AsB are in excellent

agreement with the certified values and the consensus mean for CRM710. Some variation is observed for DMA, as indicated by the fact that the values obtained for BCR627 are ~ 20% above the mean value and the mean recovery by this method compared to the consensus mean for CRM710 is ~ 11% lower.

Table 5.5 Data obtained for AsB and DMA in two certified reference materials and a candidate reference material by repeat measurements over an 8-week period. All data are quoted ± 1σ, except where indicated otherwise.

ng/g As	AsB			DMA		
	Certified Value	This work	Mean Recovery	Certified Value	This work	Mean Recovery
DORM-2 (Dogfish)	16400 ± 1100*	16300 ± 900 (n = 5)	99 %	Not certified	350 ± 40 (n = 3)	
BCR 627 (Tuna)	3900 ± 220*	3690 ± 210 (n = 6)	95%	150 ± 20*	180 ± 10 (n = 4)	120%
CRM 710 (Oyster)	32700 ± 5100 (as AsB)	31800 ± 1100 (n = 4)	97%	820 ± 200 (as DMA)	730 ± 30 (n = 4)	89%

*95% C.I.

However, the standard deviations for both data sets show a degree of overlap between the measured and certified values. The DMA value obtained for CRM710 fell within the range of data accepted for certification of this material (Figure 5.15) and is completely covered by the standard deviation of the consensus mean. This is a further indication of the suitability of this method for the accurate measurement of the arsenicals AsB and DMA.

5.2.5 Performance of the Combined ASE Extraction and LC-ICP-MS Method in International Blind Trial Intercomparisons for Arsenic Speciation

The method performance was also assessed in comparison to a number of European expert laboratories during the 'SEAS' feasibility study organised by the University of Plymouth Enterprise Limited, and sponsored by the European Union (BCR, EU)[1]. A fish material was prepared for this intercomparison by the University of Plymouth (UK) and a rice sample was provided by the Universidad Complutense (Madrid, Spain). The Istituto Superiore di Sanità (ISS) (Rome, Italy) selected the type of rice

[1] The 'SEAS' feasibility study was co-ordinated by The University of Plymouth Enterprise Limited (Plymouth, UK) under the EC contract: G6RD CT2001 00473 "SEAS" with the title: 'Feasibility Studies for Speciated CRMs For Arsenic in Chicken, Rice, Fish and Soil and Selenium in Yeast and Cereal'.

with the highest amount of arsenic as indicated by the analysis of eight different brands. The rice was processed by IRMM (Geel, Belgium) as a candidate reference material and then shipped to Madrid for homogeneity and stability testing. Because of the high moisture content, bacterial activity might have caused a modification in the content of the arsenical species. Thus, this material was deemed unsuitable and selection of a new rice sample was undertaken. In this context, four types of rice have been tested in order to identify the one with the highest content of total As, i.e., Ribe (*Japonica, fine*), Arborio (*Japonica, very fine*), Originario (*Japonica, common*), Carnaroli (*Japonica, very fine*). Six kilograms of rice of the Arborio variety, the one with the highest As content, were shipped to the IRMM to be processed and transformed into the candidate reference material.

Based on the results described above, the combined ASE extraction using methanol and subsequent LC-ICP-MS determination with chromatography A (2.2mM Ammonium hydrogen carbonate 2.5 mM Tartaric acid at pH 8.2 with 1% MeOH) was used to provide results for the matrix candidate materials during the 'SEAS' feasibility study. Subsamples (n = 12 for fish, n = 9 for rice) of these materials were extracted using the ASE method as described above on different days and the extracts were analysed by LC-ICP-MS and quantified by external calibration with ^{103}Rh added to the mobile phase to monitor fluctuations in instrument sensitivity throughout the run. Results for DMA in the rice material and AsB in the fish tissue are discussed below.

5.2.5 a) DMA in Rice

A chromatogram for an extract of the rice material is shown in Figure 5.18. Apart from the DMA peak, which was identified by retention time matching with a standard solution, a second peak was also detected. This was not identified for the purpose of this study, but elutes with the retention time of As(III). In addition to the main ion at m/z ratio 75, the chromatogram also shows the trace for m/z ratio 77, which was monitored to detect any interferences due to ArCl polyatomic ions formed in the argon plasma. Such ions can interfere with the quantitation of m/z 75 when $^{40}Ar^{35}Cl$ is formed, but if this is the case, the second isotope of Cl^- (m/z 37) must also be present and form $^{40}Ar^{37}Cl$. This is monitored at m/z 77 and if a peak occurred

simultaneously for both m/z 75 and m/z 77 this would be a good indication that interference from Cl^- is likely.

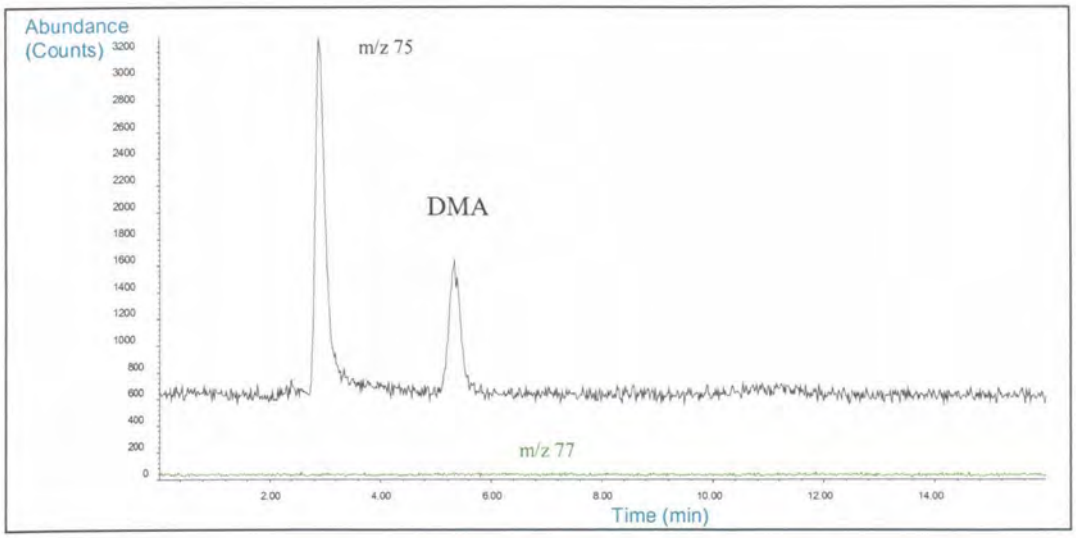

Figure 5.18 Chromatogram of an ASE extract of the rice sample provided under the SEAS feasibility study.

The results provided by all laboratories that submitted data for DMA in the rice tissue are shown in Figure 5.19.

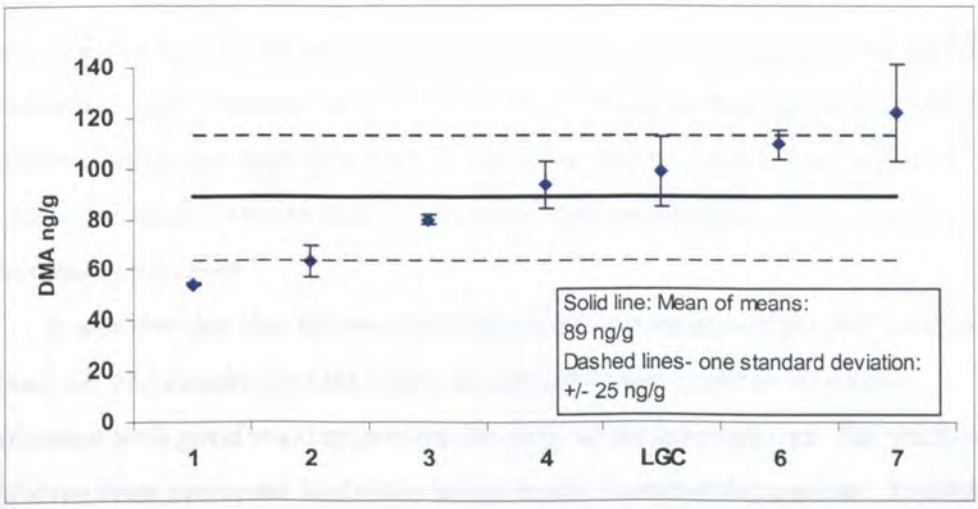

Figure 5.19 Data provided by participants for the determination of DMA in rice during the SEAS feasibility study. The data obtained by the methodology described here is referred to as 'LGC'. Error bars are ± one standard deviation of the mean.

The mean result of 99 ± 14 ng/g determined by the method developed as part of this work compared well with the mean of all results submitted of 89 ± 25 ng/g. Based on the instrumental detection limit of 0.07 ng/g DMA and the dilution factors used

during sample preparation is was estimated that the method detection limit for DMA in this rice material was in the region of 70 ng/g DMA. It was apparent that the agreement between the different laboratories was not completely satisfactory for the establishment of a certification value, as the spread of results for this analyte and matrix is in the region of 28% (RSD). This discrepancy is probably due to a combination of the low levels (ng/g) of the analyte in the matrix, which was probably near the detection limit of some of the methodologies used and the fact that no CRMs were previously available for this matrix that could have been used for method validation. Most laboratories therefore used spike recovery as a means of estimating the extraction efficiency and differences between spike recovery and actual analyte recovery from the matrix may have contributed to the spread of data.

The recommendations given in the final SEAS report (Pitts (2003), pers. comm.) regarding the certification of As-species in rice were as follows:

The study of the rice candidate material showed up many problems with the production of the candidate CRM, due to water content, and inter-conversion of species due to microbial action. These difficulties resulted in a second batch of material having to be prepared, and distribution of the material to the participating laboratories took place prior to the establishment of the homogeneity and stability of the material. These features were established during the remaining time of the study. The second batch was both dried to a much lower water content, and was also gamma-irradiated to ensure that no microbes were available to carry out any degradation processes.

It was decided that the material should be recommended for full certification for total As. The results for DMA were nearly sufficient to recommend full certification with good overlap present for most of the laboratories. The problem could have been overcome had more laboratories reported this analyte. A number of less experienced laboratories involved in this study have learned new techniques and would be capable of submitting better results in the future. As a minimum, it was felt that an indicative figure could be provided for DMA as well as for the sum of As(III), As(V) and MMA.

5.2.5 b) AsB in Fish

The data for AsB in the fish tissue (Figure 5.20) displays a much closer spread of results of about 10% after removing statistical outliers. Again, there is a good agreement between the result obtained by the combined ASE/LC-ICP-MS approach developed during this thesis of 94.9 ± 3.9 mg/kg with the mean of all data of 95.7 ± 7.8 mg/kg. The precision achieved was also satisfactory at 4.2% (RSD) for 12 subsamples from different bottles analysed on three separate days. The performance of the method in this international intercomparison is highlighted by the good agreement with data provided by several European expert laboratories with longstanding expertise in As-speciation analysis. It should also be noted that the intercomparison was carried out with a blind sample of unknown concentration rather than based on the analysis of a CRM with known certified values.

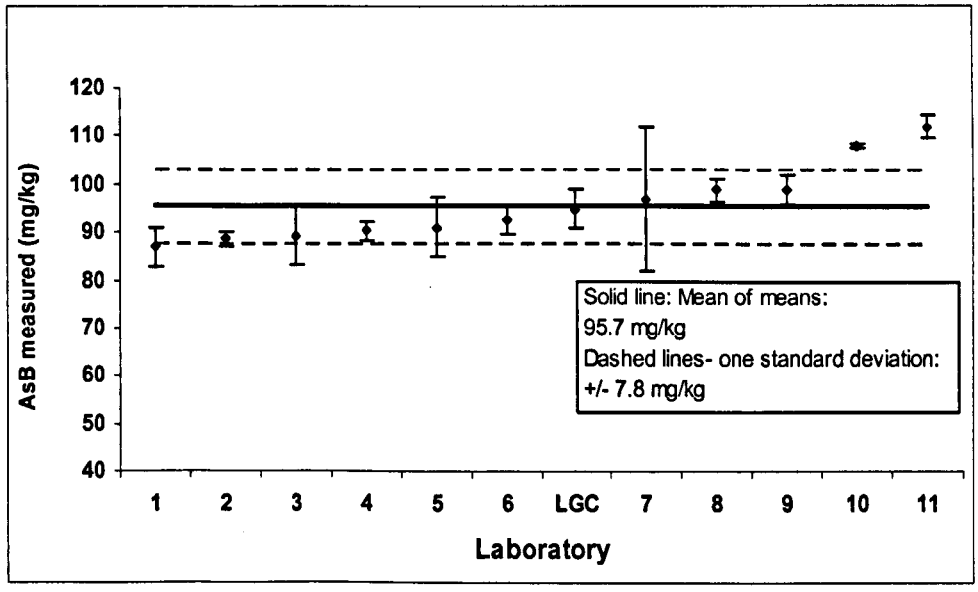

Figure 5.20 Data for the determination of AsB in the fish tissue provided during the SEAS study. The data obtained by the methodology described here is referred to as 'LGC'. (Error bars are ± one standard deviation of the mean).

The recommendations from the SEAS study are concerning this material are summarised below:

It was apparent from the results that there was adequate agreement for the material to proceed for full certification for total arsenic content and for arsenobetaine content. From discussions which particularly concerned the food scientists, it was suggested that an indicative value be given for the As(III) + As(V) + MMA would be advantageous. Although only two laboratories carried out this

determination, reasonable agreement was obtained. Since these species represent a threat to man if found in the food chain, this advice would seem to be valid, and would undoubtedly make the CRM of much greater interest to those involved in foodstuffs analysis.

The comparison of this data set with the DMA data in rice illustrates several points. The spread for the data for AsB in fish was probably much tighter because the levels are about three orders of magnitude higher and are well above the detection limits of the methodologies used in the intercomparison. The method detection limit for this work was estimated as ~50 ng/g AsB, based on an instrumental detection limit of 0.03 ng/g AsB and the dilution factors used during sample preparation. Secondly, there is a range of certified reference materials available for AsB in fish (e.g. DORM-2 and BCR 627) so that the participating laboratories had a good basis for establishing their methodologies for this part of the study.

5.2.6 Comparison with an Independent Methodology from the National Research Council, Canada for the Determination of As-Species in Marine CRMs

Under the Glazebrook Fellowship Scheme sponsored by the Department for Trade and Industry, UK a research secondment enabled a comparison to be made between independent As-speciation methodologies used by the two National Metrology Institutes (NMIs): LGC and the National Research Council, Canada (NRC). The materials used for the study were reference materials (DORM-2, DOLT-2, TORT-2) that were previously prepared and certified for the total metal content of selected elements, although only DORM-2 provides certificate values for arsenic species. Results obtained by the newly developed LGC method were compared to those from the independent method established at NRC during the analysis of arsenic species in marine based certified reference materials. An additional purpose of this study was to compile speciation data from different methods for possible certification of the arsenic species in all three materials. The NRC methodology is described in more detail below; the conditions used for the LGC method consisted of LC-ICP-MS method A and an ASE extraction comprising five 3 min. cycles at 100°C with 50% acetic acid in MeOH.

5.2.6 a) NRC As-Speciation Methodology

Extraction

A portion of each CRM (250-270mg) was weighed into an extraction/centrifugation tube and suspended in 10ml of deionised water. The samples were sonicated for 20 min and centrifuged for 20 min at 2500 rpm. The supernatant was decanted into a clean dry vial and the residue was re-extracted 3 more times with deionised water (5mL). The extracts were combined and filtered (0.45μm) prior to analysis by HPLC-ICP-MS. Eight replicate extractions for each CRM tissue were carried out on at least 3 different days.

HPLC-ICP-MS methodology

The different liquid chromatographic approaches are summarised in Table 5.6. Anion-exchange HPLC separations were carried out using a Hamilton PRP X-100 (250 x 4.6 mm x 5μm) column (Hamilton) with a PRP X-100 guard column (Hamilton) and a Supelcosil SAX (250 x 4.6 mm x 5μm) column (Supelco) with a Supelguard SAX guard column (Supelco). A Dionex BioLC, model LCM (Dionex Corp., Sunnyvale, CA, USA) fitted with a 100 μL injection loop was employed for the HPLC separations. The ICP-MS instrument used in this work was an ELAN 6000 (PE-SCIEX, Thornhill, ON, Canada) equipped with a Ryton spray chamber and cross flow nebulizer or a Meinhard nebulizer. ICP-MS measurement conditions (nebulizer gas flow, RF power and lens voltage) were optimized daily using a standard built-in software procedure. Typical examples were a nebulizer gas flow of 0.75 L/min, ICP RF power of 1100 W and a lens voltage of 8 V.

Table 5.6 Liquid chromatographic approaches used in conjunction with ICP-MS detection.

Method	Separation	Column	Dimensions	Mobile phase
LGC	Anion exchange	Hamilton PRP X-100	250mm x 4.6mm, 5μm	2.2mM ammonium carbonate/ 2.5mM tartaric acid, 1% MeOH, pH 8.4
NRC	Anion exchange	Hamilton PRP X-100	250mm x 4.6mm, 5μm	0-5 min: 5mM CH_3COOH//5mM ammonium acetate 5-30 min: 50mM CH_3COOH//50mM ammonium acetate

Quantification

A total of 8 extracts of each CRM tissue were analysed for total As and speciated As on at least 3 separate days. By performing the analyses on 3 separate days systematic errors that could be overlooked if all analyses were carried out on the same day were minimised. Random errors for the 3 days are likely to be reflected by an elevated coefficient of variation. For each chromatographic column used, organoarsenic species were quantified by standard additions at two levels. Chromatographic signals were processed using in-house software.

5.2.6 b) Comparison of Data obtained by both Methods

The data in Table 5.7 show a good agreement between the two methods for AsB regardless of the matrix material analysed. The AsB data for DORM-2 also agree well with the certified value and data reported by other groups (Goessler et al., 1998; Kirby and Maher, 2002). However, significant differences in the data for DMA were observed for all of the CRMs analysed. Generally, the ASE approach leads to higher recoveries of DMA by 27% (DOLT-2), 56% (DORM-2) and 60% (TORT-2) than ultrasonication with water.

Table 5.7 Comparison of results by both analytical protocols for the quantification of AsB and DMA in the tissues DORM-2, DOLT-2, and TORT-2. (All μg/g As).

Method	DORM-2		DOLT-2		TORT-2	
	AsB	DMA	AsB	DMA	AsB	DMA
NRC	16.72 ± 0.35	0.21 ± 0.03	5.70 ± 0.30	1.16 ± 0.25	13.47 ± 1.02	0.79 ± 0.09
LGC	16.81 ± 0.89	0.48 ± 0.07	5.22 ± 0.45	1.60 ± 0.14	14.14 ± 0.62	1.98 ± 0.10

The fact that the DMA recovery by ASE was higher in three different materials with varying concentrations of other species indicates that this was probably an extraction related effect rather than the result of false quantitation due to co-elution with other species. A comparison of the data obtained with that quoted in the literature indicated that different methods yield different DMA recoveries. The DMA data obtained for DORM-2 here by ASE (0.48 ± 0.07 μg/g As) agree very well with data reported by Kohlmeyer et al. (2002), who determined 0.49 ± 0.03 μg/g As for DMA in this material. The microwave extraction approach used by Kirby and Maher (2002) showed DMA data from DORM-2 and TORT-2 (0.280 ± 0.004 μg/g

As and 1.03 ± 0.10 μg/g As respectively) that fell in-between the sonication and ASE data presented here.

A comparison of the total As content in extracts of fish tissues by ASE and sonication has already been carried out by McKiernan and co-workers (1999). The speciation results were also given, but expressed as relative percent area rather than quantitative data that could be used for comparative purposes here. Their data showed that sonication and ASE gave comparable results for total As as well as the relative proportions of AsB/AsC and DMA in DORM-2. The difference between the DMA recoveries presented here and the data shown by McKiernan et al. (1999) could be due to the fact that the ASE procedure used for their work was carried out at ambient temperature rather than at 100°C and did not include acetic acid in the extractant.

Another outcome of the study was a lack of consistency between the total As determined and the sum of the individual species detected. The sum of the species extracted by the sonication approach and quantified by the NRC method for DORM-2 and DOLT-2 (17.3 μg/g As and 15.9 μg/g As respectively) were in good agreement with the sums of As-species species recovered by the MAE approach used by Kirby and Maher (2002; 17.5 μg/g As and 16.1 μg/g As respectively). However, there was some discrepancy between the total As determined in the aqueous sonication extracts, the sum of the species recovered, and the total certified As content. The sum of the species recovered by sonication corresponds to a maximum of 84% (DOLT-2) to 94% (DORM-2) of the total As determined in the aqueous extract. In comparison to the certified As content of the three materials, the sums of the extracted species ranged from 43% (DOLT-2) to 74% (TORT-2) and 97% (DORM-2). The low extraction efficiency and relatively high CV for the DOLT-2 tissue were probably due to the nature of the tissue, which is fibrous and has a wider range of particle sizes (when compared to DORM-2 and TORT-2, which are both fine powders).

When comparing the sum of the major species (AsB + DMA) extracted by the ASE approach to the total certified As content in the different materials, it is apparent that a quantitative recovery (96%) was only achieved with the DORM-2 material. The recoveries based on the same principle for DOLT-2 and TORT-2 range from 41% to 75% respectively. The sum of AsB and DMA recovered from DORM-2 by the MAE approach by Kirby and Maher (2002) and the sonication method here

account for 95% and 94% respectively. These are encouraging data for the ASE approach considering that only two of the 8 - 10 species quantified by the MAE and sonication approaches were used for the calculation of these sums. The quantitation of the remaining species in ASE extracts of these samples is the subject of further investigation that is currently on-going. Based on the indication that the ASE approach may recover a greater proportion of the DMA present in these tissues, it remains to be seen whether the extraction of the other As- containing species can also be enhanced.

This comparison showed that certification of the AsB content of these materials could be carried out on the basis of the data presented here, as there is very good agreement between two independent methodologies used for the analysis. The certification of DMA and other species in the three materials would currently not be possible due to the discrepancy between the data from the two methods. An evaluation of DMA results reported in the literature also indicates that the quantitation of DMA in the materials is method dependent and that a more conclusive intercomparison, preferably involving a greater range of different extraction, separation and detection methodologies, is required for a meaningful certification value. In addition to the variation in the DMA data highlighted here, a discrepancy between the sum of all detected As-containing species and the total As measured in the extracts was also confirmed during this study.

5.3 SUMMARY

Two separate anion exchange LC-ICP-MS methods using isocratic elution were developed for the speciation of AsB, DMA, MMA, As(III) and three unidentified species. Out of the different extraction methodologies examined, namely shaking, closed vessel microwave extraction and accelerated solvent extraction, the ASE method was considered to provide the best solution for fast and accurate extraction. The data shown demonstrate the accuracy of the developed ASE/LC-ICP-MS approach for the determination of AsB in fish tissues and also indicate that the DMA data generated for marine samples and rice are comparable in accuracy and precision to other European and international expert laboratories.

The method was tested during participation in a blind trial intercomparison study and also by comparison with a completely independent method carried out at

the National Research Council (NRC) Canada. Some differences in DMA data were highlighted, not only for the data generated here compared to the NRC approach but also compared to different values reported in the literature for one of the CRMs tested, but not yet certified for DMA. These differences appear to be extraction dependent in the case of the direct comparison between the LGC and the NRC methods, because the same standards were used for quantification. Therefore, in order to certify these materials for DMA as well as AsB, an intercomparison study with a greater number of laboratories may be useful to elucidate this research further.

Further work is also needed to elucidate the processes that affect the quantitation of total As in marine tissues by ICP-MS. At present it appears likely that variations in the data reported may be due to different signal responses (Larsen and Stürup, 1994) of the residual species in the extracts/ digests, and therefore care must be taken to convert all of the species to one form that can be accurately and reproducibly quantified against available As standards (Goessler and Pavkov, 2003).

CHAPTER 6

DEVELOPMENT OF A SIMULTANEOUS EXTRACTION METHOD FOR TIN, ARSENIC AND MERCURY SPECIES USING ACCELERATED SOLVENT EXTRACTION

In recent years organometallic speciation analysis involving ICP-MS detection has grown from an area of academic research interest, to the establishment of this technique as a routine monitoring tool in commercial laboratories. Whilst a lot of attention has focused on the improvement of the separation and detection methods for organometallic speciation in line with technological advances, many sample preparation methodologies still rely on time-consuming and labour-intensive methods. Therefore there can be a discrepancy in the time-efficiency of the separation and detection of organometallic species in a sample, which may be achieved in several minutes, and the extraction of those species from the matrix, which may take several hours. In order to provide analytical result in the shortest time possible, for example during toxicological monitoring studies of food products for human consumption, it is imperative that fast and preferably automated extraction methods are developed that can match the time-efficiency of modern analytical detectors such as ICP-MS. Routine monitoring methods for such applications often demand quantitative recoveries of the target analytes ranging from 70 - 110% or even 60 - 120% (e.g. Quality Controls Procedures for Pesticides Residues Analysis, 1999/2000).

Based on the results described in Chapters 4 and 5 for the extraction of organotin and arsenic species by accelerated solvent extraction, a combined simultaneous extraction of these species appeared to be feasible. In addition, the extraction by ASE of methylmercury (MeHg) was examined because this species is of toxicological concern, particularly in seafood. No method development was carried out for the separation and quantitation of this species, but existing methodologies were employed for the determination of this compound in the ASE extracts obtained.

At present the availability of environmental certified reference materials that contain species of tin and arsenic is very limited, because the scope of certification campaigns has historically been limited to different species of a single element. The BCR and other CRM producers such as NRC, Canada are increasingly trying to certify their materials for species of several elements and at the time of writing two materials (BCR 710 Oyster tissue and NRC DORM-2, Dogfish muscle) are available. The oyster tissue is certified for As species as well as organotin (MBT, DBT and TBT) and MeHg whereas DORM-2 is certified for MeHg and two As species.

6.1 SPIKE RECOVERY EXPERIMENTS

Initial work was carried out based on the extraction approach that was found to be suitable for DBT and TBT species using the recommended conditions described in Section 4.2.2.c). An experiment was set-up to determine whether spiked aliquots of calibration standards were recovered quantitatively during this procedure or whether there was any indication of analyte losses, retention or degradation under the extraction conditions tested. As shown in Table 6.1 below, the recovery for all of the species examined during this experiment was in the range of 92 - 113%. The somewhat higher recovery of DBT (127%) was linked to a contamination problem, but not further investigated due to the satisfactory results achieved for this compound with sediment CRM extractions previously.

Table 6.1 ASE Recoveries determined for spiked calibration solutions.

Recoveries (%)	MBT	DBT	TBT	MeHg	AsB	MMA	As(V)
MEAN	103	127	96	113	92	96	92
SD	6	11	4	11	1	2	2

6.2 ARSENIC SPECIATION IN BIOLOGICAL MATERIALS

Initial experimentation on the extraction of arsenicals in biological materials was carried out as described in Chapter 5. In order to test the accuracy of the developed ASE extraction and HPLC-ICP-MS method, a variety of certified and candidate reference materials of marine origin were extracted and analysed. The samples included the certified fish reference materials DORM-2 (Dogfish muscle, NRC Canada) and BCR 627 (Tuna fish, BCR EU) as well as an oyster tissue material

(BCR 710), which is pending certification. The evaluation of the ASE method was described in more detail in sections 5.2.4 and 5.2.5. Considering that satisfactory data were obtained for two of the main As-species in a variety of biological materials, the extraction conditions for the combined approach were not significantly changed with the extraction of arsenicals in mind.

6.3 ORGANOTIN SPECIATION IN SEDIMENTS AND OYSTER TISSUE

The methodology used and the results of previous work on the extraction of DBT and TBT from sediments have already been described. Briefly, the results achieved for the extraction of PACS-2 using the conditions described by Arnold et al. (1998) were 1087 ± 77 ng/g Sn for DBT and 876 ± 51 ng/g Sn for TBT (expanded uncertainties with a coverage factor of 2), which are in good agreement with the certified values of 1090 ± 150 ng/g Sn and 980 ± 130 ng/g Sn respectively.

An independent verification of the accuracy of the ASE approach and an HPLC-ID-ICP-MS method described in was obtained during the P-18 study of the CCQM for the determination of TBT in a blind trial sediment (Metrologia, 2002). The value obtained by this method of 0.657 ± 0.082 nmol/g TBT^+ (expanded uncertainty with a coverage factor of 2) was in very good agreement with the mean result of 0.680 ± 0.015 (one standard deviation) nmol/g TBT^+ obtained by 14 international expert laboratories (Sturgeon, et al., 2003).

The same analytical method has also been used for the determination of TBT in the oyster tissue BCR 710, as described by Wahlen and Catterick (2003) where a mean value and standard deviation of 131 ± 3 μg/kg (as TBT^+) (n = 7) were obtained compared to a mean value and standard deviation obtained by 9 different European laboratories of 133 ± 25 μg/kg (as TBT^+) (n = 68). Thus the quantitative value reported using this method showed an excellent agreement with the consensus mean. The extraction of MBT, MPhT, DPhT and TPhT by ASE from mussel tissue was also described in the same publication, although the recovery of these compounds was not assessed.

One of the drawbacks of using sodium acetate in the extraction medium as proposed by Arnold et al. (1998) was that it lead to precipitation of the salt on the valves of the extraction cells. This resulted in leaking of the solvent at the top of the cells and interruption of automated runs of more than 4 - 6 samples at a time.

Tropolone was also investigated for the extraction of organotin species. Although increases in the recovery of monobutyltin (MBT) and monophenyltin (MPhT) were noted, the use of this chelating agent also led to reactions with residual material in the lines of the ASE instrument which had been used for a wide range of applications. This resulted in clogging of the static valves of the instrument, which had to be replaced every time this approach was tested. This was neither practical nor cost-effective, and experiments with tropolone were therefore discontinued. The extraction medium for subsequent experiments was therefore changed to 50% acetic acid in methanol without addition of sodium acetate or tropolone.

6.4 COMBINED EXTRACTION APPROACH FOR ORGANOMETALLIC SPECIES OF TIN, ARSENIC AND MERCURY

The certified reference oyster tissue BCR 710 was used for this investigation as this material contains species of the elements As, Sn and Hg and consensus mean data for the material was available from the certification round. This material was certified during the MULSPOT certification campaign of the BCR, where it was attempted to provide a speciation CRM with certification data for species of the elements As, Hg, Se and Sn in a single CRM of relevance for environmental applications. For the production of the CRM, oysters had been collected from the Bay of Arcachon, France, which is an area of historic organotin contamination (Alzieu, 1991). Due to the choice of a marine organism, the levels of arsenicals were also expected to be suitable for certification, and MeHg contamination was also deemed a possible contaminant. The consensus mean data for different species of As, Sn and Hg are summarised in Table 6.2. No selenium species were detected at concentrations that were suitable for certification in this material.

Table 6.2 Consensus data for BCR 710 Oyster tissue from the certification campaign.

All as compounds (mg/kg)	Consensus Mean	Standard Deviation	Number of results
AsB	32.98	5.08	8
DMA	0.82	0.18	6
MMA	1.62	0.95	3
MBT	0.049	0.014	6
DBT	0.082	0.017	9
TBT	0.133	0.025	10
MeHg	0.115	0.014	10

The ASE conditions used (Table 6.3) were based on the previous results obtained, and were adjusted so as to provide a good compromise for the extraction of as many of the species present in the sample as possible for the extraction of the material. The analysis of DBT and TBT was carried out as described in Section 4.2 after ethylation of aliquots using GC-ICP-IDMS. The arsenic species were separated and determined using the HPLC-ICP-MS conditions described in Section 6.1. Calibration of AsB was performed by external calibration lines against a commercially available AsB standard, and DMA and MMA were quantified using standard addition calibration. MeHg was also analysed by HPLC-ICP-MS and calibrated using standard addition calibration. The separation and quantification of this compound was not initially intended to be part of this thesis, but due to the toxicological interest in this analyte, was included here based on a method established at LGC during the latter stage of the preparation of the thesis.

Table 6.3. Extraction conditions used for accelerated solvent extraction.

Instrument	Dionex ASE 300
Pre-Heat	2 minutes
Extraction steps	5 x 3 minutes
Temperature	100°C
Pressure	1500 psi
Solvent	50% Acetic acid in methanol

Table 6.4 shows the data obtained and these are represented diagrammatically in comparison to the consensus mean values from the certification campaign in Figures 6.1 and 6.2. It is apparent that good correlation between the data reported here and the consensus mean values for the MULSPOT certification campaign is achieved for AsB, DMA, DBT and TBT. Recoveries for these species range from 80% (DMA) to 99% for AsB. DBT and TBT were quantitatively extracted with respective extraction efficiencies of 81% and 84%. The results for TBT were somewhat lower than those measured when the material was first obtained, and this is possibly a reflection of the fact that the material has been opened frequently at room temperature, which may have lead to losses of volatile compounds like TBT. The lowest recovery for any of the species determined was obtained for MMA at 28% compared to the consensus mean value.

Table 6.4 Species determined in the oyster tissue BCR 710 by the developed ASE co-extraction method.

Species*	Measured Value (Mean ± σ)	Consensus mean (Mean ± σ)	Mean Recovery
AsB	32590 ± 3130	32980 ± 5080	99%
DMA	660 ± 32	820 ± 180	80%
MMA	450 ± 60	1620 ± 950	28%
DBT	67 ± 5	82 ± 17	81%
TBT	112 ± 10	133 ± 25	84%
MeHg	88 ± 4	115 ± 14	76%

**All data are reported as ng/g as the respective species.*

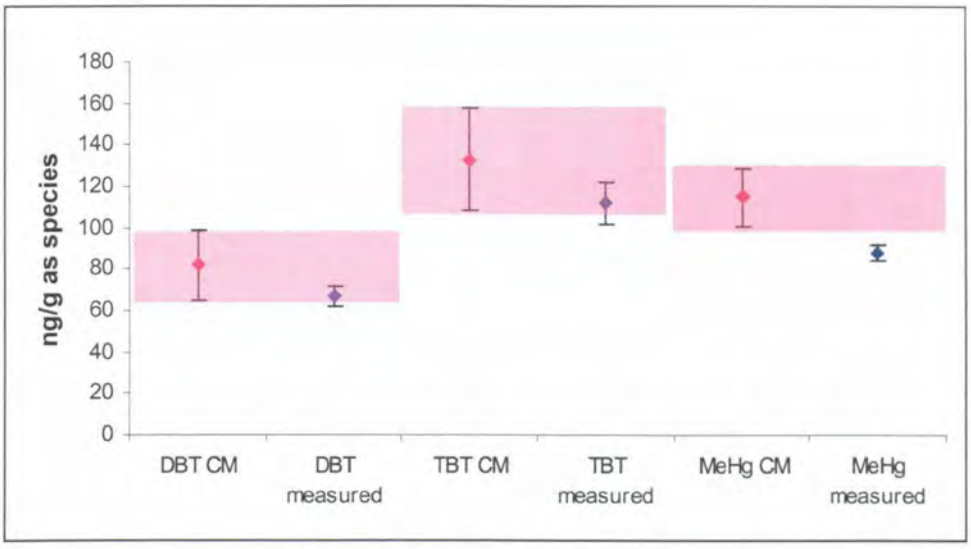

Figure 6.1 Comparison of the data obtained for the analysis of organotin species and MeHg in the oyster tissue BCR710 (blue diamonds) with the consensus mean values (marked by CM in brackets) from the certification campaign (red diamonds).

The graphs shows the good agreement between the measured values and the consensus mean data for DBT, TBT, AsB and DMA. However, the ASE method performs less well for those compounds such as MeHg and MMA for which this approach had not been validated previously. Therefore, the accuracy of the methodology is good for those compounds which had previously been determined using the separate ASE methods and quantified on the basis of individual elemental species. This is perhaps not surprising because no detailed studies had been carried out to determine the extraction behaviour of MMA and MeHg with time.

The precision of the extraction and measurement is also good for all of the analytes except MMA where the RSD exceeds 10%. This is due to the fact that the signal response for MMA and the concentration measured is lower compared to the other arsenicals determined in this material, and the measurement of the smaller peak area is therefore less reproducible. Table 6.2 also indicates that the consensus mean data for MMA is based on only three separate data sets which did not agree very well with each other, and the consensus mean value for this compound should therefore be seen as an indicative value only.

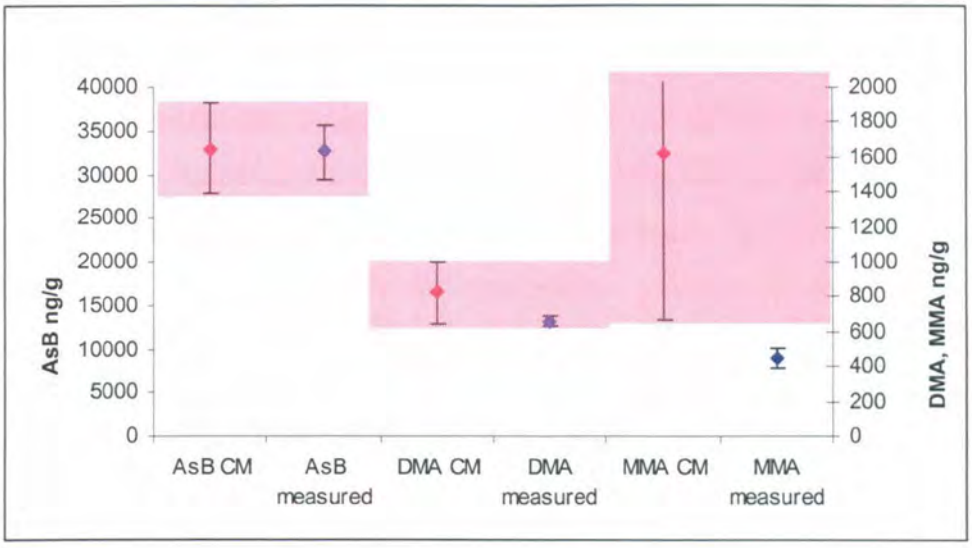

Figure 6.2 Comparison of the data obtained for the analysis of arsenic species in the oyster tissue BCR710 (blue diamonds) with the consensus mean values (marked by CM in brackets) from the certification campaign (red diamonds).

Figure 6.3 shows a chromatogram of the As-species extracted and detected in the oyster tissue by the ASE approach proposed. As can be seen the peak detected in the unspiked sample for MMA is difficult to integrate due to its small size compared to the background noise.

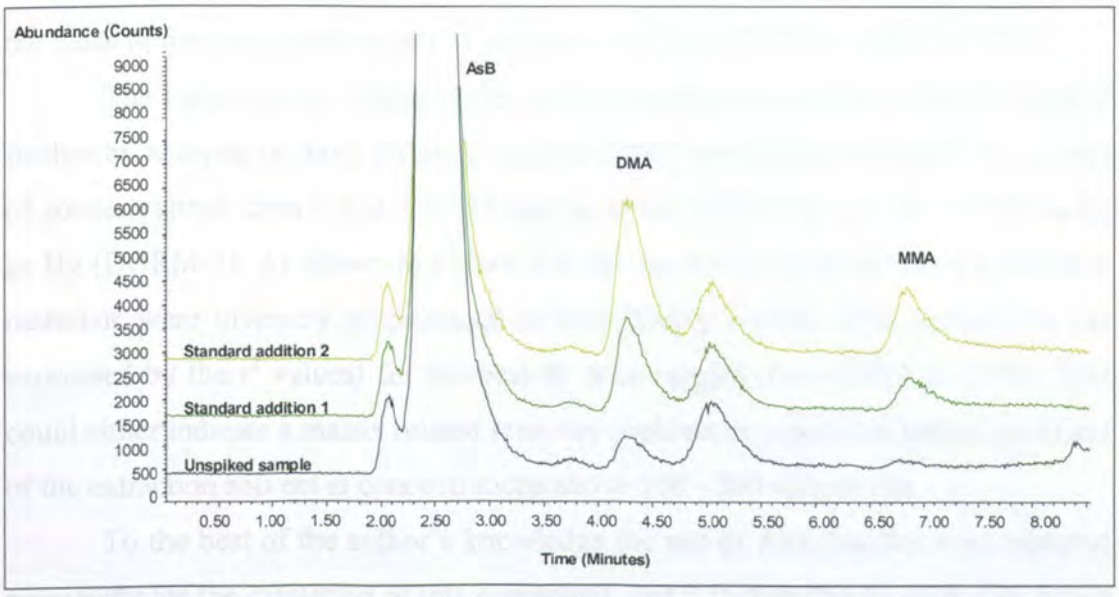

Figure 6.3 Chromatograms showing the As-species detected in the ASE extracts of BCR 710 and the standard additions performed for the quantitation of DMA and MMA.

The resolution of the peaks was affected (probably due to the increased matrix influence from the sample) when attempts were made to obtain a bigger peak and hence a more reproducible integration by reducing the dilution factor to 5-fold instead of 10-fold. Based on the mean recovery achieved here, it can be concluded that the extraction of this compound by this ASE approach from the oyster tissue matrix is not of sufficient quality for quantitative analysis. In addition to the arsenicals identified and quantified here, a further three species were also extracted from the material and separated by the LC-ICP-MS approach. These species were not identified or quantified for the purpose of this study, but are likely to include arsenosugars B and D and $TMAs^+$ according to data published by McSheehy et al. (2001) on the same material.

MeHg was included in this assessment because it is of toxicological relevance in seafood and was known to be present in the sample. The measured concentration of MeHg was somewhat lower than the consensus mean established for BCR710. However, in contrast to the As- and Sn species determined, no preliminary work on the extraction of this compound by ASE had been carried out, and therefore the conditions described are not necessarily the optimum conditions for this species. Therefore, a mean recovery of 76% was considered a promising first step, especially since the extraction method had not been specifically adapted for this compound on the basis of previous experiments as was the case for AsB, DMA, DBT and TBT.

The behaviour of MeHg under these extraction conditions was investigated further by analysis of three different marine CRMs certified for MeHg over a range of concentrations from 0.152 ± 0.013 mg/kg as Hg (TORT-2) to 4.47 ± 0.32 mg/kg as Hg (DORM-2). As shown in Figure 6.4, the recoveries obtained for the different materials were inversely proportional to their MeHg content with correlations (as expressed by the r^2 values) for the best-fit lines ranging from 0.916 to 0.995. This could either indicate a matrix related recovery problem or a possible saturation effect of the extraction solvent at concentrations above 100 - 200 ng/g as Hg.

To the best of the author's knowledge the use of ASE has not been reported previously for the extraction of this compound, and it is therefore feasible that future work could make further improvements in the recovery of this compound possible. Compared to the performance indicators used for routine methods of some pesticides where a recovery of spiked compounds in the range of 60 - 140% is required, the

recovery of MeHg from the oyster tissue can be considered adequate at least for quantitative environmental monitoring studies.

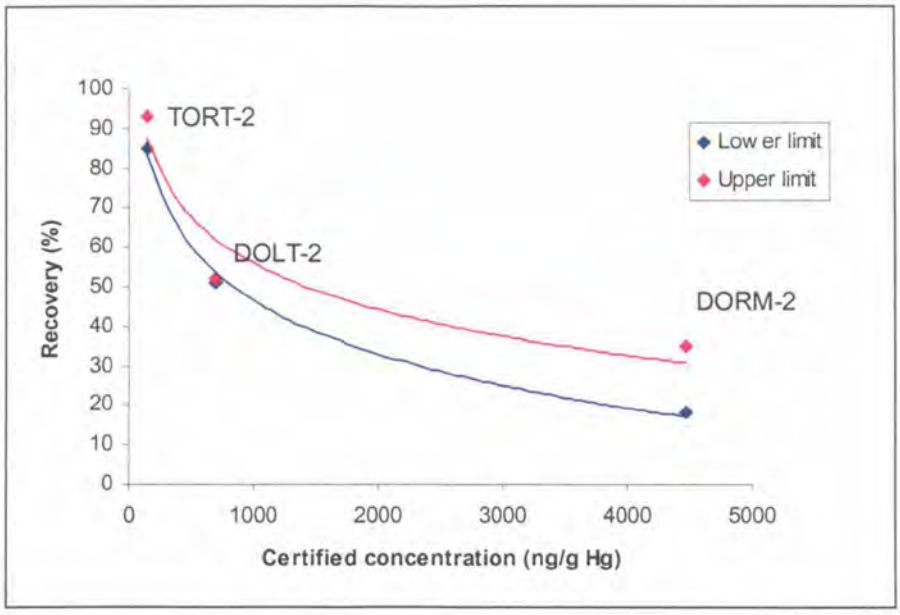

Figure 6.4 Relationship observed between the recovery obtained by accelerated solvent extraction and the certified MeHg content of the sample materials.

The simultaneous extraction approach described has produced good results for the quantitative extraction of two organotin species (DBT and TBT) and two arsenic species (AsB and DMA) from a difficult matrix material. The information obtained in this way could be used for toxicological assessments of seafood samples. In samples where DMA and AsB together constitute a major proportion of the total As content the toxicological risk can be considered to be low. DBT and TBT are also of toxicological interest in seafood, and the presence of these species can be monitored at the same time.

The extraction efficiency of ~ 76 ± 3% for MeHg shows promise for this compound at relatively low concentrations. Although this recovery does not fall within the range covered by the standard deviation about the consensus mean for the certification data, it conforms to requirements for common routine surveillance methods for applications such as pesticide residues in food commodities which often have to cover recovery ranges of 60 - 120%. Due to the fact that it is capable of extracting several species of toxicological interest simultaneously, the ASE method developed and tested here provides a significant improvement over many currently available monitoring methods for trace element speciation. The suitability of the

method needs to be verified for a greater range of applications including the analysis of soils, sediments and other biological tissues as well as herbal medicines or clinical tissues. However, so far, a good performance has been achieved for five analytes of toxicological interest using a matrix material which combines analytically challenging conditions such as a relatively high fat and organic carbon content with low analyte concentrations (e.g. DBT, TBT, MMA and MeHg).

The proposed simultaneous multi-elemental speciation extraction approach has several benefits. Firstly, it results in a significant improvement in time efficiency because the species of different elements can be extracted in a single extraction rather than by using separate procedures. This would be beneficial for environmental monitoring studies of large sample batches. As a result of the improved time efficiency, the sample turn-around time is also improved and therefore the results for critical toxicological studies or during acute pollution incidents would be available sooner. The timeliness of crucial decisions in such circumstances would obviously greatly benefit from such improvements. Secondly, the resources needed for the analysis on the whole would be minimised and this results both in financial streamlining as well as reducing negative impacts on the environment from laboratory operations.

Apart from providing the aforementioned benefits, it also provides a novel technique from an analytical viewpoint because currently only a few truly simultaneous speciation extraction approaches exist (Monperrus et al., 2003 a), whereas most other speciation methods are more limited in the scope of the elemental species that they have been shown to extract simultaneously (e.g. Aguerre et al. 2001; Monperrus et al. 2003 b).

6.5 SUMMARY

The extraction approach has been successful in general with the quantitative extraction of two organotin species (DBT and TBT) and two arsenic species (AsB and DMA) from a difficult matrix material. The extraction efficiency of ~ 76 ± 3% for MeHg also shows promise for this compound of toxicological interest at relatively low concentrations. Although this recovery does not fall within the range covered by the standard deviation about the consensus mean for the certification data, it conforms to requirements for common routine surveillance methods for

applications such as pesticide residues in food commodities, which often have to cover recovery ranges of 60 - 120%.

In light of such performance indicators, the ASE method developed and tested here provides a significant improvement in the currently available monitoring methods for trace element speciation methodologies. Although its suitability needs to be verified for a greater range of applications covering matrix materials such as soils, sediments and other biological tissues as well as herbal medicines or clinical tissues, a good performance has been obtained using a matrix material that combines analytically challenging conditions, such as a relatively high fat and organic carbon content, with some very low analyte concentrations (e.g. DBT, TBT, MMA and MeHg).

CHAPTER 7

APPLICATION OF THE ORGANOMETALLIC SPECIATION METHODS TO THE STUDY OF A SEDIMENT CORE FROM THE TILBURY DOCKS, LONDON, UK

The methodologies described in the previous chapters for the extraction and determination of organotin and arsenic species were applied to samples taken from a sediment core provided under a collaborative study with Imperial College, London in order to determine the vertical distributions of Sn and As species. The collection of the core used for this study and the dating of the core material is described in detail by O'Reilly Wiese et al. (1997 a). The samples analysed here were taken from the core referred to as 'Core 2' in that publication. So far, the core sediments have been used to obtain information on the vertical distribution patterns of trace metals (O'Reilly Wiese et al., 1997 a), inputs of polychlorinated biphenyls and organochlorine insecticides (Scrimshaw and Lester, 1997) and the solid phase partitioning of metals (O'Reilly Wiese et al. 1997 b). In this work the samples are used to assess the vertical distribution of organotin and arsenic species in the sediment.

7.1 HISTORY OF THE SITE

The Tilbury basin was built in 1880 and comprises 19 acres of mud flats in the lower-mid part of the Thames estuary. This part of the estuary is a high-turbidity zone with intermediate salinities of 11-15 psu (practical salinity unit, Harino et al., 2003). Significant influxes of water, sediments and particulate matter are received from the North Sea and the Thames drainage basin, and therefore this area is subject to rapid siltation. Materials of marine and fluvial origin are deposited on the mud flats, particularly during slack water periods when the tide changes direction.

Initially, the basin was built to provide an access route to the dockyards, but this function ceased when a new lock entrance was constructed. Until 1929 the docks at this site were used extensively, and due to the high sedimentation rates, the channels in the basin were dredged frequently until early 1965 in order to allow

access for shipping. After dredging ceased, the accumulation of sediments in the basin took place at varying rates, with rates decreasing from an initial rate of 3 m/yr (1966) to 0.08 m/yr (1966 - 67) to 0.01 m/yr (1968 to present). The area has seen intense industrial activity including brickworks, steel manufacturers, battery recycling centres, automobile manufacturers, and gas works, as well as receiving inputs from some of Europe's largest sewage treatment works (STW) at Beckton, Crossness and Tilbury. There has been a general decline in industrial activity in the area since the mid-1970s, but inputs of urban run-off and from the STW continue to the present day. With this recent history, the location provides an ideal spot for a core sample, which should reflect a profile for organometallic analytes of environmental importance from a variety of possible sources.

7.2 ORGANOTIN SPECIATION IN SURFACE AND SUB-SURFACE SEDIMENTS

Harino et al. (2003) described the findings of their research into organotin species in the Mersey and Thames estuaries a decade after UK TBT legislation. Some of the samples were taken from the Tilbury area, although the exact location was not described in detail. The data provided for butyltin species in surface sediments from this site (MBT: 0.006, DBT: 0.009 and TBT: 0.011 all as μg/g Sn dry weight) can be used to put the data obtained here into context. However, the analytical methodologies are significantly different in their respective approaches, and the method used by Harino et al. (2003) does not appear to be validated by the use of a certified reference sediment, which makes the accuracy difficult to assess. The method applied here for organotin speciation is based on the approach described in detail in Chapter 4, and is briefly summarised below.

7.2.1 Sample Preparation and Analysis

Sub-samples from different depths throughout the core were air-dried and ground at Imperial College in June 2003 and stored in clear glass vials with screw tops.

7.2.1 a) Extraction

In addition to the sediment samples from the Tilbury core, several subsamples of a certified reference sediment (PACS-2) were also analysed. Polycarbonate chips from the core liner material and extraction blanks were also taken through the analytical

procedure. A mixed ^{117}Sn enriched DBT and TBT spike was prepared as an internal standard to enable quantitation of these compounds by isotope dilution mass spectrometry.

Table 7.1 shows the depths below Ordnance Datum from which the samples were taken. The sample bottles were shaken manually for several minutes to homogenise the sediments prior to sub-samples being taken for analysis. About 1.6g of the samples were weighed into 11mL ASE cells fitted with PTFE liners and filled with a diatomaceous dispersing agent. The sample was mixed with the dispersing agent and spiked with 100μL of the isotopically labelled ^{117}Sn analogues.

Table 7.1 Sample weights and dilutions factors.

Depth below OD (cm)	Depth of core (cm)	Sample weight taken for extraction (g)	Weight of solvent after extraction (g)	Dilution factor
141-151	45 -50	1.4889	14.1442	9.5
180-190	65 -70	1.6154	14.4456	8.9
220-229	85 -90	1.6141	13.6132	8.4
259-269	105 -110	1.6295	14.8069	9.1
298-308	125 -130	1.6949	14.2349	8.4
337-347	145 -150	1.6419	14.6642	8.9
376-386	165 -170	1.6745	14.0570	8.4
416-425	185 -190	1.6319	15.0910	9.2
455-465	205 -210	1.7874	14.0183	7.8
494-504	225 -230	1.6304	14.3934	8.8
533-543	245 -250	1.6471	14.3927	8.7
572-582	265 -270	1.7634	13.7987	7.8
612-621	285 -290	1.6152	14.1542	8.8
651-661	305 -310	1.6063	14.1466	8.8
690-700	325 -330	1.6691	12.6686	7.6
739-749	350 -355	1.6586	14.2145	8.6
778-788	370 -375	1.5984	14.4075	9.0
857-866	410 -415	1.6181	14.5570	9.0
896-906	430 -435	1.6095	12.1581	7.6
935-945	450 -455	1.6613	14.6674	8.8
974-984	470 -475	1.7138	14.7480	8.6
1013-1023	490 -495	1.7072	14.7535	8.6

The samples were then extracted using Accelerated Solvent Extraction (ASE) under the conditions shown in Table 7.2. After the extraction, the extracted solvent was weighed in order to facilitate the calculation of a gravimetric dilution factor. The

extracts obtained from the core samples varied in colour from bright yellow to greenish-yellow. Of the samples extracted only one failed (Vial 11, Depth 455 – 465cm below OD) to be satisfactory. This was only apparent after the extraction as the solution was not of the same colour as the others and no OT-species were detected during the analysis of that extract.

Table 7.2 Accelerated solvent extraction parameters.

Instrument	Dionex ASE 300
Cell size	11 mL
Pre-Heat	2 minutes
Heat	5 minutes
Extraction steps	5 x 3 minutes
Temperature	100°C
Pressure	1500 psi
Flush volume	40%
Purge time	100s
Solvent	50% (v/v) $CH_3COOH/MeOH$

7.2.1 b) Derivatisation

The sample extracts were derivatised using sodiumtetraethylborate (STEB). A 1M sodium acetate buffer was prepared to adjust the extracts to pH 4.5 – 5.0. 5 mL of the sample extracts were added to 5mL of buffer solution, 2mL of hexane and 1 mL of 2% w/w STEB in amber screw-capped glass vials. The mixture was shaken manually for 10 minutes and the organic and aqueous phases were allowed to separate. The hexane layer was then transferred into a GC autosampler vial using a glass Pasteur pipette. The hexane fractions were stored in the fridge at 4°C if they could not be analysed immediately.

After phase separation, a green-brownish substance appeared in the organic hexane layer. This must have been formed during the reactions of the derivatisation process, as no solid matter was apparent in the sample extracts after ASE. The hexane fraction for analysis was collected in such a manner that this was not disturbed or taken into the autosampler vial. The amount of the substance varied between samples and occurred only in the actual sediments from the Tilbury core, but not in the extraction blanks or quality control CRM sediments, which were also taken through the procedure.

7.2.1 c) GC-ICP-MS Analysis

The GC method used followed that of Wahlen and Wolff-Briche (2003) and is described in Section 4.1.2. For the analysis described here, the instrumental detection limits based on 3 times the standard deviation of the blank signal (3σ) for each analyte were estimated as 0.25 ng/g for MBT, 0.03 ng/g for DBT and 0.03 ng/g for TBT (all as Sn). Based on typical sample weights and dilution factors used, the corresponding method detection limits obtained were 6.1 ng/g for MBT, 0.7 ng/g for DBT and 0.8 ng/g for TBT (all as Sn).

7.2.2 Results and Discussion

Table 7.3 below shows the results for MBT, DBT and TBT determined in the CRM PACS-2. The data for DBT and TBT compare well with the certified values of 1090 ± 150 ng/g and 980 ± 130 ng/g as Sn respectively. These compounds were quantified using species-specific isotope dilution analysis (SS-IDMS) and the methodology used has been tested with good results in a number of international intercomparison studies with other expert laboratories.

Table 7.3 Data for MBT, DBT and TBT in PACS-2. All ng/g (as Sn).

Replicate	MBT	DBT	TBT
1	239	1014	875
2	235	966	885
3	231	993	862
Mean	235	991	874
SD	4	24	12
Recovery (%)	52	91	89

The method has not been tested for the determination of MBT and the certified value (450 ng/g ± 50 ng/g as Sn) for this reference sediment has been shown to be debatable by a number of independent publications (e.g. Chiron et al., 2000; Rodriguez-Gonzalez et al., 2003). The actual MBT content appears to be closer to 600 ng/g, although this compound has not yet been re-certified. The MBT recovery compared to the certified value is low (52%) and compared to the value quoted in the

literature a mean recovery of ~ 40% is obtained for this compound. Based on these data it was decided to apply a recovery correction factor of 2.5 for MBT in the samples provided and to quote the data obtained for DBT and TBT by SS-IDMS uncorrected.

The reasons for the low recovery of MBT from the reference sediment could be due to several factors. Firstly, this compound was quantitated by single-point external calibration rather than species-specific isotope dilution. Therefore, if there were differences in the derivatisation yield for this compound between the extracted samples and the calibration standard used due to interference from matrix components, the accuracy of the result would be affected. Secondly, the affinity of the different butyltin species for charged clay particles in sediments increases with a decreasing number of butyl-chains, in-line with increasing polarity of the compounds. The extraction method used here has been developed and tested for DBT and TBT but has not been adapted for MBT. These factors together may result in the lower recoveries of MBT compared to DBT and TBT.

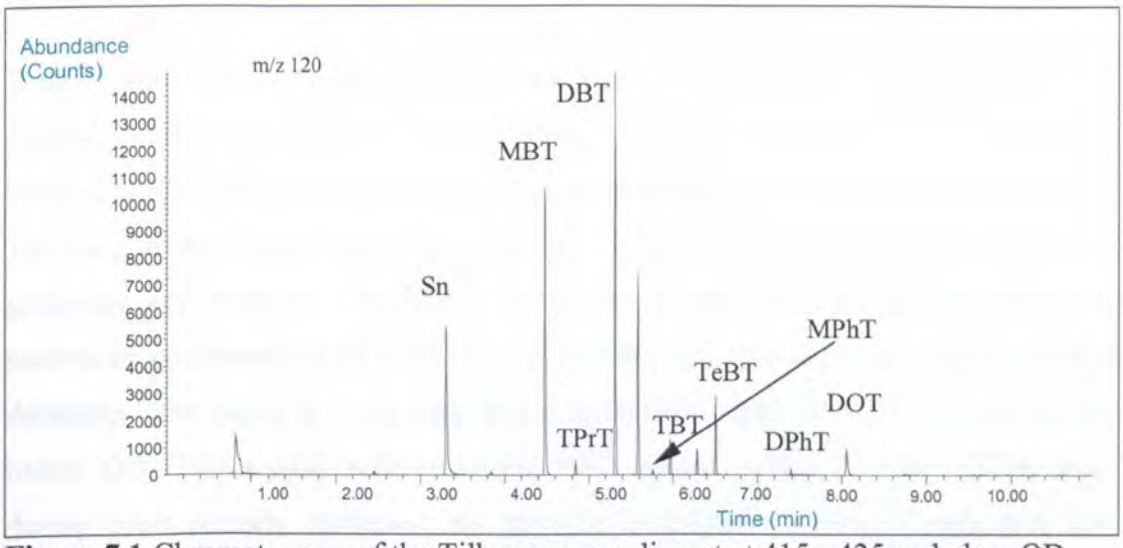

Figure 7.1 Chromatogram of the Tilbury core sediment at 415 - 425cm below OD.

A total of 10 organotin species were detected in samples from the sediment core at the Tilbury Docks (Figure 7.1). Out of these, eight (MPhT, DPhT, MBT, DBT, TBT, TeBT, TPrT, DOT) were identified by retention time matching with standard solutions and three (MBT, DBT, TBT) were quantified by single point external calibration (MBT) or species-specific isotope dilution analysis (DBT, TBT). The

species-specific isotope dilution approach provided further confirmation of the species identity for DBT and TBT. Two species could not be identified with the standards available in the laboratory. Table 7.4 shows the results for MBT, DBT and TBT determined in the samples provided. The distribution of these species is shown diagrammatically in Figure 7.2.

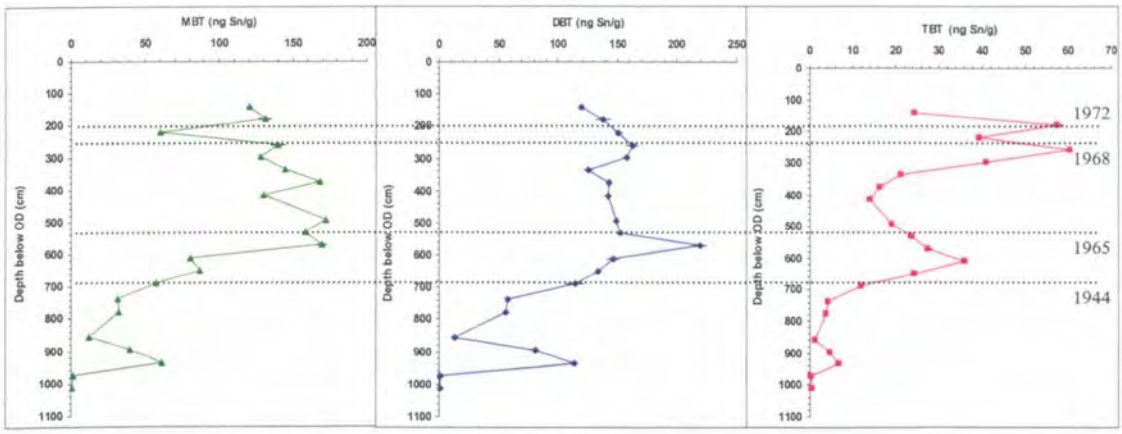

Figure 7.2 Distribution of MBT, DBT and TBT in the sediment core with depth (cm below Ordnance Datum). The error bars indicate one standard deviation of duplicate injections of the same extract.

Broadly speaking, the patterns for the vertical distribution of DBT and MBT are similar and this is reflected in a fairly strong correlation coefficient of 0.73 for their respective concentrations with depth. The concentrations of DBT and MBT are about 150 ng/g as Sn in the region between 100 - 600cm below OD and then decrease gradually with depth to ~ 20 ng/g at 850cm below OD. The TBT profile displays a maximum concentration of ~ 60 ng/g at a depth of ~ 265cm below OD and then decreases with depth to ~ 14 ng/g before increasing again to ~ 36 ng/g at 600cm below OD. This would indicate higher TBT inputs during the early 1970s than during other periods. However, the actual concentration is not so high that this signifies a major input.

Another feature of interest in the distribution of several OT species is the relatively high concentrations detected in the sample at ~ 896 - 945cm below OD. Here, there are significantly higher levels of DBT, MBT, TeBT and an unidentified species compared to the samples corresponding to 200cm above this depth. Such an increase is also noticeable in the Cu, Pb and Zn concentrations in profiles from the same core shown by O'Reilly Wiese et al. (1997). This increase coincides with the

top of an impermeable clay layer that starts at ~ 955cm below OD (Scrimshaw and Lester, 1997). There are several possible reasons for this 'anomaly'.

First, it could be due to deposition of sediment contaminated with these species in line with the accumulation of sediments in this location with time. Given that the sediment layer at ~780cm below OD is dated as ~ February 1944 (O'Reilly Wiese et al. 1997 a) and that organotin compounds were not widely used in industrial applications until after the Second World War, this explanation seems unlikely. A second explanation and one which could in part explain the somewhat convoluted pattern of butyltin species in the core shown in Figure 7.2 is that there may be some vertical transport of certain metals and their organometallic species or complexes through the interstitial water of this sediment. The impermeable clay layer would represent a boundary to such transport and if the main vertical flux was in a downward direction this could lead to accumulation of these analytes in this area. No organotin species were detected in the clay layer itself, which may also support this theory.

Table 7.4 Data for MBT, DBT and TBT (all as ng/g as Sn) in the core sediments

Depth below OD (cm)	MBT	SD (duplicate injections)	RSD (%)	DBT	SD (duplicate injections)	RSD (%)	TBT	SD (duplicate injections)	RSD (%)
141-151	120	2	1.3	119	2	1.2	24	1	0.6
180-190	131	5	3.5	138	6	4.4	58	1	2.1
220-229	60	2	3.8	150	3	2.0	39	1	0.5
259-269	140	5	3.4	162	5	2.7	61	1	0.2
298-308	128	1	0.6	157	3	1.8	41	1	1.4
337-347	144	1	0.1	125	4	2.8	21	1	3.0
376-386	168	2	0.9	142	1	0.2	16	1	1.7
416-425	130	1	0.1	142	1	0.4	14	1	1.5
494-504	172	1	0.4	149	1	0.2	19	1	1.5
533-543	158	2	1.2	152	1	0.7	24	1	3.3
572-582	170	3	1.9	219	5	2.4	28	1	3.3
612-621	81	1	1.5	146	5	3.2	36	1	0.6
651-661	87	1	1.2	134	3	2.0	25	1	1.2
690-700	58	1	0.0	114	7	6.3	12	1	3.6
739-749	32	1	1.7	57	1	0.7	4	1	0.0
778-788	33	1	0.5	55	1	0.6	4	1	1.9
857-866	12	1	0.0	12	1	2.3	1	1	0.0
896-906	40	1	3.1	81	1	0.8	5	1	1.6
935-945	61	1	2.3	113	1	1.2	7	1	2.1
974-984	< 6.1			< 0.7			< 0.8		
1013-1023	< 6.1			< 0.7			< 0.8		

Due to the tidal nature of the location and the great number and variety of possible sources, the interpretation of the vertical profiles of the different butyltin species in this core is considerably more difficult than that of cores taken from inland lake locations. This is because a) the site where the core was taken from has been dredged regularly up to 1966, b) the sediment in the top layer has not accumulated uniformly since dredging stopped, c) the sediment surface is exposed to the atmosphere at low tide and atmospheric processes therefore may also affect organotin distributions, and d) the site undergoes significant fluxes of sediment, particulates and water throughout the tidal cycle and therefore horizontal fluxes as well as vertical deposition affect the distribution pattern of the analytes.

For example, evaporative losses of TBT to the atmosphere during the sediment exposure at low tide and vertical movement within the interstitial water through the sediment column are processes that may affect the vertical distribution of the organotin species determined. Arnold et al. (1998) described TBT data in a sediment core taken near the harbour of Wädenswill on Lake Zürich, Switzerland. The pattern shown indicates maximum concentrations of TBT in the mid-1980s of up to 80 ng/mL (depth of 14 - 16 cm), and decreasing concentrations towards the top of the core up to a dated point of 1997 when concentrations were below 10 ng/mL. This pattern can clearly be linked to decreasing use of TBT in line with legislation prohibiting its use on pleasure craft. Another interesting difference between the core from the lake and that described here are the significantly different sedimentation rates. The sediment accretion rate in the core examined by Arnold et al. (1998) was about 15cm in 11 years, i.e. an annual average accumulation of ~ 1.4 cm/year. Over a similar time period, the accretion rates for the Tilbury basin are estimated as being of the order of ~ 10 cm/year.

The origins of the sediments in both cores are also significantly different. In a lake location without tidal influences, the slow accretion rates indicate that the main source of sediment accumulation is sedimentation from the overlying water column. In a tidal estuary on the other hand, significant inputs of marine sedimentary particles are due to horizontal fluxes throughout the tidal cycle, as well as fluvial inputs of terrestrial origin. In addition, the mixing of saline seawater with freshwater of low salinity leads to the formation and deposition of colloidal material (Libes, 1992), which is not formed at comparable rates in lake waters of fairly constant

hydrochemical composition. A combination of these reasons probably result in the somewhat convoluted patterns displayed in Figure 7.2. This makes it more difficult to use the data from this core to draw firm conclusions about historical usage and deposition patterns of organotin species in the Thames estuary.

In general it can be said that the levels of the butyltin species determined are low compared to more polluted harbour sediments (e.g. PACS-2). The fact that DBT and MBT are the predominant species in the samples possibly indicates a source other than anti-fouling paint from ships. DBT is the main butyltin compound detected and is present at concentrations, which are ~ 3-fold greater than TBT throughout the core, so the breakdown of TBT is not considered to be a likely source of the former. A more likely explanation is a source due to terrestrial inputs such as industrial uses, sewage effluents or leaching from PVC materials. This may be supported by the fact that dioctyltin (DOT), another PVC stabilising compound, was also detected in the samples and a good correlation exists between DBT and DOT (r^2 = 0.79) with depth. The maximum TBT concentrations coincide with a period when this compound was extensively used in antifouling paints applied to ships, but the actual levels determined are low compared to sediments from contaminated major harbours which usually fall in the μg/g as Sn range (Chiron et al., 2000).

The number of samples analysed gives reasonable vertical resolution to determine the distribution pattern throughout the core, and the weight of the sub-samples taken from each depth was designed to address possible problems associated with homogeneity of the core at a particular depth. However, it would be useful in future studies to take more sub-samples from the same depth and estimate the horizontal variation across the diameter of the core at the particular depth. This would enable further conclusions about the small-scale variability in the material to be drawn.

7.3 ARSENIC SPECIATION IN THE SEDIMENT EXTRACTS

The speciation of arsenic in sediments usually depends on a number of different factors. These include the quality of the overlying water column and the pH, redox potential, microbiological activity, and the amount and characteristics of sorbent materials in the sediment itself. In general, total As concentrations are higher in sediments than in the water column. In marine sediments the As levels typically fall

in the μg/g range. The water soluble As is usually inversely proportional to concentrations of Fe, Mn, Al and Ca, which are elements that As can interact with. The main forms of As in sediments and soils are the inorganic species As(III) and As(V). An equilibrium usually exists between these two major species and this is dependent mostly on the redox conditions (Xu et al., 1991). In oxidative environments the equilibrium shifts towards As(V), whereas As(III) is the predominant form in reductive sediments.

Biotransformations of arsenic species in sediments can occur via several different mechanisms, namely reduction, oxidation, methylation and degradation. At present there are about 20 different strains of fungi, yeasts and bacteria that have been shown capable of transforming As-species in-situ. Several of these have been isolated from raw sewage (Cox and Alexander, 1973). This capability is seen as a partly protective mechanism against arsenic-toxicity and allows some of these organisms to persist under extremely high As- concentrations (Da Costa, 1972). The volatilisation of inorganic As by methylation to different forms of arsines (e.g. $(CH_3)_3As$, $(C_2H_5)(CH_3)_2As$) which have a characteristic garlic odour is seen as a major mechanism for removal of As from soil and sediments to the atmosphere. The degradation of AsB to As(V) by microbial activity has also been proposed with TMAO, DMA and MMA as intermediary breakdown products (Hanaoka et al., 1995). For marine sediments it has been shown that no methylated As-species were detectable (Maher, 1984), and it has been proposed that the presence of methylated species must be due to anthropogenic inputs such as agricultural pesticides or industrial activity rather than microbial activity.

7.3.1 Analytical Methodology

The same extracts that were prepared for the organotin determinations described above were used to determine the distribution of arsenic species in the core. The sample pre-treatment and extraction were carried out as described in Section 7.1, except that no ethylation was carried out.

The HPLC-ICP-MS conditions were used as described in Chapter 5. The extracted samples were diluted in the mobile phase (pH 8.2) rather than water to increase the pH of the resulting mixture and reduce the possibility of retention time shifts for the target species, because the solvent used for the extraction of the

sediment samples from the core material contained acetic acid. The chromatographic run-time was also increased in order to monitor the elution of As(V), which was found to be the last eluting compounds in the sediment extracts. Calibrations were performed by external calibration curves for As(III) and As(V).

7.3.2 Results and Discussion

As shown in Figure 7.3, up to 6 different arsenic species where detected in some of the sediment extracts. Out of these, AsB, DMA, MMA, As(III) and As(V) were identified by retention time matching with standards and/or spiking of the sediment extracts with individual analyte solutions. The chromatogram indicates that as expected, the inorganic arsenicals are the main species in the extracts.

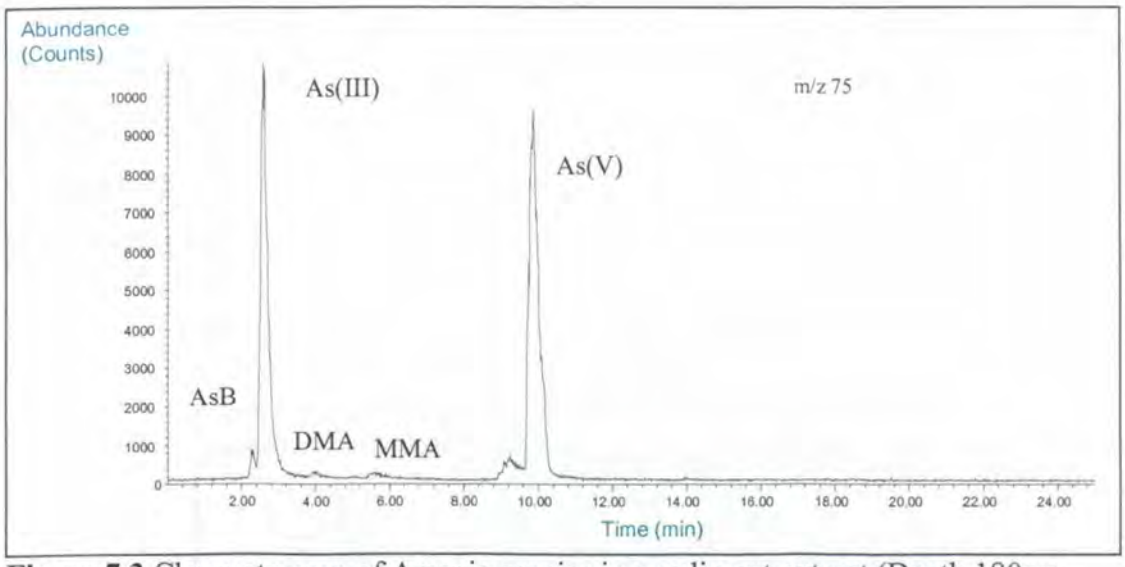

Figure 7.3 Chromatogram of Arsenic species in a sediment extract (Depth 180cm below OD).

Although this is true for all of the samples analysed, the minor species detected, namely AsB, DMA and MMA also contributed to the total As content in varying proportions (1.4% - 6.8% of total peak area) through the core. This pattern is shown in Figure 7.4. Although the pattern is quite variable with depth, there is a general decline from proportions of greater than 3% (AsB at a depth of 220cm below OD) to 0% for all minor species in the clay layer (974cm - 1013cm below OD). This is indicative of inputs of these species from the overlying water column and subsequent transformations to one of the inorganic species after deposition in the sediment. If the presence of these species was due to in-situ methylation of inorganic species by

microbial activity, the general trend would be expected to be one of increasing proportions with depth from the surface.

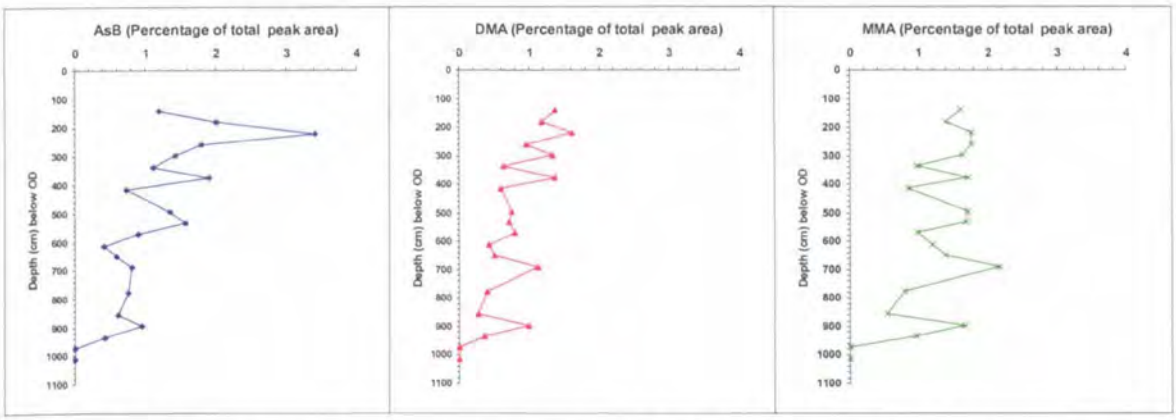

Figure 7.4 Proportions of minor arsenic species (AsB, DMA and MMA) detected in samples from the Tilbury core expressed relative to the total peak area.

The minor species were not individually quantified because the focus of this work was the identification and quantitation of the major As-species in order to determine their distribution pattern with depth, which would allow conclusions about redox characteristics of the core to be drawn if persistent patterns were observable.

Table 7.5 Data for the inorganic arsenical determined in the core sediment samples (ng/g As).

Depth below OD (cm)	As(III)	As(V)	Sum inorganic As
141-151	514	489	1002
180-190	376	442	818
220-229	164	235	399
259-269	504	361	865
298-308	377	326	703
337-347	470	411	881
376-386	287	343	631
416-425	738	458	1196
494-504	416	447	864
533-543	474	435	909
572-582	741	472	1213
612-621	2286	611	2898
651-661	1558	871	2429
690-700	1045	529	1574
778-788	2937	1160	4097
857-866	4201	1136	5337
896-906	772	455	1228
935-945	2163	1412	3575
974-984	213	385	598
1013-1023	201	209	410

As shown by the data in Table 7.5 and Figure 7.5 there is a distinct pattern in the concentrations of both inorganic As-species that can be observed.

The core profile can be divided into three distinct zones in terms of the inorganic As distribution. The 'top layer' ranging to a depth of ~600cm below OD, the 'middle layer' in the region from 600cm to 974cm below OD and the 'clay layer' below 974cm below OD.

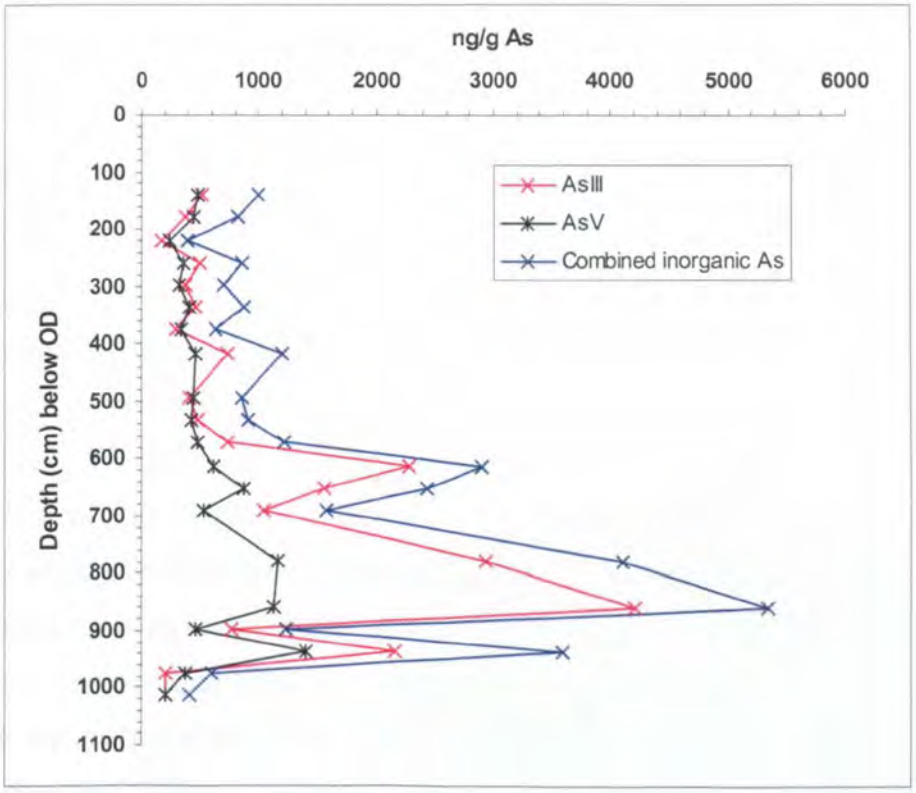

Figure 7.5 Vertical distribution of arsenite (As(III) and arsenate (As(V)) throughout the Tilbury core.

In the top 600cm, concentrations of both of both As(III) and As(V) fall between 160 and 740 ng/g As, and in this part of the core both species are present at similar concentrations. The sum of both inorganic arsenicals in the top part of the core ranges from ~ 400 ng/g to ~ 1200 ng/g as As. Below this depth, arsenite becomes the dominant species with concentrations up to 4200 ng/g As and generally exceeding the levels of As(V) by 1.5 to 3.7 fold. The combined inorganic As concentration in this region varies between ~ 1200 ng/g and 5400 ng/g as As, i.e. on average the sum is about 3.5 times greater in this part of the core than in the overlying 600cm. The distinction between these two layers could indicate that the

sediments in the 'middle layer' form a more reductive environment than those above it. This could be due to the fact that they were deposited at a time prior to improvements in water quality in the 1950 - 1960s, when the dissolved oxygen content was critically low (< 5%) and sewage inputs significantly higher than afterwards. The sediments in the top layer were generally deposited after improvements to the water quality of the Thames had been achieved due to the construction of the major sewage treatment works and in addition, this 'top layer' was more prone to reworking and aeration during the intermittent dredging operations.

In the clay layer (974cm - 1013cm below OD), the combined inorganic arsenic species represented some of the lowest levels in the whole of the core ranging from 400 - 600 ng/g as As. Although this zone is not distinctly different from the 'top layer' in terms of the actual concentrations measured it is significantly different from the 'middle layer' above it.

No certified reference sediment was available at the time to verify the accuracy of this methodology for the determination of inorganic As species in a sediment matrix by an approach that had initially been developed and verified for the analysis of organic arsenicals in biological materials. However, a 'rough' estimate of the method recovery could be calculated by comparing the sum of the inorganic As species to the certified total As content in the PACS-2 sediment that was taken through the analytical procedure. This calculation indicated that the ASE extraction with 50% CH_3COOH in MeOH recovers ~ 61% of the total As determined by a complete sediment digestion using hydrofluoric acid for the certification. Although, this value appears to be quite a low recovery, it would not be expected that a leaching extraction with the aim of maintaining the integrity of the molecular species would completely recover all of the strongly bound As- which is incorporated into the mineral structure of sedimentary particles.

The main question that this estimate of total As recovery cannot answer, is the recovery of individual species and possible transformations during the extraction procedure and subsequent storage and sample preparation. However, all of the samples were treated in the same way and the storage duration was kept to a minimum period under suitable conditions (dark at +4°C), that any conversions taking place should affect all of the samples in the same way. Although it was

therefore not possible to guarantee the absolute accuracy of the data generated here, the data still provide a valuable insight into the general distribution pattern of the inorganic As-species throughout the core. This is also supported by the good agreement between the arsenite and arsenate patterns observed between the top and middle layers and the anticipated distribution according to other research on the behaviour of these species under reducing and oxidising conditions.

7.4 SUMMARY

The data described here provide a valuable insight into the distribution of organometallic species of anthropogenic as well as natural origin in a core taken from the Thames estuary. They highlight the effectiveness of the new speciation methodologies to monitor species of tin and arsenic in an important environmental core sample. The data acquired enables the trends to be followed over several decades (~ 60 years). From this information it is possible to deduce the most likely sources of environmental contaminants. This in turn provides us with a better understanding of the sources and risks associated with different anthropogenic activities.

The study of the speciation of organotin compounds in the sediments using the highly sensitive technique of GC-ICP-MS enable the detection of up to 10 different organotin species in the sediments. Out of these, eight could be identified and the butyltin species MBT, DBT and TBT were quantified to allow the determination of their vertical distribution throughout the core profile. It appears that the main sources of organotin compounds are due to terrestrial inputs, for example effluents containing PVC stabilisers such as DBT or octyltin species as opposed to being due to marine inputs such as TBT from anti-fouling paints on ship's hulls. This is confirmed by the fact that DBT constitutes the major species determined and TBT concentrations are minor in comparison even at depths that coincide with periods of significant use of this compound in antifouling paints.

The overall distribution of organotin species in this core is complex, especially in comparison to sediment cores taken at inland lake locations. This is thought to be a result of the continuously changing tidal inputs as well as dredging operations that have led to the removal of material and hence the disruption of a clear

historical 'profile' The concentrations of the main butyltin species correspond well with data for other sediments from the Thames estuary.

The distribution of arsenicals in the core material also conformed closely to other research in that the data shown here indicates significantly greater levels of As(III) in the deeper sediments below 600cm below OD compared to the overlying top layer of the core. It is therefore likely that the sediments in the middle part of the core represent a more reducing environment than the top-layer. This may be due to the longer time since deposition to the oxygenated surface layer as well as lower levels of dissolved oxygen in the water column at the actual time of deposition. A total of six As-species were detected by the methodology employed, and five of these were identified by retention time matching with available standards. The predominant arsenicals detected throughout the core were arsenite and arsenate at combined concentrations ranging from ~ 400ng/g to 5400 ng/g as As. The presence of organic arsenic species such as AsB, DMA and MMA predominantly in the upper part of the core is a possible indication of anthropogenic inputs rather than in-situ formation due to microbial activity. The contributions of these species were relatively minor in comparison to the inorganic species and ranged from 0 - 6.8% of the total peak area measured.

The bottom part of the core consists of a clay layer and there are no significant concentrations of either organotin species or organic arsenicals, indicating that this may represent an effective boundary to migration of anthropogenic inputs to the underlying geological formations.

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

A number of chromatographic separation methodologies were developed for organotin compounds by gas and liquid chromatography coupled to ICP-MS detection and arsenic speciation by anion-exchange LC-ICP-MS.

Advantages over previously developed organotin methods include improvements in analyte resolution, reductions in analysis time, increased analyte capabilities and/or reduced limits of detection. An existing LC-ICP-MS method for speciation of DBT, TBT, DPhT and TPhT was improved to enable baseline resolution of previously undetected organotin species in a mussel tissue CRM. The addition of gases such as O_2 and N_2 during GC-ICP-MS measurements of organotin species significantly decreased the detection limits for MBT, DBT and TBT in standard solutions from ~ 0.4 - 0.7 ng/mL to 0.006 - 0.01 ng/mL. The use of a novel GC-interface with heated transfer line and injector enabled the separation and detection of more than 13 organotin compounds after ethylation.

GC and LC separations were compared for the IDMS measurement of TBT in sediment extracts. This highlighted the benefits of GC-ICP-MS such as superior analyte capabilities and measurement precision, which were linked to the greater resolution power, higher sensitivity and more reproducible peak integration by this technique. However, both methods delivered equally accurate results and LC-ICP-MS was considered to be a more time-efficient option for certain analytes, because no derivatisation was required during sample preparation, which significantly increases sample throughput by this method. Two separate anion-exchange chromatographies were developed for the separation of arsenicals using isocratic elution. Good resolution was achieved for up to seven analytes in oyster tissue, particularly between AsB and As(III).

The developed methodologies formed the basis for evaluating a range of extraction techniques (sonication, mechanical shaking, microwave extraction and accelerated solvent extraction) for the extraction of organotin and arsenic species from environmental and biological matrices, including sediments and oyster, mussel and fish tissues. Although several of the techniques examined delivered promising results for the extraction of Sn species and arsenicals in a range of available CRMs, accelerated solvent extraction (ASE) was selected for further method development in

an attempt to develop a novel simultaneous extraction approach for organometallic species of different elements (As, Hg and Sn). ASE was chosen because this technique provided the greatest scope for automated high sample throughput extractions, and because accurate data for separate extractions of organotin species from sediments and oyster tissue as well as arsenicals from a range of marine CRMs had been obtained during method validation previously.

A methanolic mixture of 50% acetic acid was used for extraction of an oyster tissue candidate reference material (BCR710) known to contain species of Sn, As, and Hg. Promising results for this simultaneous extraction approach with recoveries ranging from 76 - 99% were obtained for some organotin (DBT and TBT) and arsenic species (AsB and DMA) as well as for MeHg, whilst the low recovery (28%) of MMA requires further investigation.

Species-specific isotope dilution using ^{117}Sn enriched species was used for the accurate and precise determination of organotin species during several certification campaigns for the production of new speciated CRMs (e.g. BCR710 oyster tissue; P-18/HIPA-1 sediment). In addition, the labelled analogues of DBT, TBT and TPhT were also applied to elucidate the processes affecting these species during extraction and derivatisation. Species transformations during ASE extractions of sediments were monitored using labelled DBT, and the derivatisation efficiency of ethylation with sodiumtetraethylborate was verified in mussel tissue extracts by a comparison between SS-IDMS and external calibration data. This indicated that in the extracts concerned, TPhT and TBT had been fully derivatised, whereas DBT had not been. In a matrix-free calibration standard the order of derivatisation efficiency was established as TBT, DBT, MBT, TPhT, DPhT, MPhT, which had indicated an influence of the molecular structure of the species on the derivatisation efficiency, in addition to the number of ethylgroups required for full derivatisation.

The combined extraction and separation methodologies were employed for a study of the organotin and arsenic species in a sediment core from the Thames estuary (UK). In total, more than nine organotin species and six arsenicals were detected at varying levels throughout the core. The major organotin compound was DBT at maximum concentrations of 160 ng/g Sn. The vertical distributions of MBT, DBT and TBT were determined and there were significant variations in all three species with depth. The determination of the different sources of the respective

compounds was difficult to assess due to the fact that sediment deposition had not been uniform and the site had been dredged repeatedly. However TBT is present at relatively low levels compared to sites from polluted harbours with significant inputs of anti-fouling compounds, which suggests that terrestrial sources need to be considered, especially for compounds such as DBT and DOT which are used in PVC stabilisation processes. The main arsenic species detected were the inorganic arsenicals As(III) and As(V). Concentrations generally increase with depth, especially below 600cm below OD and are generally in the range of ~160 - 4200 ng/g As. Methylated arsenic species such as AsB, DMA and MMA were also detected throughout the core, although at significantly lower levels.

Recommendations for further work have been highlighted in the relevant chapters of this thesis. In particular, it would be worthwhile to investigate the simultaneous ASE approach further for the extraction of methylmercury from biological tissues. As discussed in Chapter 6, there seems to be a concentration or matrix dependent saturation effect for this compound, and further investigation into the exact extraction behaviour of this toxic species would make the application of the method for this compound more robust.

The extraction and quantitation of MBT in a wide range of environmental samples would also warrant further investigation, as the recovery of this compound from the CRM sediment PACS-2 was not satisfactory, although it was not conclusively determined whether this was due to extraction or derivatisation related effects. Since this is one of the main breakdown products of the more toxic compounds DBT and TBT, it is useful to monitor this species in environmental samples.

A fuller assessment of the accuracy of the determination of inorganic As species in sediments and soils using a range of suitable CRMs would also be a useful study, which would shed further light on the exact concentration distribution of the major species throughout the Tilbury core.

The study of organometallic compounds with regards to clinical and human health related applications is also a growing research field, and work is currently underway to determine the suitability of the methods developed here for environmental applications to sample types such as urine, serum and whole blood.

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APPENDIX

APPENDIX 1. REGULATIONS ON ANTIFOULING PAINTS WORLDWIDE

APPENDIX 2. CERTIFIED REFERENCE MATERIALS USED

APPENDIX 1. REGULATIONS ON ANTIFOULING PAINTS WORLDWIDE

Country	Year	Regulations
Austria	Not specified	Banned the use of TBT antifouling paint in fresh water lakes.
Australia	1989	Prohibited the use of TBT-based paints on vessels less than 25 metres (m) in length. Maximum leaching rate of 5 micrograms per square centimetre per day ($\mu g/cm^2/day$) for vessels greater than 25 m in length. All drydocks must be registered with the Environmental Protection Agency because of discharges. All antifoulants must be registered.
Canada	1989	Prohibited the use of TBT-based paints on vessels less than 25 m in length, except for aluminum-hulled vessels. Maximum leaching rate of 4 $\mu g/cm^2/day$ for vessels greater than 25 m in length. All antifoulants must be registered.
Commission of the European Communities (EC)	1991	Prohibited the use of TBT-based paints on vessels less than 25 m in length. TBT antifoulants available only in 20 litre (L) containers.
Europe (non-EC members)	Varies	Prohibited the use of TBT-based paints on vessels less than 25 m in length (most countries).
Finland	1991	Prohibited the use of TBT-based paints on boats less than 25 m in length.
France	1982	Prohibited the use of TBT-based paints on vessels less than 25 m in length, except for aluminum-hulled vessels.
Germany	1990	Prohibited the use of TBT-based paints on vessels less than 25 m in length. Ban on retail sale. Ban on its use on structures for mariculture. Regulation for the safe disposal of antifouling paints after removal.
Hong Kong	Not specified	All TBT antifoulants must have a valid permit for import/supply. All antifoulants must be registered.
Ireland	1991	Prohibited the use of TBT-based paints on vessels less than 25 m in length. TBT antifoulants available only in 20-L containers. All antifoulants must be registered.
Japan	1990 1992	TBT banned for all new vessels. TBT banned for all vessels.
The Netherlands	1990	Prohibited the use of TBT-based paints on vessels less than 25 m in length. Washing/blasting slurry used to prepare TBT antifoulants may be treated as hazardous waste. TBT antifoulants available only in 20-L containers. All antifoulants must be registered.

APPENDIX 1. CONTINUED: REGULATIONS ON ANTIFOULING PAINTS WORLDWIDE

Country	Year	Regulations
New Zealand	1989	The application of TBT copolymer antifouling paint is banned with three exceptions: hulls of aluminum vessels, the aluminum outdrive, or any vessel greater than 25 m in length. The application of TBTO free-association paints is banned. Maximum leaching rate of 5 $\mu g/cm^2/day$ for vessels greater than 25 m in length. All antifoulants must be registered.
	1993	Use of any organotin containing antifouling paint was totally prohibited.
Norway	1989	Prohibited the use of TBT-based paints on vessels less than 25 m in length.
South Africa	1991	Prohibited the use of TBT-based paints on vessels less than 25 m in length. TBT antifoulants available only in 20-L containers. All antifoulants must be registered.
Sweden	1989	Prohibited the use of TBT-based paints on vessels less than 25 m in length.
	1992	Maximum leaching rate of 4 $\mu g/cm^2/day$ for vessels greater than 25 m in length. All antifoulants must be registered.
Switzerland	1987	The use of TBT-based antifouling paints is banned in fresh water lakes. All antifoulants must be registered.
United Kingdom	1985	Restricted the sale of TBT-based paints, effectively banning all TBTO free-association paints.
	1987	Prohibited the use of TBT-based paints on vessels less than 25 m in length and on fish-farming equipment. TBT antifoulants available only in 20-L containers. All antifoulants must be registered as pesticides; Advisory Committee on pesticides must approve sale and use. Washing/blasting slurry may be treated as hazardous waste.
United States	1988	Prohibited the use of TBT-based paints on vessels less than 25 m in length, except for aluminum-hulled vessels. Maximum leaching rate of 4 $\mu g/cm^2/day$ for vessels greater than 25 m in length.
	1990	All antifoulants must be registered. TBT-based antifouling paints can only be applied by certified applicators.

Source:
http://ortepa.org/pages/r2.htm
October 2003

APPENDIX 2. CERTIFIED REFERENCE MATERIALS USED

Name	Supplier	Material	Species Certified*
CRM477	BCR	Mussel tissue	MBT: 1.50± 0.28 mg/kg DBT: 1.54 ± 0.12 mg/kg TBT: 2.20 ± 0.19 mg/kg
CRM626	BCR	AsB in solution	AsB: 1031 ± 6 mg/kg
CRM627	BCR	Tuna fish	AsB: 52 ± 3 µmol/kg DMA: 2.0 ± 0.3 µmol/kg
CRM710	BCR	Oyster tissue[a]	MBT: 0.049 ± 0.014 mg/kg DBT: 0.082 ± 0.017 mg/kg TBT: 0.133 ± 0.025 mg/kg AsB: 32.98 ± 5.08 mg/kg DMA: 0.82 ± 0.18 mg/kg MeHg: 0.115 ± 0.014 mg/kg
DOLT-2	NRC	Dogfish liver[§]	MeHg: 0.693 ± 0.053 mg/kg Hg
DORM-2	NRC	Dogfish muscle	AsB: 16.4 ± 1.1 mg/kg As $TMAs^+$: 0.248 ± 0.054 mg/kg As MeHg: 4.47 ± 0.32 mg/kg Hg
P-18/HIPA-1	NRC, LGCPromochem	Marine sediment	TBT: 80.6 ± 10.6 ng/g Sn
PACS-2	NRC	Marine sediment	MBT: 0.45 ± 0.05 mg/kg Sn DBT: 1.09 ± 0.15 mg/kg Sn TBT: 0.98 ± 0.13 mg/kg Sn
TORT-2	NRC	Lobster hepatopancreas[§]	MeHg: 0.152 ± 0.013 mg/kg Hg

* Concentrations given as species unless otherwise stated.
[a] Concentrations based on consensus mean values and one standard deviation from certification round.
[§] Certification of arsenic species in these materials is currently underway.

Supplier contact details

BCR:
Community Bureau of Reference
Commission of the European Communities
Rue de la Loi, 200
B-1040 Brussels
Belgium

LGC Promochem:
Queens Road
Teddington
Middlesex
TW11 0LY
United Kingdom

NRC:
National Research Council of Canada
Institute for National Measurement Standards
M-12 Montreal Road
Ottawa, Ontario, Canada K1A 0R6