Development of a multi-species method by GC-coupling with inductively coupled plasma isotope dilution mass spectrometry for the simultaneous determination of alkylated lead, mercury and tin compounds

Dissertation
zur Erlangung des Grades
"Doktor der Naturwissenschaften"
(Dr. rer. nat.)

Fachbereich Chemie, Pharmazie und Geowissenschaften
der Johannes Gutenberg-Universität Mainz

Nataliya Poperechna
geboren in Khmelnytsky, Ukraine

Mainz 2005

Abstract

An accurate and sensitive species-specific GC-ICP-IDMS (gas chromatography inductively coupled plasma isotope dilution mass spectrometry) method for the determination of trimethyllead and a multi-species-specific GC-ICP-IDMS method for the simultaneous determination of trimethyllead, methylmercury, and butyltins in biological and environmental samples were developed. They allow the determination of corresponding elemental species down to the low ng g^{-1} range. The developed synthesis scheme for the formation of isotopically labeled $Me_3{}^{206}Pb^+$ can be used for future production of this spike. The novel extraction technique, stir bar sorptive extraction (SBSE), was applied for the first time in connection with species-specific isotope dilution GC-ICP-MS for the determination of trimethyllead, methylmercury and butyltins. The results were compared with liquid-liquid extraction. The developed methods were validated by the analysis of certified reference materials. The liquid-liquid extraction GC-ICP-IDMS method was applied to seafood samples purchased from a supermarket. The methylated lead fraction in these samples, correlated to total lead, varied in a broad range of 0.01-7.6 %. On the contrary, the fraction of methylmercury is much higher, normally in the range of 80-98 %. The highest methylmercury content of up to 12 µg g^{-1} has been determined in shark samples, an animal which is at the end of the marine food chain, whereas in other seafood samples a $MeHg^+$ content of less than 0.2 µg g^{-1} was found. Butyltin species could only be determined in samples, where anthropogenic contaminations must be assumed. This explains the observed broad variation of the butylated tin fraction in the range of <0.3-49 % in different seafood samples. Because all isotope-labelled spike compounds, except trimethyllead, are commercially available, the developed multi-species-specific GC-ICP-IDMS method has a high potential in future for routine analysis.

Zusammenfassung

Eine genaue und nachweisstarke spezies-spezifische GC-ICP-IDMS (gas chromatography inductively coupled plasma isotope dilution mass spectrometry) Methode für die Bestimmung von Trimethylblei sowie eine multi-spezies-spezifische GC-ICP-IDMS Methode für die simultane Bestimmung von Trimethylblei, Monomethylquecksilber und drei Butylzinnspezies in biologischen und Umweltproben bis in den ng g^{-1} Bereich hinein wurden entwickelt. Das entwickelte Syntheseschema für einen isotopenangereicherten $Me_3{}^{206}Pb^+$-Spike kann für die zukünftige Herstellung dieses Spikes verwendet werden. Die neuartige Extraktionstechnik, SBSE (stir bar sorptive extraction), wurde zum ersten Mal in Verbindung mit einer spezies-spezifischen GC-ICP-IDMS für die Bestimmung von Trimethylblei, Monomethylquecksilber und Butylzinnverbindungen verwendet. Die Ergebnisse wurden mit der Flüssig-Flüssig-Extraktion verglichen. Die entwickelten Methoden wurden mittels der Analyse von zertifizierten Referenzmaterialien validiert. Die Flüssig-Flüssig-Extraktion GC-ICP-IDMS Methode wurde zur Analyse von Seafoodproben aus dem Supermarkt verwendet. Der Anteil von Trimethylblei in diesen Proben schwankt im breiten Bereich zwischen 0,01 % und 7,6 %. Entgegengesetzt ist der Anteil von Methylquecksilber wesentlich höher, üblicherweise im Bereich von 80-98 %. Die höchste Methylquecksilberkonzentration bis zu 12 µg g^{-1} wurde in Haifischproben, Tieren am Ende der Meeresnahrungskette, bestimmt wobei in anderen Proben ein Methylquecksilbergehalt geringer als 0,2 µg g^{-1} gefunden wurde. Butylzinnspezies konnten nur in Proben bestimmt werden, wo anthropogene Kontamination vorauszusetzen war. Dies erklärt die beobachtete breite Variation von Butylzinnanteil im Rahmen von <0,3-49 % in unterschiedlichen Seafoodproben. Da alle isotopenangereicherten Spikeverbindungen, außer Trimethylblei, kommerziell erhältlich sind, hat die entwickelte multi-spezies-spezifische GC-ICP-IDMS Methode in der Zukunft ein hohes Potential für die Routineanalyse.

Quality is never an accident. It is always the result of high intention,
sincere effort, intelligent direction, and skillful execution.
It represents the wise choice of many alternatives.

William Foster

Index

1 General part

1.1 Introduction

Metals have been the focus of applied atomic spectroscopy from the very beginning of its use. The interest in trace metals has led to the determination of their concentrations at successively lower levels in a variety of samples. The quest for the background level of platinum in blood, for the levels of gold in open ocean water, or for femtogram traces of Roman lead in the two-thousand-years-old Arctic snow and ice layers is no longer a fiction. Inorganic trace analysis entered the 21st century as a well-established field of analytical chemistry. In the real world, metals seldom exist as free ions. Usually, they occur as different species resulting from a series of natural bio(geo)chemical processes. The species carries a message which is vital for the evaluation of the metal bioavailability, and hence its toxicity or essentiality, and for the understanding of mechanisms controlling biological life. Therefore information on a metal which is based uniquely on its total concentration has been recognized not only to have little meaning but often to be misleading [1].

The harmful effects of the chemical forms of trace metals in toxicology were brought to broad public attention in the late 1950s, when it was discovered that the widespread occurrence of neurological disease among residents in Minamata Bay and along the Agano River, Japan, was linked to methylmercury poisoning. Local chemical industries producing acetaldehyde and vinyl products were eventually pinned down as pollution sources. In the manufacturing processes used, catalytic inorganic mercury compounds were unintentionally converted into methylmercury that was discharged with process wastewater to nearby fishing grounds. Additionally inorganic mercury was also transformed to methylmercury through biomethylation by microorganisms and algae. Methylmercury was accumulated through the aquatic trophic levels and the people in these areas, who depended heavily on a fish-rich diet, were exposed to considerable amounts of methylmercury. By the end of November 1999, nearly 2,300 diagnosed cases of methylmercury poisoning (Minamata disease) had been identified in Kumamoto and Kagoshima Prefectures [2]. Other severe cases of alkyl mercury poisonings have occurred, for example in Iraq during the early 1970’s, when flour made from methyl- and ethylmercury treated seed-grain was used to

prepare bread. An epidemiologic follow-up suggested that as many as 40,000 individuals may have been poisoned [3].

Tetramethyllead, tetraethyllead and the corresponding mixed methyl-ethyl compounds were used for a long time in Western Europe and the US as antiknock additives in petrol. The spillage of tetraalkyllead in the Mediterranean Sea, caused by a crash of the M/S Cavteat, made the analytical community sensitive to organic forms of lead. These toxic volatile compounds undergo degradation processes in the atmosphere and environmental waters, forming inorganic lead and trialkyllead as relatively stable species. Even if leaded gasoline is still used in many countries and particularly in Africa and Asia, where it causes a health risk to the local population and ecosystem [4], trimethyllead in the marine environment is usually of biogenic origin today (Chapters 2.1).

Extinction of the oyster population in the Arcachon Bay in southern France stimulated interest in the possible release of tributyltin from antifouling paints [5]. This compound is very toxic to marine organisms at very low concentrations. Tributyltin can be transformed under influence of light and microorganisms in aqueous media into the less toxic dibutyltin and monobutyltin species. These butylated tin species are quite stable in sediments, where they are accumulated. They are also bio-accumulated by living organisms from the water-sediment interface, causing severe and long-term toxic effects on the local fauna with impact on biodiversity and human health [6].

Because of the emerging importance of elemental speciation and its relevance to bioscience, material science, environmental chemistry, occupational health, eco- and clinical toxicology, foodstuff, pharmaceutical industry and other issues of societal importance, efforts are being directed toward the development of new instruments and techniques, that can provide information not only about the elemental composition of a sample, but also about the chemical forms, in which each element is found. Due to the high toxicity of methylmercury, trimethyllead and butyltin compounds (Chapter 2.2) and their possible accumulation in sediments and marine animals, the development of a corresponding accurate and simultaneous multi-species method is of high importance for the quality assurance of seafood and the environmental control. Sensitive and selective analytical techniques must be applied to detect the usually low concentration level of these organometallic species in biological and environmental samples. Analytical methods for alkylmetal determinations, as described in literature, include different sample preparation steps,

e.g. derivatization, extraction and chromatographic separation (Chapter 2.3). Each of these analytical steps can affect the accuracy of the analytical result, which can also be influenced by transformation of the elemental species.

The use of certified reference materials (CRM) is one usual way to validate analytical procedures. Unfortunately, there are only a few CRMs available for individual elemental species and different matrices. No reference material is available for a simultaneous determination of trimethyllead, monomethylmercury, and the butyltin species. The species-specific isotope dilution mass spectrometric analysis (IDMS) with its high accuracy, precision and its ability to control possible species transformations during sample preparation is an important alternative to the application of reference materials. IDMS is an analytical method, based on the measurement of isotope ratios in samples, where its isotopic composition has been altered by the addition of a known amount of an isotopically enriched element (species). Isotope dilution methods were developed for a wide range of applications in elemental speciation during the last years. Investigation on the evolution of number of publications on isotope dilution analysis for elemental speciation shows a continuous increase, particularly over the last two years (Figure 1). This demonstrates the tremendous importance which species-specific and species-unspecific IDMS is acquiring nowadays in the growing field of elemental speciation analysis.

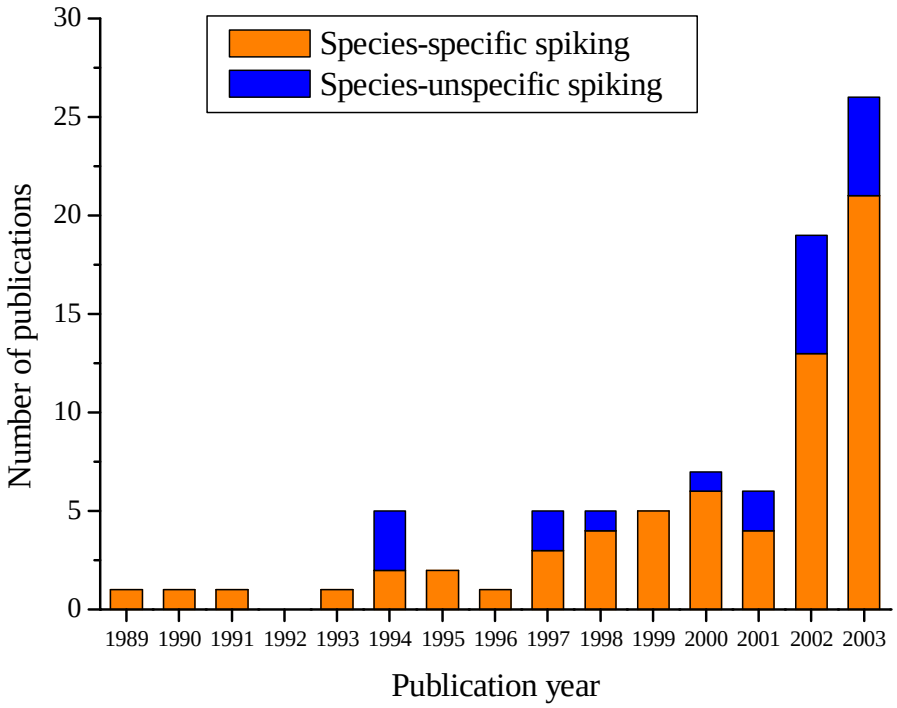

Figure 1: Evolution of the number of publications on isotope dilution analysis in elemental speciation by species-specific and species-unspecific spiking modes [7]

The first purpose of this work was the development of a sensitive species-specific IDMS method for the determination of trimethyllead in environmental and biological samples by GC-ICP-MS coupling. Because an isotopically enriched trimethyllead spike is not commercially available, its synthesis in the milligram scale followed by stability studies and characterization is one of the main and important tasks for GC-ICP-IDMS determination. The next purpose was the development of a multi-species-specific GC-ICP-IDMS method for simultaneous determination of trimethyllead, monomethylmercury, mono-, di- and tributyltin.

Suitable sample preparation procedures, which guarantee total mixing of natural and isotope-enriched species, should be developed for environmental (soil, dust, sediment) and biological (seafood) samples. Degradation and transformation of the species being analyzed during the sample preparation procedure should be controlled. GC and ICP-MS conditions should be optimized for good separation and sensitive determination of lead, mercury and tin species. Achievement of accurate and precise analytical results from trace elemental speciation analysis

by isotope dilution mass spectrometry requires accurate and precise isotope ratio measurements on fast transient signals, produced by gas chromatography. Therefore parameters, affecting the accuracy and precision of the measured isotope ratios, should be taken into account and must carefully be optimized.

Besides the classical liquid-liquid extraction, a relatively novel solid phase extraction technique, stir bar sorptive extraction, should be applied for GC-ICP-IDMS determination of trimethyllead, methylmercury and butyltins. Advantages and disadvantages of both approaches should be compared and discussed in consideration of their routine application.

The developed GC-ICP-IDMS method should be validated by the analysis of different reference materials, certified for individual species. The accuracy and precision of the analytical results should be discussed in comparison with results from literature, obtained by other methods.

Another purpose of this work was the application of the developed method for multi-species determination of trimethyllead, methylmercury and butyltins in different representative seafood samples. Besides the concentrations of alkylated metal species, the total content of lead, mercury and tin was determined to get more knowledge on the contamination profile of this type of food. This includes development and optimization of microwave-assisted sample preparation procedure for multi-element determination of lead, mercury and tin by ICP-IDMS and the characterization of the corresponding spike solutions.

1.2 Summary of results

In this work an accurate and sensitive species-specific GC-ICP-IDMS method for the determination of trimethyllead and a multi-species-specific GC-ICP-IDMS method for the simultaneous determination of trimethyllead, methylmercury, and butyltins in biological and environmental samples were developed. They allow the determination of corresponding elemental species down to the low ng g^{-1} range.

A synthesis scheme for the formation of isotopically labeled $Me_3{}^{206}Pb^+$ with high total yield (75 %) was developed which can be used for future production of a corresponding spike compound. A synthesized isotope-enriched trimethyllead spike was characterized with respect to isotopic composition, purity and concentration. A study of the spike stability by reverse isotope dilution analyses showed no significant change of the $Me_3{}^{206}Pb^+$-concentration within 18 months of storage at 4˚C under exclusion of light.

The novel extraction technique, stir bar sorptive extraction (SBSE), was applied for the first time in connection with species-specific isotope dilution GC-ICP-MS for the determination of trimethyllead, methylmercury and butyltins. The results were compared with liquid-liquid extraction. Detection limits for hexane extraction and SBSE (given in brackets) for biological samples are comparable: 1.4 (0.9) ng g^{-1} for $MeHg^+$, 0.06 (0.04) ng g^{-1} for Me_3Pb^+, 0.3 (0.6) ng g^{-1} for $BuSn^{3+}$, 1.2 (2.3) ng g^{-1} for Bu_2Sn^{2+} and 0.3 (0.2) ng g^{-1} for Bu_3Sn^+. Similar detection limits were obtained for environmental samples. They are sufficient for the routine determination of methylmercury, trimethyllead and butyltin compounds in most biological and environmental samples. The SBSE requires additional desorption equipment, cryofocusing of the analytes prior to GC separation and is more time consuming than classical liquid-liquid extraction. Therefore hexane extraction was applied for the analysis of seafood samples.

Significant demethylation of methylmercury was observed during pretreatment with tetramethylammonium hydroxide, which is used for digestion of biological samples. But this species transformation has no influence on the final result if species-specific isotope dilution is used for quantification. In the case of environmental samples (like sediments) artifact formation of methylmercury in the presence of high excess of inorganic mercury was found. This must be taken into account and necessary corrections must especially be made for samples in which the $MeHg^+$ / Hg^{2+} ratio is extremely low. A separation of Hg^{2+} from organomercury prior to derivatization and extraction was found to reduce the artifact effect and can be used as an alternative solution for this problem.

The developed GC-ICP-IDMS method was validated by the analysis of certified reference materials demonstrating the reliability of the method. GC-ICP-IDMS results were also compared with results from literature, obtained by other methods. The precision of 1.1-4.3 % by GC-ICP-IDMS was much better compared with other methods (7.1-13.9 %).

The species-specific GC-ICP-IDMS method can be applied as a reference method for other analytical techniques, because it offers high accuracy, good precision and the possibility to control possible species transformations during sample preparation. It may also push the certification of trimethyllead in other materials than the only available certified reference material, urban dust. Meanwhile the obtained results for the trimethyllead concentrations in reference materials, not certified for this elemental species, can be used as reliable indicative values, because these materials are internationally available.

The liquid-liquid extraction GC-ICP-IDMS method was applied to analyze trimethyllead, methylmercury and butyltin compounds in seafood samples, purchased from a supermarket. Additionally the total content of lead, mercury and tin was determined by ICP-IDMS. As an important result it was found that the methylated lead fraction correlated to total lead in these samples varied in a broad range of 0.01-7.6 %. On the contrary, the $MeHg^+$ fraction is much higher, normally in the range of 80-98 %. The highest methylmercury content (up to 12 $\mu g\ g^{-1}$) was determined in shark samples, an animal which is at the end of the marine food chain. Other seafood samples showed a $MeHg^+$ content of less than 0.2 $\mu g\ g^{-1}$. The methylmercury concentration correlated well with the corresponding mercury content by a correlation coefficient of 0.998. On the contrary, no correlation was observed between trimethyllead and total lead in the investigated samples. All these results for seafood are in excellent agreement with those for corresponding reference materials and indicate different up-take mechanisms for methylated lead and mercury by marine animals. Butyltin species could only be determined in samples, where anthropogenic contaminations must be assumed. This explains the observed broad variation of the butylated tin fraction in the range of <0.3-49 % in different seafood samples. All results for alkylated metal species in real samples could be determined with similar good precision as in the reference materials (1.1-11.8 %).

The developed multi-species-specific GC-ICP-IDMS method covers the most important toxic alkylated heavy metal species in the marine environment and is therefore a reliable tool for the environmental control and the analytical quality assurance of seafood, which will be of increasing importance in the future. The developed method has a high potential in the future for routine analysis, presuming that an isotopically enriched trimethyllead spike will be commercially available.

2 Fundamentals

2.1 Organometallic compounds in the environment

2.1.1 Anthropogenic sources of alkylated lead, mercury and tin species

Organometallic compounds are found in the environment because they are naturally formed there or they have been introduced by anthropogenic sources. The first documented human activity involving organic lead compounds dates back to the 1850's, when ethylated lead compounds were first synthesized. From these tentative beginnings organolead chemistry has developed into one of the largest areas of organometallic chemistry, largely prompted by the discovery in 1922 that tetraethyllead (Et_4Pb) can act as an efficient antiknock agent for petrol engines. An industry of tetraalkyllead compounds, including Et_4Pb, tetramethyllead (Me_4Pb) and methyl-ethyl mixed compounds introduced later, quickly developed thereafter. The consumption of lead in this industry occupied as much as 15% of the total world lead consumption in the early 1970s (2.5 million tons) and tetraalkyllead was known to be one of the largest volumes of organic compounds being produced at that time [4].

In the 1970s public health specialists addressed serious concern over the adverse health aspects, particularly neurotoxic effects, of inorganic lead emitted into the environment via the combustion of leaded gasoline. From a technical viewpoint, leaded gasoline was found not to be suitable for catalytic converters, a new technology introduced in the 1970s to reduce hydrocarbon, nitrogen dioxide and carbon monoxide emission from the exhaust of cars. This led in 1975 to the use of leaded-free gasoline in the USA and Japan and later also in many European countries. However, leaded gasoline is still used in many countries, particularly in Africa and Asia, and this causes a health risk to the local population and ecosystem [4]. These volatile compounds undergo degradation processes in the environment, forming inorganic lead but also trialkyllead as relatively stable species.

The first methylmercury compounds were synthesized in the 1860's. Apparently, two of the laboratory technicians involved in the experiment died of methyl mercury poisoning. As a result, the scientific community avoided organic mercury compounds until the beginning of the 20th

century when it was found that methyl- and other short-chained alkyl- or phenylmercury derivatives had excellent anti-fungal properties [3]. Organic mercury compounds were from there on used in numerous applications where such properties were desired. For example, methyl- and ethylmercury compounds were efficiently used globally for a long time as a cereal seed dressing and also in the paper pulp industry where phenylmercury acetate was used as a fungicidal additive in the pulp.

Mercury, as inorganic mercury or as organomercury compound, has been used in a wide variety of industrial applications, e.g. as catalyst, in the pulp and paper industry, in the chlor-alkali industry, in batteries, in fungicides and pharmaceutical (e.g. thimerosal) [8]. Besides anthropogenic sources, monomethylmercury and trimethyllead are also produced by algae and bacteria in aquatic systems (Chapter 2.1.2).

The first organotin compound was prepared over 150 years ago. Now total organotin production is more than 50,000 t per year, with about one-quarter of this being triorganotin biocides. About 4,000 t per year of tributyltin derivatives are manufactured, as wood preservatives, antifoulant and disinfectant biocides [9]. The consumption of tin for the production of organometallic tin compounds is 7% of total tin consumption. Other major industrial applications of organotin compounds are heat and light stabilizers for rigid polyvinyl chloride (PVC), homogenous catalysts for silicones, polyurethane forms and transesterification reactions, precursor for forming SnO_2 films on glass, as well as fungicides and acaricides in agriculture. In environmental terms, the role of tributyltin is the most important among the different tin species. Tributyltin was extensively used as wood preservatives and a biocide in antifouling paints. It was first used in “free association” formulations where the biocide is physically mixed with the other paint ingredients. In 1974 the methyl methacrylate-tributyltin copolymer systems had been developed which release Bu_3Sn^+ steadily from the top few nanometres, exposing a free tributyltin-containing polymer surface below. The basic advantages are that release of Bu_3Sn^+ is controlled, constant and just sufficient for the purpose. Large vessels in harbour and inshore small fishing boats and pleasure craft have given rise to the main tributyltin environmental problem. A large vessel lying at anchor in port for 3 days might lose about 200 g of tributyltin to the waters, but 600 g if freshly painted. This can lead to concentrations in large marinas or dockyards of 100-200 ng Sn L^{-1} in the water [6]. The focus on control of Bu_3Sn^+ has been on near shore effects where the reported environmental damage is greatest. Prohibitions for use have therefore been focused on smaller vessels (< 25 metres in length, i.e. inshore craft). At

present only Austria, Switzerland and New Zealand have totally banned tributyltin. On the other hand many other countries still have little or no control over this compound. Bu_3Sn^+ can be transformed in aqueous media under the influence of light and microorganisms into the less toxic Bu_2Sn^{2+} and $BuSn^{3+}$ as relatively stable species. There is some evidence for direct input of Bu_2Sn^{2+} and $BuSn^{3+}$ from PVC materials to the environment, but most comes from tributyltin degradation.

2.1.2 Biomethylation of lead, mercury and tin in the environment

Biomethylation refers to the process where living organisms cause direct linkage of methyl groups (of other alkyl groups much rarer) to metals or metalloids, thus forming metal(loid)-carbon bonds. This process has been extensively studied in nature and biomethylation activity has been found in soil, but mainly occurs in sediments from environmental waters such as estuaries, harbors, rives, lakes and oceans. The attachment of a methyl group to a metal(loid) changes the chemical and physical properties of this element, which in turn influences toxicity, mobility and geological cycling. Biomethylation play, therefore, an important role in biogeochemical cycles of elements, in health hazards and environmental pollution. The organisms responsible for biomethylation are almost exclusively microorganisms. Anaerobic bacteria are thought to be the main agents of biomethylation in sediments and other anoxic environments. Some aerobic and facultatively anaerobic bacteria, as well as certain fungi and algae, have also been shown to be capable of biomethylation.

Biomethylation (i.e. methyl transfer) in organic molecules – such as proteins, nucleic acids bases, polysaccharides and fatty acids – occurs in all living cells and is an essential part of the normal intracellular metabolism. The three main biological methylating agents for organic molecules – S-adenosylmethionine, methylcobalamine and N-methyltetrahydrofolate – have all been shown to be involved in the biomethylation of metal(loid)s. The methyl group in biochemistry is transferred as an intermediate radical ($CH_3^{\bullet}$) or as a carbonium ion (CH_3^+) and thus the recipient must be a nucleophile. N-methyltetrahydrofolate and S-adenosylmethionine are thought to transfer methyl groups as carbonium ions or as an intermediate radical, whereas the transfer potential of S-adenosylmethionine is higher. Conversely, for N-methylcobalamine – a derivative

of vitamin B_{12} – the methyl group is transferred as a carbanion (CH_3^-) and the recipient must be electrophilic. Although methylcobalamine appears to be the sole carbanion-donating natural methylating agent, in the natural environment the carbanion can also be transferred to metals from other organometallic species that may be present [10].

Biological methylation of mercury by microorganisms in aquatic sediments was first demonstrated by Jensen and Jernelöv [11]. Although humic substances may act as methyl donors in abiotic methylation of Hg, the inability of autoclaved samples to methylate mercury has been observed in a number of studies, and it is generally accepted that Hg methylation in nature is principally a biological process [12]. A great number of different bacterial cultures such as *Neurospora*, *Clostridium*, *Pseudomonas*, *Bacillus*, *Enterobacter aerogenes*, *Escherichia coli* etc. can produce methylmercury from added Hg^{2+} [13]. However, the most important Hg methylators in the environment are sulphate reducing bacteria (SRB). In order to elucidate the role of these microorganisms in Hg methylation in natural sediments specific metabolic inhibitors, addition of sulphate, and coincident measurement of sulphate reduction rate and methylmercury production were studied. Through a series of inhibition-stimulation experiments of anoxic estuarine sediment Compeau and Bartha found that the strain *Desulfovibrio desulfuricans* LS displayed very high mercury methylating potential [14]. In subsequent studies [15], the same research group showed that mercury methylation in *Desulfovibrio desulfuricans* LS occurred via donation of a methyl group from methylcobalamine and further suggested that the reaction was mediated through enzymatic catalysis in the acetyl-CoA pathway [16,17]. However, the biochemical pathway for methylation of inorganic mercury in SRB is still somewhat unclear since methylcobalamine and acetyl-CoA mechanisms are not at all unique to SRB and it is likely that another, so far unidentified, component is most likely also involved in this process. Recently it was also shown that some SRB strains, which do not utilize the acetyl-CoA pathway, are also capable of mercury methylation and possibly even without the presence of methylcobalamine [18]. At present there seems to be no clear answer why SRB methylate mercury. In living organisms there are a number of biochemical pathways performing methylation on a variety of substrates, e.g protective methylation of DNA, and it is likely that mercury methylation is a coincidental result of inorganic mercury competing with intended substrates to be methylated. Apart from pure culture mercury methylation experiments, the importance of SRB for the production of methylmercury in different environmental compartments has been further verified in a number of studies [19,20,21]. The measurements of sediment microbial community

composition and activity in combination with inorganic mercury radiotracers have shown correlation between the abundance and respiration of SRB populations and rates of mercury methylation. Microorganisms have also been found capable of producing dimethylmercury, however, with much lower rate than the monomethylation rate [22].

In contrast to mercury, the biomethylation of inorganic or ionic organic lead compounds has been a topic of controversy, with no conclusive explanation of the mechanism of the reactions that might lead to the corresponding alkyl lead compounds [23,24]. The following factors contribute to this situation:

1. Monomethyllead compounds decompose so rapidly at ambient temperatures that they have not been isolated up to now;
2. The methyl-lead linkage is very labile, and the methyl group can be readily transferred to other acceptors, especially mercury. Such transfer may contribute to the instability of monomethyllead intermediates (e.g. through reductive elimination).

Such reactivity would ordinarily prevent a monomethyllead intermediate from existing sufficiently long to allow the introduction of additional methyl groups. If a potential Pb(II) substrate was complexed by appropriate ligands, it might undergo biomethylation to form a sufficiently long-lived monomethyllead(IV) intermediate to receive a second methyl group and form a stable dimethyllead(IV) compound. On the contrary to mercury, methylation of lead by methylcobalamine (carbanion donor) has not been successful carried out [25]. Craig and Rapsomanikis examined lead methylation by environmentally realistic CH_3^+ donors, such as methyl iodide, S-adenosylmethionine and betaine, and found that only methyl iodide could methylate inorganic and metallic lead to tetramethyllead [26]. Lead methylation by methyl iodide was also reported by Ahmad et al. [27]. Biomethylation of lead was observed for bacterial cultures of *Alcalinges*, *Acinetobacter*, *Flavobacterium*, *Aeromonas*, *Pseudomonas*, and *Genus bacillus* [28, 29]. Trimethyllead like monomethylmercury and dimethylmercury are also produced through natural biomethylation by polar marine bacteria and macroalgae [30,31].

Naturally occurring organotin compounds are mostly products of the methylation of inorganic tin. These compounds were observed in natural and waste waters [32,33], the atmosphere [34] and landfill gases [35], sediments [36] and biological tissues [37]. Mixed methylbutyltin compounds have also been detected [38,39], with methyltributyltin being the most abundant

species. They were attributed to the methylation of butyltin species because such compounds are not produced by industry. The main mechanisms for methylation (through methyl carbanion or methyl carbonium ion) were demonstrated to be successful for tin.

Organometallic compounds, anthropogenically introduced to the environmental waters or naturally formed there, are rapidly bio-accumulated in tissues of marine organisms causing severe, long-term toxic effects on local fauna with repercussions on biodiversity and human health. That makes their accurate determination of special importance for seafood quality assurance.

2.2 Toxicity of organic lead, mercury and tin compounds

Toxicity of metals is strongly dependent on the chemical form in which they appear. Speciation of an element is more important than the total elemental concentration for the evaluation of its toxicity and bioavailability. With the exception of organoarsenic compounds, organometallic species are more toxic than their inorganic salts (Table 1). The reason for the enhanced toxicity of organometallics compared with the inorganic derivatives is due to the existence of lipophilic or hydrophobic groups (R) on the same species also having a hydrophilic dipole. This allows transport in aqueous body fluids, and also solubility and transport through fatty tissue and cell walls by diffusion [40].

Table 1: LD_{50} (the quantity of substance per unit body weight given as a single dose required to kill half the exposed population within 14 days) of lead, mercury and tin compounds in experimental animals (from [40,41,42,43])

Compound	LD_{50} mg kg^{-1} body weight	
	Mouse	Rat[b]
Me_3Pb^+	6.8[a]	<36
Pb^{2+}	-	150
$MeHg^+$	47[a]	10
Hg^+	-	210
$BuSn^{3+}$	1400[b]	2140
Bu_2Sn^{2+}	35[b]	150
Bu_3Sn^+	117[b]	129

[a] – intraperitoneal; [b] – oral administration route

Usually the toxic effects are at a maximum for the singly-charged species (e.g. R_3Pb^+, R_3Sn^+, $MeHg^+$). It should be noted that the toxic effects of the saturated organometallics (R_4Sn, R_4Pb etc.) are usually due to R_3M^+, the metabolic products in organisms. In general alkyl groups are more toxic than aryl groups when attached to metals. The most toxic alkyl groups (attached to a common metal) vary from organism to organism, but methyl, ethyl and propyl groups tend to be the most toxic. For tin compounds toxicity of the organotin cations is at a maximum for the trialkyl series ($R_3Sn^+ > R_2Sn^{2+} > RSn^{3+} \geq R_4Sn$). For trialkyltins methyl and ethyl groups are the most toxic to mammals, with higher alkyl groups being more toxic to bacteria, invertebrates and fish. Similar to trialkyltin compounds, trialkylleads are most toxic and tetraalkylleads the next, while dialkyllead species and inorganic lead are the least toxic ($R_3Pb^+ > R_4Pb > R_2Pb^{2+} \geq Pb^{2+}$).

Mechanisms of toxicity are various, but good coordination to base atoms such as S, O, N on enzyme sites seems to be the main one. Coordination of the organometallic species to the enzyme blocks the sites and prevents the reaction with the biological substrate. The other consequence of organometallic introduction into vertebrates is a diminution of the myelin coating of nerve fibres. In addition, water accumulation and oedema in the central nervous

system may occur. Other toxic effects are due to coordination to non-enzyme sites: thiols, histidine residues of proteins, hemoglobin, cytochrome P_{450}, cerebral receptors. Fundamental interferences with DNA, protein synthesis, mutagenicity and genotoxicity have also been reported [44].

The main result of organometallic poisoning in vertebrates is damage to the central nervous system, leading to various symptoms including coma, ataxia, hyperactivity, convulsion, incoordination, speech problems and psychological-attitudinal changes. Trialkyltin toxicity, for example, arises through disruption of calcium and mitochondrial functions, membrane damage, disruption of ion transport and inhibition of adenosine triphosphate (ATP) synthesis. Dialkyltins also inhibit oxygen uptake in mitochondria and inhibit α-ketoacid oxidation. Acute organic lead poisoning is focused on the central nervous system, with pathological changes to the brain being found, including neuron destruction and degeneration of nerve tracts. Trialkyllead and -tin compounds, together with monomethylmercury, all bind to sulphide residues. They can destroy the normal pH gradient across mitochondrial membranes, thereby uncoupling oxidative phosphorylation [10].

The results of organomercury poisoning with respect to brain function are quite similar to those considered above. Rapid penetration of the blood-brain barrier and binding by sulphydryl groups lead to sensory disturbance, tremor, ataxia, visual and hearing difficulties. Methylmercury inhibits protein synthesis and RNA synthesis and causes particular damage to the developing brain. Since fish and seafood products is the main source of exposure to methylmercury for most individuals and for the foetus (through the maternal diet), the consumption limitations of some fish types are strongly recommended for pregnant women, women intending to become pregnant and young children [45,46,47]

2.3 Analytical methods for the determination of alkylated metal species

The need to perform organometallic speciation studies has become of growing importance over the last few years, because of their significant role in pollution monitoring, waste-site remediation, evaluation of metal bioavailability and because of high toxicity of alkylated metal species (Chapter 2.2). The environmental and economic impact of elemental speciation has led to the development and use of a wide variety of analytical techniques for the determination of the chemical forms of metals in different kinds of samples.

The most common strategy to carry out the separation and determination of different organometallic species is the hyphenation of a powerful separation technique such as liquid chromatography (LC) [48,49], gas chromatography (GC) [50,51,52], supercritical fluid chromatography (SFC) [53,54] or capillary electrophoresis (CE) [55,56] with element-specific detection techniques such as atomic absorption (AAS), atomic emission (MIP-AES and ICP-AES), atomic fluorescence (AFS) or mass spectrometry (ICP-MS). An overview of hyphenated techniques used for determination of organometallic species is presented in Figure 2. The choice of an adequate separation technique is determined by the physicochemical properties of the analyte (volatility, charge, polarity), while the detection technique is determined by the level of analyte in the sample and by the necessity of mono- or multi-element analysis.

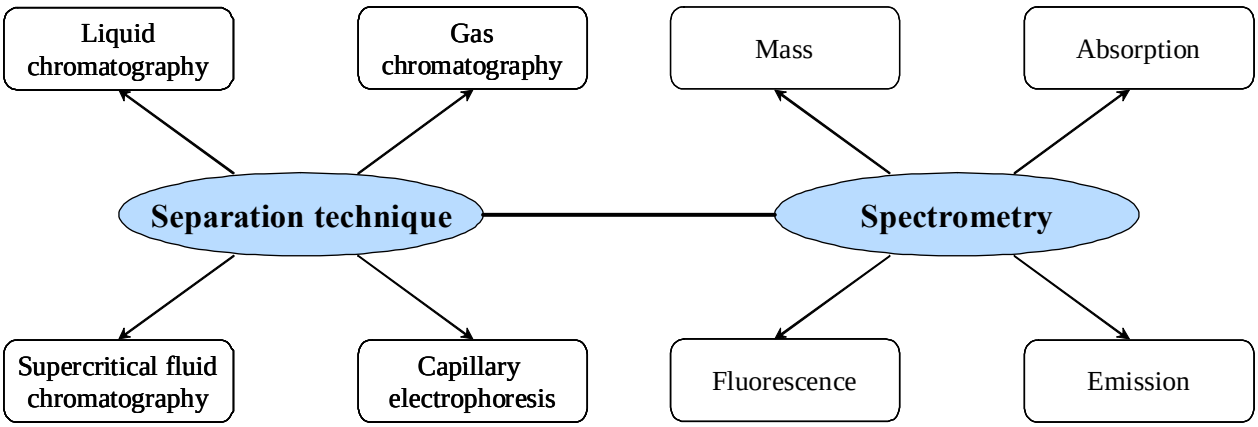

Figure 2: Hyphenated techniques used for determination of organometallic species

Gas chromatography and liquid chromatography have been predominantly used for the separation of alkylated metal species. Separations by high-performance liquid chromatography (HPLC) offer the advantage that derivatization (transformation of ionic species into volatile compounds) is not required, which eliminates a potential source of uncertainty in the final results and can reduce analysis time. However, the range of compounds that can be analyzed in a single run is limited compared to GC. Sensitivity and resolution capability of liquid chromatography is generally poor than for GC analysis. An overview of different hyphenated techniques applied for the analysis of organometallic compounds is given in different review articles [57,58,59].

Flame AAS was the first element-specific detector applicable for both GC and LC but with low sensitivity. The use of an electrically heated quartz furnace (QAAS) for atomization dramatically increased the sensitivity and GC-QAAS has long been the predominant analytical method for organolead and -tin compounds in the environment. More recently atomic emission detectors with inductively coupled argon plasma (ICP) and microwave induced helium plasma (MIP) have been used. ICP-AES is suitable for both LC and GC. MIP-AES is only suitable for GC but it is more sensitive. The main advantage by the use of AES as GC or LC detector is the fact that it allows simultaneous (or high-speed sequential) multi-element analysis in one chromatographic run.

Inductively coupled plasma mass spectrometry (ICP-MS) has emerged as a new high sensitive multi-element detector. Now it is widely applied for elemental speciation studies as an element-specific detector in liquid and gas chromatography, capillary electrophoresis, supercritical fluid chromatography [60]. The combination of capillary GC with ICP-MS has become an ideal methodology for the speciation analysis of organometallic compounds in complex environmental and biological samples because of the high resolving power of GC and the sensitivity and specificity of ICP-MS. The first investigations on the coupling of a GC with ICP-MS were carried out using packed columns [61]. A real breakthrough of this coupling technique appeared when capillary columns became commercially available. Many applications of capillary GC coupled to ICP-MS have been published since that time [62,63,64].

Though the coupling of a gas chromatograph to an ICP-MS instrument is not as simple as in the case of coupling for liquid chromatography, there are several advantages which make GC-ICP-MS favorable in comparison with HPLC-ICP-MS systems:

- High chromatographic efficiency (much narrower peak widths in comparison with HPLC);
- High sensitivity;
- High sample introduction efficiency (100% in comparison to 2-3% by typical nebulizer / spray chamber assemblies used in HPLC couplings);
- Separation of the solvent from the analytes during the chromatographic run (stability of the plasma during elution of the analytes);
- Efficient use of plasma energy for atomization and ionization (no need to volatilize and atomize solvent molecules);
- Stable plasma conditions during the chromatographic run (temperature programming instead of gradient elution). This means that the sensitivity remains constant during the separation, a stable baseline is obtained and, hence, low detection limits can be achieved;
- Low spectral interferences. The use of a dry plasma and the absence of any solvent lead to much less spectral interferences in comparison with solution nebulization.

Before separation and detection of organometallic compounds a number of discrete analytical steps including extraction, cleanup, preconcentration and derivatization procedures must often be carried out. Pretreatment of the matrix with acids or bases at room temperature, using microwave- or ultrasound-assisted systems is the most popular extraction method. For GC separation derivatization of non-volatile, ionic species into thermally stable volatile compounds is necessary. The analytical characteristics of three derivatization reactions (hydride generation, aqueous alkylation by $NaBEt_4$ or $NaBPr_4$ and Grignard reagents) currently used for the speciation of organometallic compounds are compared in Table 2. As can be seen alkylation in aqueous solutions offer the best analytical characteristics. This is also justified by the increasing number of publications on elemental speciation using this type of derivatization.

For preconcentration of analytes capillary purge-and-trap or liquid-liquid extraction, solid phase microextraction and stir bar sorptive extraction can be used. Each analytical step can become a source of error. Therefore accurate quantification by isotope dilution mass spectrometry may play a crucial role for quality assurance in trace elemental speciation of environmental and biological samples.

Table 2: Analytical characteristics of some derivatization reactions used for speciation of organometallic compounds [65]

Characteristic	Hydride generation	Aqueous alkylation	Grignard reagents
Reaction yield	High for small ions but lower for trialkylated species	High, except for inorganic species	Very high
Reproducibility	Low due to foam formation	High	High
Interferences	Severe in samples with high organic or metallic content	Low matrix effects except in the presence of chloride	No interferences due to matrix separation
Stability of the reagents	High	Decompose slowly under radiation and humidity	Require an inert and dry atmosphere
Ease of handling	High (pH ≈ 2)	High (pH ≈ 5)	Very low due to extraction into an organic phase, inert atmosphere and destruction of the excess of reagent
Speed of reaction	Instantaneous, allows on-line derivatization	High, can be accelerated using microwave irradiation	Low, the global process requires several reactions steps
Limitations	Only suitable for small organometallic species in simple matrices	$NaBEt_4$ not suitable for some applications, use of $NaBPr_4$ instead	None
Cost of reagents	Low	High	High

2.4 Fundamentals of isotope dilution mass spectrometry for organometallic species

Isotope dilution mass spectrometry (IDMS) is playing an increasingly important role in trace element determination as well as in organic and biochemical analysis, especially in analytical fields where the sample matrix influences the accuracy of results, for example in clinical and environmental chemistry [66,67,68]. Since 1991 the number of publications on elemental IDMS using TIMS (thermal ionization mass spectrometry) and especially ICP-MS increases yearly (Figure 3).

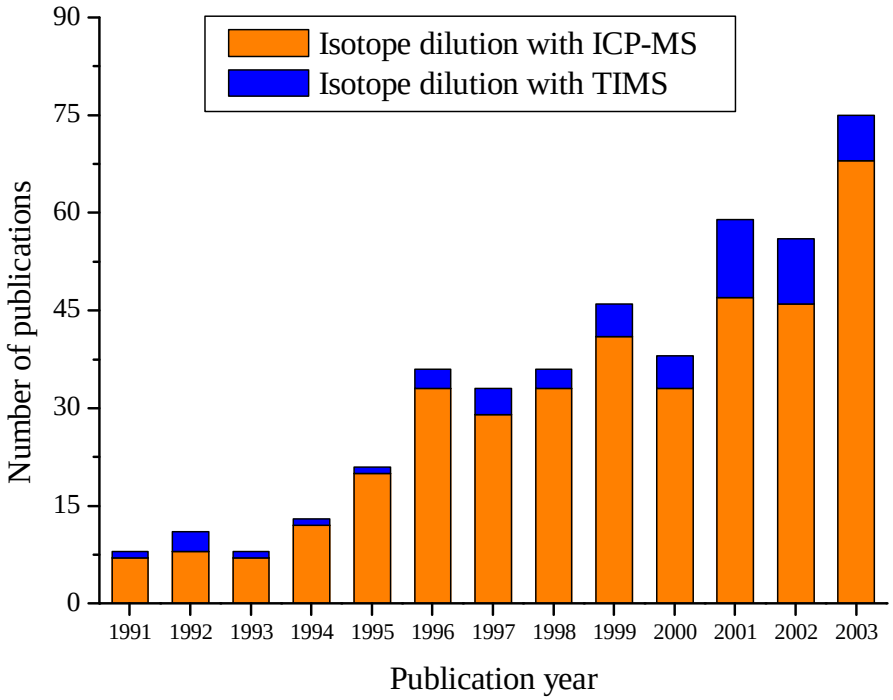

Figure 3: Evolution of the number of publications on elemental isotope dilution analysis using ICP-MS and TIMS [7]

The application of isotope dilution methodologies for trace metal speciation has also increased significantly over the last decade (Figure 1) [7,69,70,71,72]. As first reported by Rottmann and Heumann [73], the application of IDMS for elemental speciation can be performed under two different modes: the so-called “species-specific” and “species-unspecific” spiking mode. In the species-unspecific spiking mode, the addition of spike is carried out after a complete separation

of the different species in the sample has taken place (post-column spiking). In this case the spike may exist in any chemical form, which can be different from the species to be determined. This mode is especially useful if the structure and composition of the analyzed species is not exactly known or if the corresponding isotopically labeled compounds are not commercially available or cannot be synthesized. However, species-unspecific mode does not offer the full range of advantages of IDMS described below. In the species-specific spiking mode, the spike solution containing the species to be analyzed in an isotopically labeled form is added to the sample at the beginning of the analytical procedure and all traditional advantages of IDMS can be fully exploited.

The basis of a species-specific IDMS analysis is the application of an isotopically labeled analogue of the analyte as an internal standard. IDMS is based on the measurement of isotope ratios *R* in samples, where its isotopic composition has been altered by the addition of a known amount of an isotopic analogue (named "spike"). The element of the species to be analyzed must therefore have at least two stable or long-lived radioactive isotopes, able to be measured in a mass spectrometer free of spectrometric interferences. This principle is illustrated in Figure 4 for trimethyllead analysis.

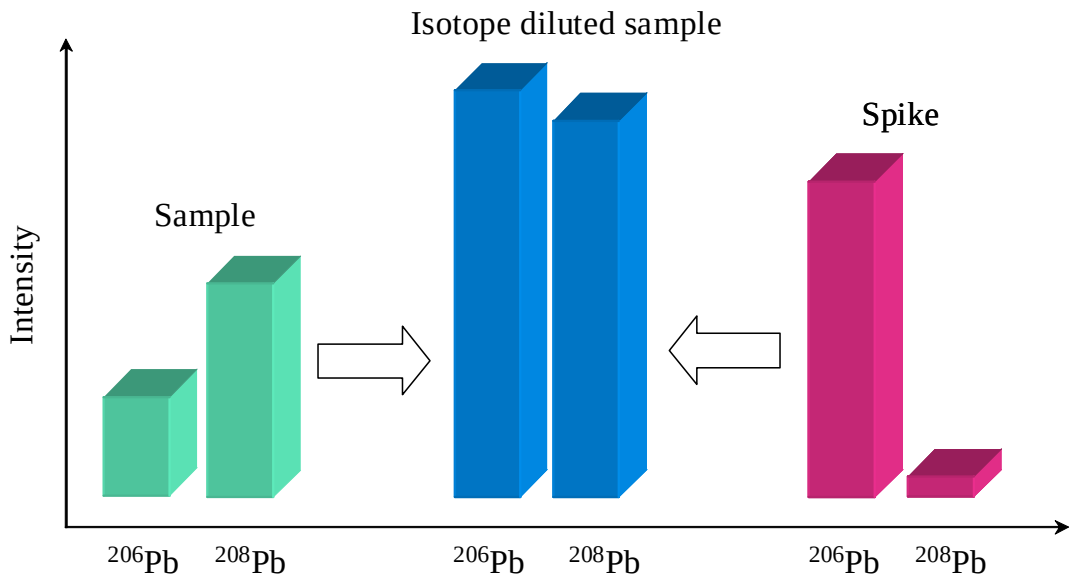

Figure 4: Principle of isotope dilution technique for trimethyllead analysis by application of a $Me_3{}^{206}Pb^+$ spike

An accurately known amount of the spike with known isotopic abundance is added to the exactly weighed sample of natural isotopic abundance and unknown concentration of analyte. After the spike is added, the sample and the spike species must be mixed completely. The corresponding isotope ratio *R* of the isotope diluted sample is measured by mass spectrometry and used for calculation of the analyte concentration. The two isotopes selected for the measurements of *R* are the spike isotope and usually the isotope with highest abundance in the sample (reference isotope), if there is no interference from an isobaric nuclide of another element. The isotope ratios ^{206}Pb/^{208}Pb, ^{200}Hg/^{202}Hg, ^{119}Sn/^{120}Sn with ^{206}Pb, ^{202}Hg, and ^{119}Sn as spike isotopes and the others as reference isotopes were selected in this work for the GC-ICP-IDMS determination of trimethyllead, methylmercury and butyltins. The following isotope dilution equations are presented for the case of trimethyllead analysis. The numbers of atoms for spike and reference isotopes are equal to the sum of the sample portion and the spike portion:

$$R = \frac{^{206}Pb}{^{208}Pb} = \frac{N_S \cdot {}^{206}h_S + N_{Sp} \cdot {}^{206}h_{Sp}}{N_S \cdot {}^{208}h_S + N_{Sp} \cdot {}^{208}h_{Sp}} \quad (1)$$

where *N* is the number of atoms, *h* the isotope abundance (%), and subscripts *S* and *Sp* indicate sample and spike, respectively.

If one solves Equation 1 for N_S, it follows

$$N_S = N_{Sp} \frac{{}^{206}h_{Sp} - R \cdot {}^{208}h_{Sp}}{R \cdot {}^{208}h_S - {}^{206}h_S} \quad (2)$$

Equation 2 is the most basic form of the isotope dilution equation. As can be seen, if one knows the number of atoms added with the spike and the isotope composition of both sample and spike one can easily calculate the number of atoms in the sample only by measuring *R* in the mixture. This expression can be converted in concentrations (mass/mass) instead of number of atoms by using the expressions:

$$N_S = \frac{c_S \cdot m_S}{M_S} \cdot N_A \quad (3)$$

$$N_{Sp} = \frac{c_{Sp} \cdot m_{Sp}}{M_{Sp}} \cdot N_A \quad (4)$$

where N_A is the Avogadro constant; c_S and c_{Sp} the concentrations of the species in the sample and the spike (as metal); m_S and m_{Sp} the mass taken from sample and spike in the mixture; M_S and M_{Sp} the atomic weights of the element in the sample and the spike, respectively.

By substitution of Equations 2-4, the final isotope dilution equation is obtained:

$$c_S = c_{Sp} \frac{m_{Sp}}{m_S} \cdot \frac{M_S}{M_{Sp}} \cdot \frac{{}^{206}h_{Sp} - R \cdot {}^{208}h_{Sp}}{R \cdot {}^{208}h_S - {}^{206}h_S} \qquad (5)$$

This equation does not take the blank correction into consideration. There are two possible approaches for the blank correction:

- If the blank gives no appreciable signal in comparison with the samples, then a simple blank subtraction can be performed on the individual MS measurements;
- If the blank does contain significant amounts of analyte then separate quantification of the blank level (by IDMS) is necessary.

A simplified approach for accurate correction of the blank during the trace analysis, when a significant blank is encountered in both IDMS and reverse IDMS processes, is in detail described by Yang and Sturgeon [74].

In Equation 5 the concentration of the species in the sample c_S is determined just by measuring R by mass spectrometry as all other parameters in the equation are known. The content and isotopic abundances of the spike used in Equations 2 and 5 are available from the certificate (e.g. $Me^{202}Hg^+$ spike and ^{119}Sn enriched butyltin mix, Chapter 3.8.3) or can be determined by reverse IDMS with standard solution of known concentration and natural isotope abundances (e.g. $Me_3{}^{206}Pb^+$ spike, Chapter 3.5.2). For determination of the spike concentration by reverse IDMS Equation 1 should be solved for N_{Sp}.

To achieve the best precision for the isotope dilution results an optimum range of spike addition should be taken into account [68,75]. The high precision in isotope ratio measurement can usually be achieved for ratios of approximately unity. The optimum value of R (R_{opt}) can be calculated from the following formula:

$$R_{opt} = \sqrt{\frac{^{206}h_{Sp}}{^{208}h_{Sp}} \cdot \frac{^{206}h_{S}}{^{208}h_{S}}} \qquad (6)$$

IDMS offers unique advantages in comparison to other calibration techniques, such as external calibration or the standard addition method. First, instrumental instabilities, such as signal drift or matrix effects have no influence on the final concentration of the species in the sample. Second, once species of the sample and the spike were completely mixed, possible loss of analyte during sample preparation has no influence on the final result. Third, derivatization, clean up and extraction procedures do not need to be quantitative and no recovery corrections have to be applied. Finally, if species transformations are taking place during sample preparation, they can be detected by species-specific IDMS. As a result of all these advantages high precision and accuracy of final results are achieved. Therefore IDMS is internationally accepted as a definitive method.

Nevertheless, several requirements must be fulfilled to make these statements true. First, one has to take into account that any loss of substance (either sample or spike) before complete mixing takes place may pose an important source of error. Additionally, no isotope exchange between different species must take place during sample preparation. On the other hand, the measurement of the intensities of the reference and spike isotopes performed by the mass spectrometer must be free of spectral interferences and the factors affecting the accuracy of the isotope ratio measurements, such as mass bias and detector dead time, must be under control. Finally, an exhaustive control of the blank values is also required since any kind of contamination affecting the isotope diluted sample leads to biased *R* values.

3 Experimental

3.1 Chemicals

Contaminations from the surrounding area, chemicals or equipment can essentially influence the analytical results of trace metal determinations. Therefore, all glassware, PFA (perfluoroalkoxy) bottles as well as Teflon vials, used for microwave digestion of samples, should be thoroughly cleaned before use and checked by means of a blank determination. To keep blank levels low, the equipment was boiled in 10% nitric acid and then in ultrapure water, after that dried at 70°C and wrapped in PE-foil for storage. Polyvinyl chloride (PVC) materials have to be avoided as they may contain dibutyltin which can be leached by water along with monobutyltin and inorganic tin [76].

In the case of alkylated metal species, contamination from the ambient air is less probable. But cross contaminations can be caused by parallel sample preparation or used standard solutions. In order to diminish the risk of these contaminations, sample preparation was carried out in single-use 7 mL glass vials. Chemicals can be one of the contamination sources too. Therefore chemicals were chosen with the best available purity grade to keep the blank levels as low as possible. These blanks were determined with each new batch of reagent. The applied chemicals, their purity and sources of supply are summarized in Table 3.

Nitric acid was purchased at the quality level “pro analysis” (p.a.) and additionally purified by distillation under sub-boiling conditions in a quartz apparatus. Ultrapure water with a conductivity of less than 0.05 µS was obtained from a Milli-Q water purification system (Millipore, Eschborn).

Table 3: Applied chemicals

Chemicals	Purity	Supplier
Liquids/solutions:		
acetic acid	suprapure, 100 %	Merck
butyltin mix (^{119}Sn enriched)	-	ISC
butyltin trichloride	97 %	Acros Organics
n-hexane	purris., ≥99.5 %	Fluka
hydrogen peroxide	suprapure, 30 %	Merck
iodine monochloride	98 %	Acros Organics
iodomethane	99 %	Acros Organics
methanol	ECD tested	Acros Organics
methyllithium	1.6 mol L^{-1}	Acros Organics
methylmercury chloride (^{202}Hg enriched)	-	IRMM
nitric acid	p.a., 65 % (distilled)	Acros Organics
tetramethylammonium hydroxide (TMAH)	purum, 25 %	Fluka
tributyltin chloride	96 %	Aldrich
water	MQ	Milli-Q system
Solids:		
dibutyltin dichloride	96 %	Aldrich
lead (^{206}Pb enriched)	-	Euriso-top
methylmercury chloride	98.4 %	Sigma-Aldrich
sodium acetate	suprapure	Merck
sodium tetraethylborate	98 %	Galab Technologies
sodium tetrapropylborate	98 %	Galab Technologies
trimethyllead chloride	98 %	ABCR
Gases:		
argon	4.6	Westfalen
helium	4.6	Westfalen
oxygen	4.6	Westfalen

Stock solutions of trimethyllead and methylmercury were prepared by dissolving the corresponding compounds in 0.5 % HNO_3 (m/m) and stored in the dark at 4˚C. Diluted solutions in Milli-Q water, adapted to the necessary concentration for analysis, were prepared prior to each series of measurements. Solutions of tributyltin chloride, dibutyltin dichloride, butyltin trichloride and other tin compounds used for identification of the species were prepared in methanol.

It should be noted that organic lead, mercury and tin compounds are extremely toxic and appropriate safety precautions must always be taken. The permeability of the used gloves, e.g. “Silvershield” (North Safety Products, USA), must cover the requirements for these toxic substances. Good ventilation in the working area is absolutely necessary. Iodomethane, used for the synthesis of trimethyllead, is also highly toxic and may cause cancer. It is fatal if this substance is inhaled, swallowed or absorbed by the skin. Therefore safety glasses, gloves and good ventilation at the working place are obligatory for the trimethyllead synthesis.

3.2 Certified reference materials

A series of certified reference materials presenting different species concentrations and matrices such as biological tissues (fish, mussel, krill), sediments (estuarine and freshwater) and dust was used to validate the developed methods but also to receive some representative data for homogenized samples of typical seafood and sediments (Table 4). DORM 2 (dogfish muscle) and CRM 463 (tuna fish) were used to validate the method for total metal content as well as for the methylmercury determination. For the validation of the multi-species method of the analysis of butyltins CRM 477 (mussel tissue) and BCR 646 (freshwater sediment) were applied. Only one reference material CRM 605 (urban dust) is available where trimethyllead is certified. All other biological and sediment reference materials, listed in Table 4, were analyzed for Me_3Pb^+ content by species-specific GC-ICP-IDMS.

Table 4: Reference materials analyzed for trimethyllead content and used to validate the developed methods

Material code	Matrix	Certified compound	Agency[a]
DORM 2	Dogfish muscle	Trace elements and methylmercury	NRCC
CRM 463	Tuna fish	Total mercury and methylmercury	IRMM
CRM 422	Cod muscle	Trace elements	IRMM
CRM 477	Mussel tissue	Butyltin compounds	IRMM
CRM 278	Mussel tissue	Trace elements	IRMM
MURST-ISS-A2	Antarctic krill	Trace elements	ISS
CRM 580	Estuarine sediment	Total mercury and methylmercury	IRMM
BCR 646	Freshwater sediment	Butyltins and phenyltins	IRMM
CRM 605	Urban dust	Trimethyllead	IRMM

[a] NRCC - National Research Council, Canada; IRMM - Institute for Reference Materials and Measurements, Belgium; ISS - National Institute of Health, Italy.

Results for the total and alkylated metal content of reference materials were always calculated on dry sample material. The moisture content of reference materials was assessed by drying 100 mg

portions at 105˚C for 24-30 h until constancy in their weight. A difference of less than 0.2 mg between two weighings was defined to constant.

Results presented in Table 5 show that the water content in the reference materials varies from 0.84 % in BCR 646 (freshwater sediment) to 12.33 % in CRM 463 (tuna fish). Biological reference materials are obviously more hygroscopic than sediments and dust.

Table 5: Water content in certified reference materials (n=3)

Reference material	Water content
DORM 2 (fish)	9.35 ± 0.10
CRM 463 (fish)	12.33 ± 0.31
CRM 422 (fish)	10.11 ± 0.11
CRM 477 (mussel)	8.36 ± 0.09
CRM 278 (mussel)	5.53 ± 0.14
CRM 580 (sediment)	1.64 ± 0.05
BCR 646 (sediment)	0.84 ± 0.04
CRM 605 (dust)	2.29 ± 0.06

Before opening a bottle of a reference material, it was thoroughly homogenized by shaking it manually. The minimum sample intake recommended in the instructions for use (from 0.1 g to 1 g) was always taken for analysis. The bottles were stored under exclusion of light at -25°C or +4°C, depending on the certified species and the type of reference material.

3.3 Principle and optimization of the quadrupole ICP-MS

The inductively coupled plasma mass spectrometer (ICP-MS) with a quadrupole mass filter (model HP 4500, Agilent Technologies) was used in the scope of this work. Its schematic construction is presented in Figure 5.

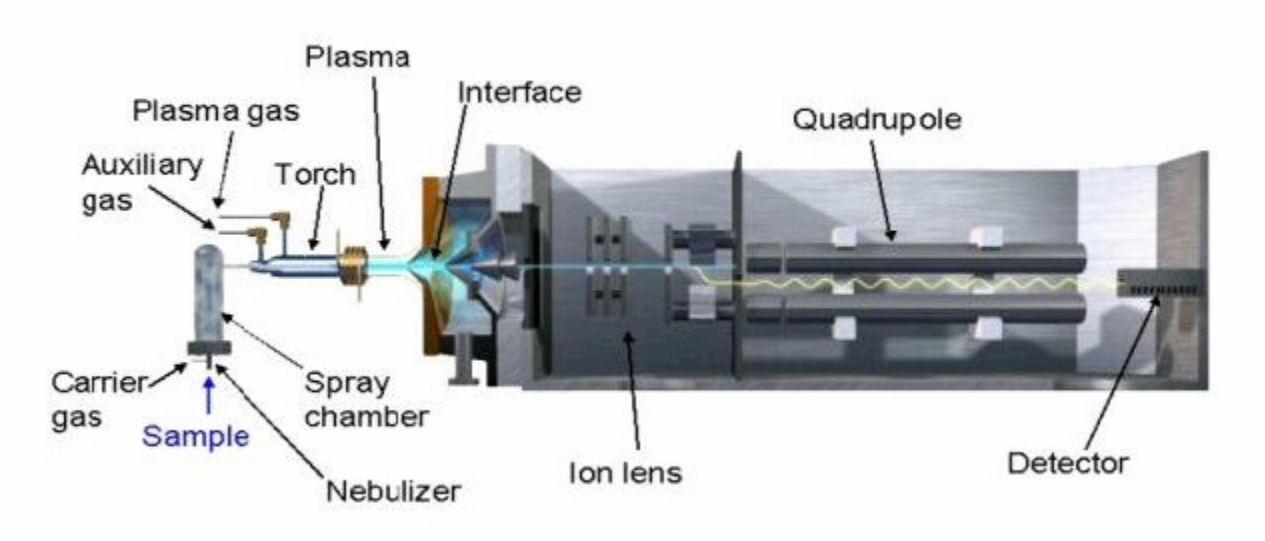

Figure 5: Schematic figure of the quadrupole ICP-MS instrument [77]

A solution is converted into an aerosol by means of a pneumatic nebulizer (microflow). In order to ensure a stable plasma and an efficient atomization and ionization in the ICP, the larger droplets (>10µm) are removed from the aerosol by means of a spray chamber (Scott Type). Subsequently, the aerosol is transported by the argon carrier gas into the center of the plasma, which is generated at the end of a quartz torch by high frequency alternating current (27 MHz, 600-1600W) of a load coil. Two more gas flows, the plasma gas and the auxiliary gas, are supplied through the torch. During residence time within the plasma, droplets are desolvated, atomized and ionized. The high temperature of the argon plasma (6000-10000 K) offers an ionization efficiency of more than 90% for the majority of elements. Since the ICP is operated under atmospheric pressure, whereas in the mass spectrometer a high vacuum is required, an interface between both components is necessary. This interface consists of two successive, coaxial and water-cooled cones, with a small central aperture – the sample cone (1mm) and the skimmer cone (0.4 mm). A beam consisting of ions, electrons and neutral particles is succed by vacuum through the aperture of the sample and skimer cone inside the quadrupole mass

spectrometer. A negatively charged extraction lens selectively attracts the positive ions, which are subsequently transported and introduced into the quadrupole mass analyzer. It consists of four equidistant, parallel hyperbolic rods onto which is applied both high frequency alternating current and direct current electric fields. The four rods are arranged in such a way that they form two pairs and rods on the opposite side have the same potential. By matching the two fields, only ions of a particular mass are able to resonate at the correct frequency which allows them to pass through the quadrupole and reach the detector (electron multiplier).

The number of ions, which reaches the detector, essentially depends on the torch position and ion lens potentials. These parameters can be changed and should therefore be controlled and optimized regularly in order to receive high sensitivity and resolution. For optimization a calibration standard solution containing ^{7}Li, ^{89}Y, ^{140}Ce and ^{205}Tl (10 ng g^{-1} in 0.2 % HNO_3) was applied. The operating conditions of the ICP-MS are summarized in the Table 6.

Table 6: ICP-MS operating conditions for the direct determination of the total metal content

Nebulizer	microflow (PFA) / at 100 µL min^{-1}
Spray chamber	Scott Type (quartz glass)
Spray chamber temperature	2°C
Cones material	Nickel
Radio frequency power	1200 W
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	1.20 L min^{-1}
Carrier gas flow rate	1.05 L min^{-1}
Torch position (“Sampling depth”)	6 mm
Points per mass	3
Integration time per point	0.6 s
Repetitions	10
Detection mode	pulse counting

3.4 Determination of total metal content in biological samples

3.4.1 Microwave digestion

Since the past decade microwave digestion plays an important role in analytical chemistry, because it offers better reproducibility, more accuracy and it is less time-consuming than conventional digestion methods on hot plates in open beakers [78]. Microwave systems usually work with closed vials, which enable low blank levels, because they avoid contaminations from the ambience. Furthermore, the volume of reagents can be reduced, which again can have a positive influence on the blank. In this work the microwave system MLS ETHOS plus from MLS GmbH (Mikrowellenlaborsysteme, Leutkrich) was used.

The organic matrix of biological samples can be oxidized to carbon dioxide and water (Equation 7). For the ICP-MS analysis oxidizing reagents used for digestion should not produce fragments that have the same m/z values as the ions of trace elements of interest. In comparison to sulphuric and perchloric acid, nitric acid produces only one major isobaric interference $^{40}Ar^{14}N^{+}$ on ^{54}Fe. Therefore it was used in this work.

$$C_{org} + 2\,HNO_3 \rightarrow CO_2\uparrow + 2\,NO_x\uparrow + H_2O \qquad (7)$$

Nitric acid concentrations in the solutions used for analysis should be limited to approximately 10% to avoid rapid and severe corrosion of the ICP-MS cones. Dilution of the digested samples with Milli-Q water is therefore usually carried out because of the high sensitivity of ICP-MS. However, any dilution has direct influence on the detection limit. Addition of 30% hydrogen peroxide was applied to minimize the volume of nitric acid for the digestion process. Hydrogen peroxide has the advantage of no interferences. Furthermore, addition of hydrogen peroxide enables essential reduction of nitrogen oxides formation during microwave digestion:

$$2\,NO_x + 3\,H_2O_2 \rightleftharpoons 2\,HNO_3 + 2\,H_2O \qquad (8)$$

A smaller quantity of produced gas keeps the pressure inside the vials lower, which means that higher digestion temperatures are possible or an increased sample amount can be dissolved. Besides this the equilibrium of the digestion reaction (Equation 7) is shifted to the right that

increases velocity and quality of the digestion. Therefore, mixtures of concentrated nitric acid and suprapur 30% hydrogen peroxide were used for all further experiments. The nitric acid (p.a.) was additionally purified in a quartz sub-boiling distillation unit to keep blank levels low.

The sample preparation procedure for the ICP-IDMS determination of lead, mercury and tin in biological samples is schematically presented in Figure 6.

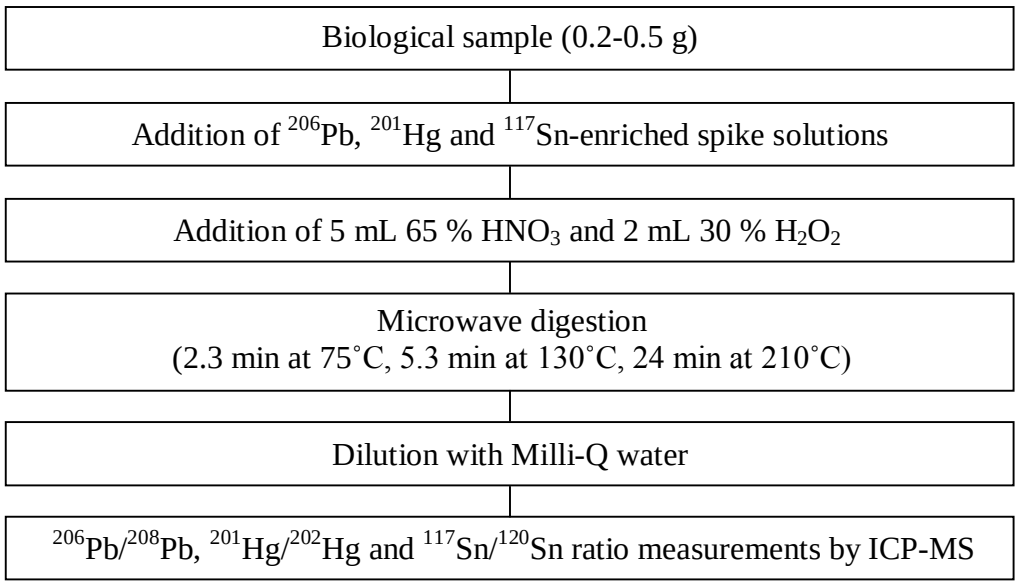

Figure 6: Schematic diagram of the sample preparation procedure for the determination of total metal content in biological samples by ICP-IDMS

Optimum amount of dry sample for 80 mL microwave vessels is between 0.2 g and 0.5 g. This limit was exceeded only in the case of wet shark samples (up to 0.7 g). Sample and spike solutions (0.1-0.6 g of diluted ^{206}Pb-, ^{201}Hg- ^{117}Sn-spike solutions) were precisely weighed into the Teflon microwave vessel and treated with 5 mL of 65 % HNO_3 and 2 mL of 30 % H_2O_2. After digestion (2.3 min at 75˚C, 5.3 min at 130˚C, 24 min at 210˚C) and cooling down to room temperature, the Teflon vessels were opened and the solutions were diluted with Milli-Q water (1:5 or 1:100 depending on the element concentration). A shorter digestion time and lower temperature of the last step of microwave program (16 min at 200˚C) resulted in undissolved particles for some samples. The diluted solutions were measured by ICP-MS using a PFA microflow-nebulizer under conditions described in Chapter 3.3.

3.4.2 Determination of total lead, mercury and tin in biological samples by ICP-IDMS

The ICP-IDMS method was used for the determination of the total metal content in biological samples after microwave digestion, described in the previous chapter. IDMS is internationally accepted as a definitive method of proven high accuracy and precision. The principles and advantages of this method are described in Chapter 2.4.

^{206}Pb-, ^{201}Hg- and ^{117}Sn-enriched spike solutions were characterized by a reverse ICP-IDMS method, using corresponding standard solutions of natural isotopic composition. Results of the isotope abundance measurements of these spike and standard solutions are presented in Tables 7-9, where spike and reference isotopes, selected for the isotope dilution technique, are bold marked. Corrections of the isobaric interferences ^{204}Pb – ^{204}Hg were made for accurate determination of isotope abundances of the lead and mercury spike and standard solutions. Other interferences like ^{196}Hg – ^{196}Pt, ^{198}Hg – ^{198}Pt, ^{112}Sn – ^{112}Cd, ^{114}Sn – ^{114}Cd, ^{115}Sn – ^{115}In, ^{120}Sn – ^{120}Te etc. were negligible.

Table 7: Isotope abundances of lead standard and spike solutions (n=3)

Isotope	Abundance of standard, %	Abundance of spike, %
^{204}Pb	1.283 ± 0.007 (0.5%)	0.029 ± 0.001 (4.9%)
^{206}Pb	**27.115 ± 0.110 (0.4%)**	**90.524 ± 0.080 (0.1%)**
^{207}Pb	20.338 ± 0.058 (0.3%)	6.671 ± 0.026 (0.4%)
^{208}Pb	**51.264 ± 0.132 (**0.3%)	**2.776 ± 0.058 (2.1%)**

Table 8: Isotope abundances of mercury standard and spike solutions (n=3)

Isotope	Abundance of standard, %	Abundance of spike, %
^{196}Hg	0.157 ± 0.001 (0.5%)	0.0006 ± 0.0001 (16.7%)
^{198}Hg	10.207 ± 0.025 (0.2%)	0.088 ± 0.002 (2.3%)
^{199}Hg	16.965 ± 0.016 (0.1%)	0.130 ± 0.001 (0.6%)
^{200}Hg	22.930 ± 0.029 (0.1%)	0.495 ± 0.004 (0.8%)
^{201}Hg	**13.282 ± 0.026 (0.2%)**	**97.922 ± 0.004 (0.004%)**
^{202}Hg	**29.517 ± 0.058 (0.2%)**	**1.274 ± 0.006 (0.5%)**
^{204}Hg	6.942 ± 0.025 (0.4%)	0.090 ± 0.001 (1.0%)

Table 9: Isotope abundances of tin standard and spike solutions (n=3)

Isotope	Abundance of standard, %	Abundance of spike, %
^{112}Sn	0.928 ± 0.007 (0.7%)	0.072 ± 0.001 (1.8%)
^{114}Sn	0.662 ± 0.009 (1.4%)	0.053 ± 0.001 (2.4%)
^{115}Sn	0.348 ± 0.006 (1.6%)	0.028 ± 0.002 (5.7%)
^{116}Sn	14.389 ± 0.026 (0.2%)	2.487 ± 0.009 (0.4%)
^{117}Sn	**7.634 ± 0.034 (0.4%)**	**85.939 ± 0.015 (0.02%)**
^{118}Sn	23.981 ± 0.065 (0.3%)	6.350 ± 0.015 (0.2%)
^{119}Sn	8.581 ± 0.012 (0.1%)	1.034 ± 0.002 (0.2%)
^{120}Sn	**32.636 ± 0.051 (0.2%)**	**3.118 ± 0.015 (0.5%)**
^{122}Sn	4.750 ± 0.010 (0.2%)	0.408 ± 0.004 (1.0%)
^{124}Sn	6.091 ± 0.025 (0.4%)	0.511 ± 0.002 (0.3%)

Determination of the spike concentrations was carried out under the same ICP-MS conditions as the isotope abundance measurements of the spike and standard solutions (Table 10). In this case mass discrimination effects in the ICP-MS were compensated by using always the measured but not corrected isotope abundances or isotope ratios for the calculation of concentrations by the IDMS equation [79].

Table 10: Concentration of the ^{206}Pb, ^{201}Hg and ^{117}Sn spike solutions determined by reverse ICP-IDMS

Analysis №	^{206}Pb spike, ng g^{-1}	^{201}Hg spike, ng g^{-1}	^{117}Sn spike, ng g^{-1}
1	539.2	55.45	412.7
2	534.6	54.17	413.5
3	532.0	55.81	413.9
Average ± SD	535.3 ± 3.6 (0.7%)	55.1 ± 0.9 (1.6%)	413.4 ± 0.6 (0.15%)

For multi-element isotope dilution analysis it is important to ensure, that spikes are not contaminated by other elements to be analyzed in the sample. This was controlled by the ICP-MS measurements, which showed no significant contamination.

Results of the ICP-IDMS determination of total lead, mercury and tin content in seafood samples are presented in Table 11.

Table 11: Total lead, mercury and tin content in seafood samples

Sample	Total Hg, ng g^{-1}	Total Pb, ng g^{-1}	Total Sn, ng g^{-1}
Mussels	179.9 ± 3.6	2505 ± 113	71.4 ± 6.7
Prawns	50.8 ± 2.8	116.4 ± 4.7	58.9 ± 6.0
Tuna fish	1184 ± 27	5.1 ± 1.1	43.9 ± 6.5
Plaice	186.1 ± 6.3	33.0 ± 4.5	31.2 ± 4.2
Pollock	784 ± 27	18.1 ± 3.6	16.9 ± 2.3
Shark 1	3996 ± 344	85.1 ± 8.8	96.9 ± 5.7
Shark 2	10859 ± 351	81.7 ± 11.5	113.7 ± 10.3

3.4.3 Validation of the ICP-IDMS method for determination of total metal content

The method for the determination of total lead, mercury and tin was validated by the analysis of two biological reference materials, DORM 2 (dogfish muscle) and CRM 463 (tuna fish). As can be seen from Table 12, ICP-IDMS values for lead and mercury are in good agreement with certified values. On the other hand, the ICP-IDMS value for tin was higher by a factor four compared to the indicative value. However, this is in excellent agreement with the ICP-MS result of 0.09 $\mu g\ g^{-1}$, obtained for tin in DORM 2 by Rosal et al. [80].

Table 12: Determination of lead, mercury and tin in biological reference materials

Element	Reference material	Certified / indicative value $\mu g\ g^{-1}$	ICP-IDMS value $\mu g\ g^{-1}$
Pb	DORM 2	0.065 ± 0.007	0.061 ± 0.006
Sn	DORM 2	0.023	0.091 ± 0.006
Hg	CRM 463	2.85 ± 0.16	2.86 ± 0.05

3.5 Synthesis and characterization of ^{206}Pb-enriched trimethyllead spike

3.5.1 Synthesis of $Me_3{}^{206}Pb^+$ spike

For the species-specific ICP-IDMS, it is essential that the added spike of enriched isotope and the indigenous elemental species for determination are present in the same chemical form. Thus, despite the substantial benefits on offer, IDMS has remained an under-exploited method of analysis, primarily because of the lack of commercially available certified and isotopically labeled species standards. In many cases, it is necessary to synthesize the relevant species.

The high costs of enriched starting material for synthesis of isotope-enriched compounds necessitate the use of small quantities. Synthetic methods are available, but application of these methods on a small scale (mg) revealed several difficulties. For the same economic reason, purification processes involving wasteful separation methods should be avoided.

In the case of trimethyllead, it is necessary to synthesize the corresponding species-specific spike. The methods for trialkyllead halide preparation are described in the literature [81]. In small-scale isotope-enriched trimethyllead and triethyllead have been synthesized by Brown et al. [82] for species-specific HPLC-ICP-IDMS. They used the reaction of $PbCl_2$, prepared from ^{206}Pb-enriched metallic lead, with the Grignard reagents MeMgI and EtMgI, respectively. In this work an alternative synthesis with methyllithium in the presence of an excess of iodomethane was selected, because it enables a quantitative yield of tetramethyllead without formation of metallic lead and has the advantage of being a fast reaction at room temperature [83,84].

110 mg ^{206}Pb-enriched metallic lead was dissolved by heating (oil bath at 150°C) with 7 mL 47% HBr. After evaporation of the acid the precipitate was washed once with ethanol and twice with diethylether and then dried under vacuum conditions. Under argon atmosphere, the corresponding $PbBr_2$ product was suspended in 10 mL ether, which included an excess of iodomethane (0.4 g) compared to the stoichiometric reaction. Under intensive stirring and ice-water cooling 2.4 mL of 1.6 mol L^{-1} methyllithium (MeLi) solution in ether were dropwise added. The reaction mixture became darker and was nearly black (precipitation of Pb) when half of the total amount of MeLi had been added. The color slowly disappeared with further addition of MeLi. When all MeLi was added the solution had become clear and colorless, showing a

quantitative formation of Me_4Pb. After stirring at room temperature (about 5 h) the ether phase was washed with water and then dried over anhydrous sodium sulphate. The ether phase was placed in a dry ice-acetone bath (-60°C) and 0.13 g I_2, dissolved in ether, were slowly added under stirring within 5 hours.

Pure products were obtained as can be seen from the GC-ICP-MS-chromatograms for Me_4Pb solution, obtained after reaction with MeLi, and for the same solution after reaction with iodine (Figure 7).

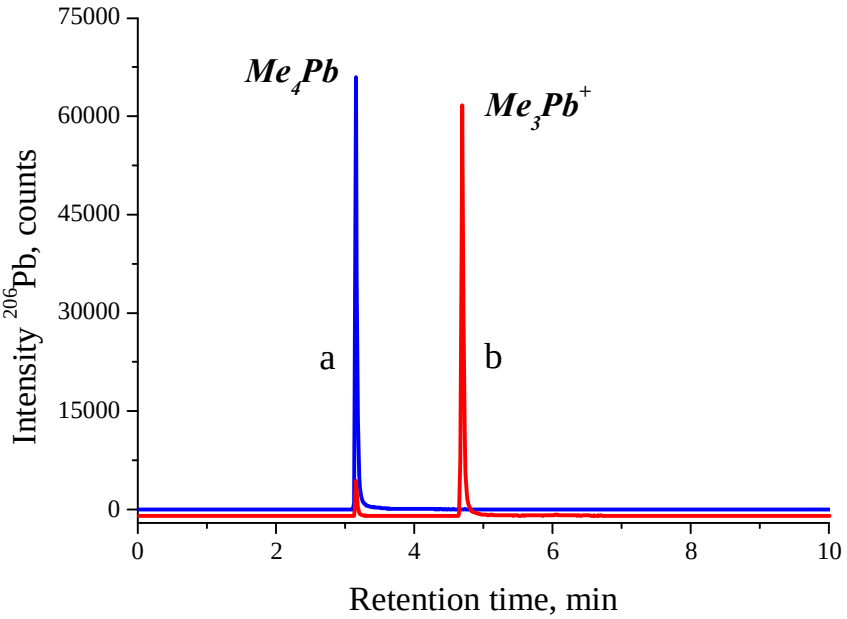

Figure 7: GC-ICP-IDMS chromatograms (a) for Me_4Pb solution obtained after reaction with MeLi (dilution 1:20000) and (b) for the same solution after reaction with iodine (dilution 1:20000, derivatization by $NaBEt_4$)

After evaporation of ether the precipitate was air-dried. Drying under vacuum conditions should be avoided, because this causes degradation of trimethyllead to lead iodide. The yield was 185 mg ^{206}Pb-enriched product, which was dissolved in 250 mL of 0.5 % HNO_3 and kept in the refrigerator at 4°C in the dark. This stock spike solution was diluted prior to analyses in a way

that the corresponding concentration fits the optimum range of spike addition for the isotope dilution technique [68].

3.5.2 Characterization of $Me_3{}^{206}Pb^+$ spike by reverse ICP-IDMS

Synthesized isotope-enriched trimethyllead spike needs to be characterized with respect to isotopic composition, purity and concentration. The isotopic analysis of the $Me_3{}^{206}Pb^+$ solution was performed after ethylation with $NaBEt_4$ by GC-ICP-MS, described in Chapter 3.6.1, as well as by direct ICP-MS measurements (Chapter 3.3). The final results are summarized in Table 13. Isotope abundances measured by GC-ICP-MS are in good agreement with those obtained from direct measurement by ICP-MS and with the analysis certificate of ^{206}Pb-enriched lead, used for synthesis of trimethyllead spike. This means that no isotopic fractionation occurred during the derivatization and synthesis procedure. Isotope abundances of the trimethyllead and inorganic lead standards were simultaneously measured after ethylation in the mixture by GC-ICP-MS (Table 14).

Table 13: Isotopic composition of the $Me_3{}^{206}Pb^+$ spike solution in comparison to the certified data for ^{206}Pb-enriched metallic lead, used for synthesis of the spike

Isotope	Certified data for ^{206}Pb, %	Measured isotope abundances (n=5)	
		ICP-MS, %	GC-ICP-MS, %
^{204}Pb	<0.01	0.016 ± 0.002	0.03 ± 0.02
^{206}Pb	99.3	99.21 ± 0.02	99.30 ± 0.14
^{207}Pb	0.5	0.48 ± 0.01	0.42 ± 0.03
^{208}Pb	0.2	0.29 ± 0.01	0.25 ± 0.04

Table 14: Isotopic composition of Me_3Pb^+ and Pb^{2+} standards in comparison to IUPAC data

Isotope	IUPAC data, %	Isotope abundances by GC-ICP-MS (n=5)	
		Me_3Pb^+ standard, %	Pb^{2+} standard, %
^{204}Pb	1.4	1.38 ± 0.03	1.37 ± 0.03
^{206}Pb	24.1	23.32 ± 0.11	23.21 ± 0.26
^{207}Pb	22.1	22.09 ± 0.15	22.02 ± 0.27
^{208}Pb	52.4	53.21 ± 0.20	53.40 ± 0.44

Figure 8 shows the GC-ICP-MS chromatograms obtained for ^{206}Pb and ^{208}Pb of a Me_3Pb^+ standard solution of natural isotopic composition and of the spike, respectively. Besides the Me_3EtPb peak, caused by ethylation of trimethyllead, a second small peak was observed in the spike chromatogram, which corresponds to Pb^{2+} derivatized by $NaBEt_4$ to Et_4Pb.

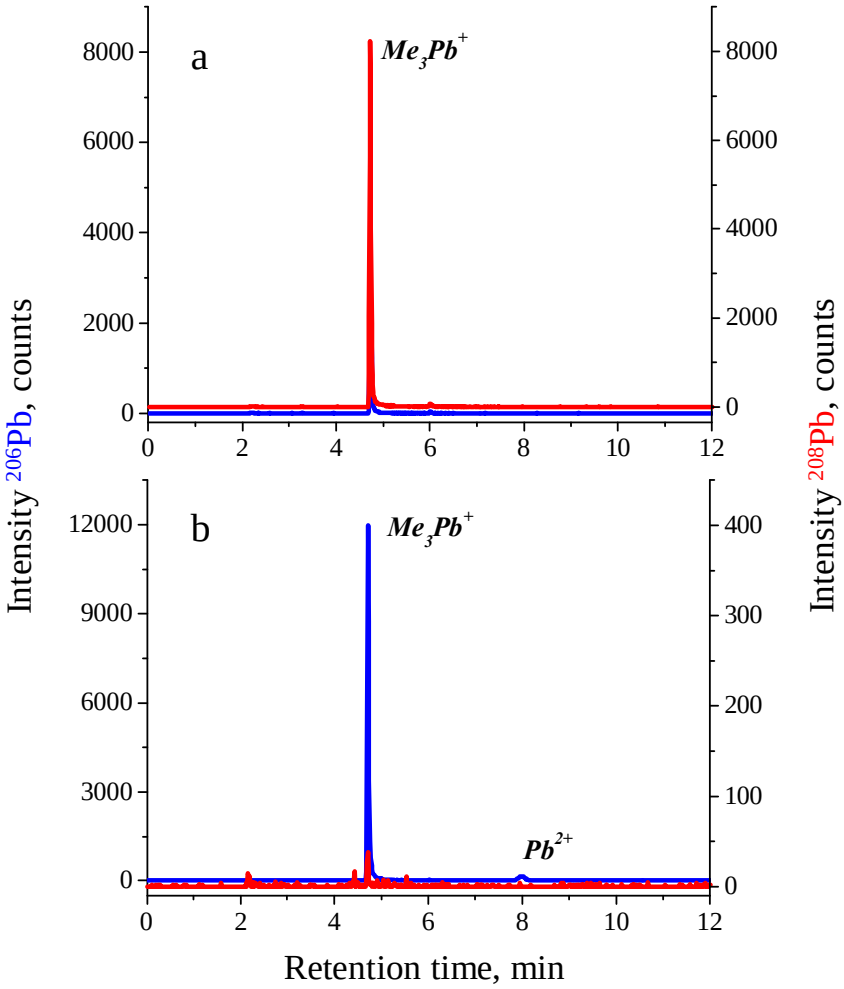

Figure 8: GC-ICP-MS chromatograms of ^{206}Pb and ^{208}Pb (a) of a Me_3Pb^+ standard solution of natural isotopic composition and (b) of the synthesized spike solution

Concentrations of trimethyllead and inorganic lead in the $Me_3{}^{206}Pb^+$ spike solution can be calculated according to the reverse IDMS equations, using isotope abundances of the spike, the Me_3Pb^+ and the Pb^{2+} standard, as well as known concentrations of the standard solutions and used amounts of two solutions. Three independent reverse isotope dilution experiments were carried out and each solution was injected three times to evaluate the precision on the isotope ratio measurement and the precision of the whole procedure. As can be seen from Table 15, the relative standard deviation (RSD) for trimethyllead determination for each triplicate injection and for the triplicate isotope dilution procedure was about 1 %. In the case of inorganic lead the relative standard deviation is worse (from 4.3 % to 9.3 % for triplicate injection and 3.4 % for the whole procedure), because of the much smaller and wider peaks for inorganic lead.

Table 15: Determination of the $Me_3{}^{206}Pb^+$ spike concentration by the reverse GC-ICP-IDMS (n=3)

Analysis №	Me_3Pb^+ as Pb, µg g^{-1}	Pb^{2+}, µg g^{-1}
1	310.5 (1.1)[a]	65.4 (7.6)
2	306.9 (1.0)	63.7 (9.3)
3	310.8 (1.3)	61.1 (4.3)
Average ± SD	309.4 ± 2.2 (0.7)	63.4 ± 2.2 (3.4)

[a] RSD in % (in brackets)

The average concentration of three independently performed analyses was 309.4 µg g^{-1} for Me_3Pb^+ and 63.4 µg g^{-1} for Pb^{2+} (both calculated as Pb). Taking into account that the spike contains some inorganic lead iodide, the total yield for the synthesis of the ^{206}Pb-enriched trimethyllead iodide was 75 %. The peak areas of the two lead species in Figure 8 do not represent the determined concentration ratio, because the inorganic lead peak is much too small. This is due to an incomplete derivatization of Pb^{2+} by $NaBEt_4$. However, this has no influence on the accuracy of the species-specific GC-ICP-IDMS determinations, because only the isotope ratio of the corresponding isotope diluted species represents the exact amount of a species. Only the precision is affected by the low signal intensity of the Pb^{2+} peak.

For all further determinations of trimethyllead in real samples, the $Me_3{}^{206}Pb^+$ spike solution was characterized only for trimethyllead. The determination of inorganic lead using this spike is also possible. But as far as the content of inorganic lead in most of the real samples is much higher than the trimethyllead concentration, an optimum range of spike addition for the isotope dilution determination for both species cannot be achieved.

3.5.3 Study on the $Me_3{}^{206}Pb^+$ spike stability

Long-term storage can have significant influence on alteration of the relative proportions of different chemical species. Reasons can be chemical reactions between species, effects of the container material, microbial activity, temperature, pH and light. Therefore, investigations on long-term stability of the synthesized spike solution were performed at different storage conditions. A $Me_3{}^{206}Pb^+$ spike solution with a concentration of 480 ng g^{-1} was prepared by dilution of the stock spike solution in MQ-water and was stored in a Teflon bottle (solution 1). Decomposition of tetraalkylleads in water is known to be also promoted by various cations [85]. Since the solution of the synthesized spike contains Pb^{2+} ions, its influence on the trimethyllead stability was also studied. A standard of inorganic lead was therefore added to the trimethyllead spike, and the corresponding solution (480 ng g^{-1} $Me_3{}^{206}Pb^+$, 100 ng g^{-1} $^{206}Pb^{2+}$ and 1 µg g^{-1} Pb^{2+}) was stored in a Teflon bottle (solution 2). Additionally, the 480 ng g^{-1} solution of $Me_3{}^{206}Pb^+$ spike was stored in a polyethylene (PE) bottle (solution 3). All solutions were stored at 4˚C under exclusion of light. Reverse isotope dilution analyses (Chapter 3.5.2), using trimethyllead standard solution of natural isotopic abundances, were carried out to determine the concentration of trimethyllead in solutions 1-3 several times within a period of 1.5 years. The results (Figure 9) show no significant change of the $Me_3{}^{206}Pb^+$ concentration within 18 months of storage. This is in agreement with results described by Quevauviller et al. [86]. The isotopic composition of the spike solutions was also found to be stable over 18 months.

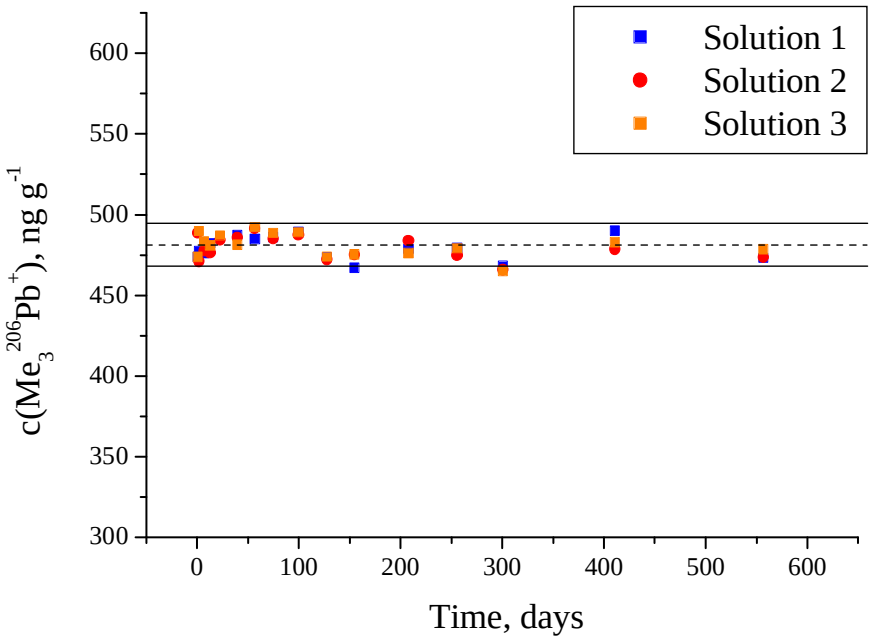

Figure 9: Dependence of $Me_3{}^{206}Pb^+$ spike concentration on the storage time at different conditions (Solution 1: 480 ng g^{-1} $Me_3{}^{206}Pb^+$, 100 ng g^{-1} $^{206}Pb^{2+}$ (Teflon); Solution 2: 480 ng g^{-1} $Me_3{}^{206}Pb^+$, 100 ng g^{-1} $^{206}Pb^{2+}$ and 1 µg g^{-1} Pb^{2+} (Teflon); Solution 3: 480 ng g^{-1} $Me_3{}^{206}Pb^+$, 100 ng g^{-1} $^{206}Pb^{2+}$ (PE))

3.6 Development of species-specific GC-ICP-IDMS method for trimethyllead determination

3.6.1 GC-ICP-MS coupling system

A gas chromatograph (model HP 6890, Agilent Technologies), fitted with a split/splitless injector and a HP-1 capillary column (cross-linked methyl siloxane; 21 m length, 0.32 mm i.d., and 0.17 µm film thickness), was used for separation of the volatile alkyllead compounds after derivatization. The gas chromatograph was coupled with the ICP-MS HP 4500 (Chapter 3.3) via a home-made transfer line. The GC-ICP-MS coupling unit is schematically represented in Figure 10.

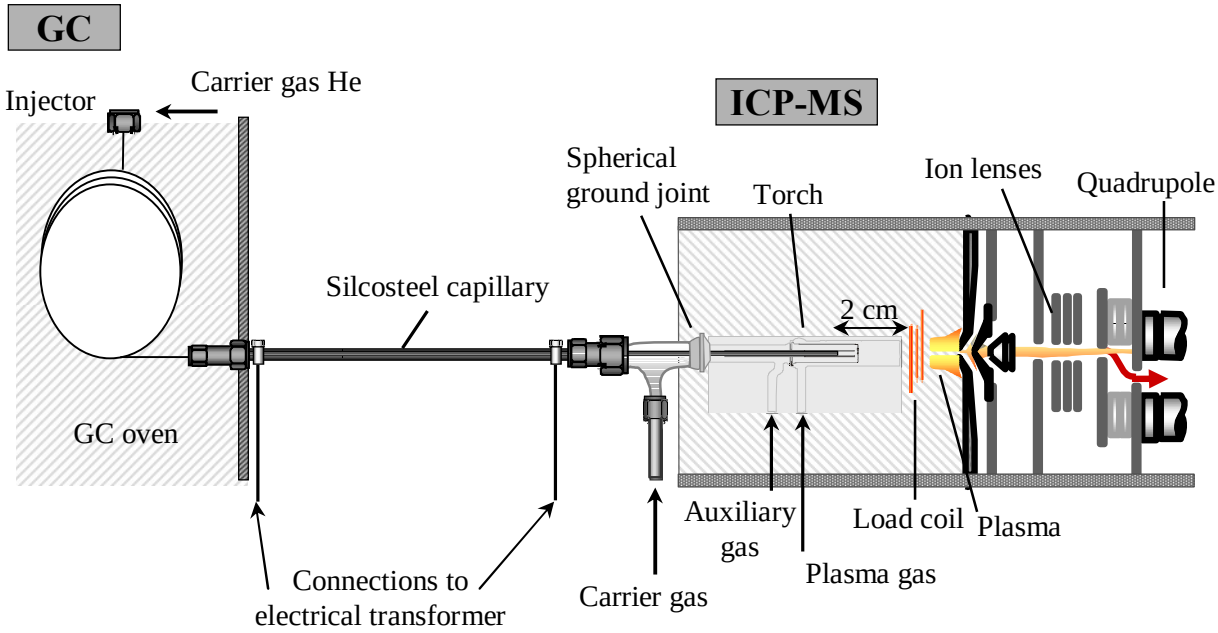

Figure 10: Schematic configuration of the GC-ICP-MS coupling system used for trimethyllead determination [87]

The transfer line is made of a Silcosteel stainless steel tube (Resteck; 0.51 mm i.d., 1.59 mm o.d.), coated with polysiloxane deactivation layer, which is inert to low polar organic compounds. No interaction and consequently no separation of compounds take place in the capillary using this coating. High mechanical stability as well as good thermal conductivity and stability up to 400°C are additional advantages of the used transfer tube. It was connected to GC capillary column via a Swagelok connector. On the ICP-MS side, the transfer line ends in the plasma torch about 2 cm in front of the load coil of the ICP (Figure 10). Coupling via a flexible glass interface with a spherical ground joint allows movement of the torch during the automatic torch positioning routine. This configuration makes coupling of the GC with the ICP-MS quick and simple. The carrier argon gas was introduced through a side arm of the glass interface. The transfer line was directly heated by aid of an electrical transformer (type 3234D, Statron, Fürstenwalde), which has the advantage of a good and fast heat transfer. No insulation is necessary using this direct heating system to hold the temperature constant. For safety reasons the transfer line is covered with a PTFE tube. All connections are sealed by metal or graphite ferrules. Such a coupling system was also successfully used in the past for other investigations by GC-ICP-MS [88].

3.6.2 Optimization of GC-ICP-MS conditions

The GC-ICP-MS conditions were optimized in order to obtain narrow peaks and maximum sensitivity for a precise lead isotope ratio measurement of the trimethyllead peak under the dry plasma conditions. Temperature and position of the Silcosteel capillary inside the torch have significant influence on the shape of the peaks. Substances can be adsorbed on the capillary walls if it is not sufficiently heated. This causes formation of so called “cold spots” with the effect of broadened peaks. A current of 7 A was sufficient to avoid those effects. The position of the end of transfer line has a tremendous influence on the detected peak shape. A positioning in 15 cm distance to the load coil obviously causes adsorption effects at the walls of the ICP torch. Result is a broadened and flat peak (Figure 11). A close distance of 2 cm to the load coil enables sharp peaks and therefore good detection limits. At this position the sample is brought directly to plasma and the trimethyllead peak is correspondingly narrow and intense (Figure 11).

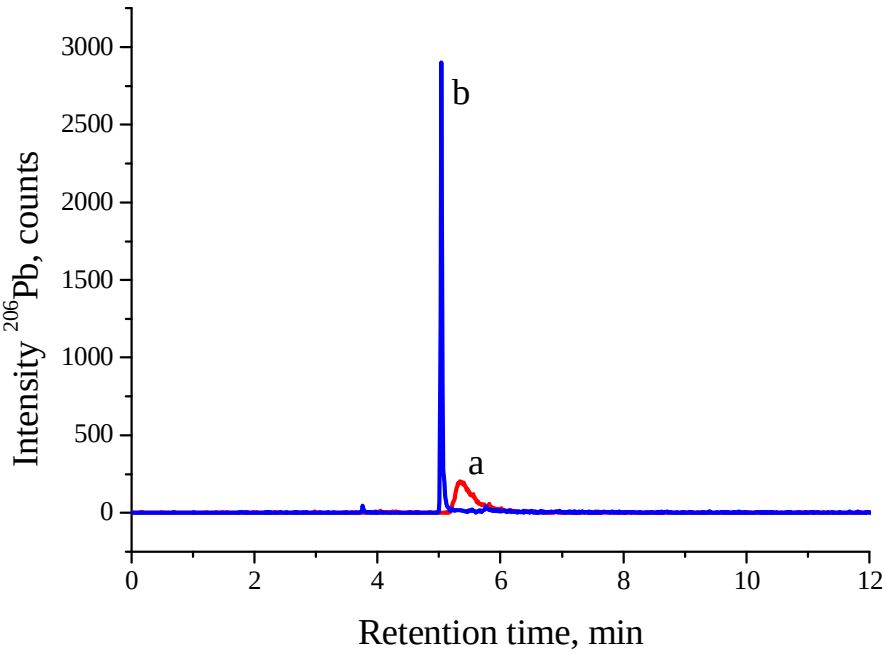

Figure 11: GC-ICP-MS chromatograms of a Me_3Pb^+ standard solution obtained at the transfer line distance from the load coil of 15 cm (a) and 2 cm (b)

Parameters such as radio frequency power (RF-power), the flow rate of carrier gas and the torch position have also significant influences on the chromatogram quality. The radio frequency power of ICP-MS has no influence on the transport of species through the transfer line, but it affects significantly the signal intensity. Optimum RF-power for the trimethyllead analysis was determined by repeated measurements of the trimethyllead standard (Figure 12). Improvement in the sensitivity for trimethyllead was observed by an increase of the RF-power. A maximum signal intensity for lead isotope ratio measurements of the trimethyllead peak was observed at a RF-power of 1200-1400 W as shown in Figure 12. Therefore, a plasma power of 1300 W was chosen for all further experiments.

In addition, the signal intensity increased by a factor of 7-8 when the argon carrier flow rate was increased from 1.0 L min^{-1} to 1.25 L min^{-1} at 1300 W RF-power (Figure 13). At higher flow rates (above 1.3 L min^{-1}) the signal decreased again. All further measurements were carried out at the argon carrier flow rate of 1.25 L min^{-1}.

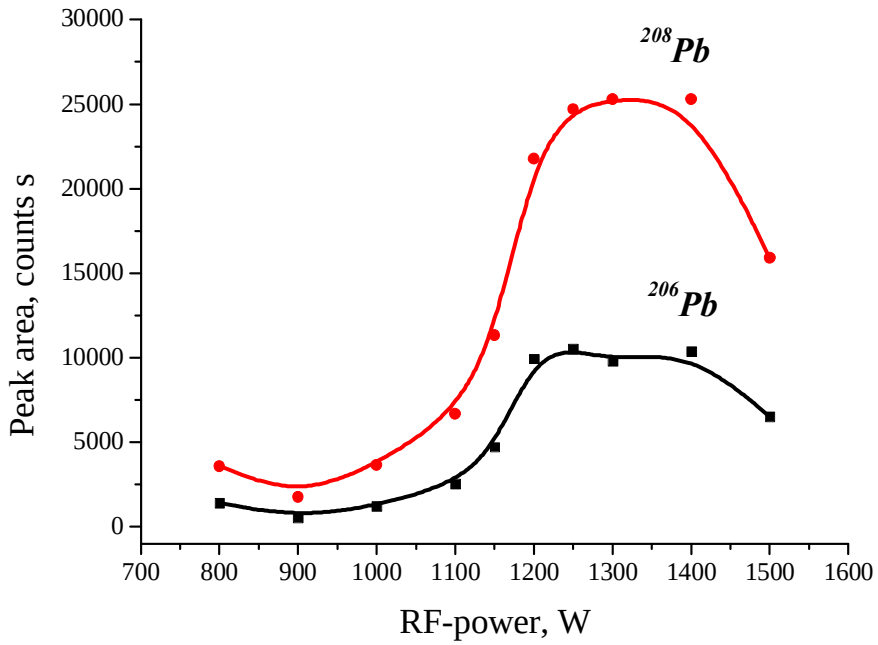

Figure 12: The effect of the RF-power on the signal intensity of lead isotopes measured in the trimethyllead peak

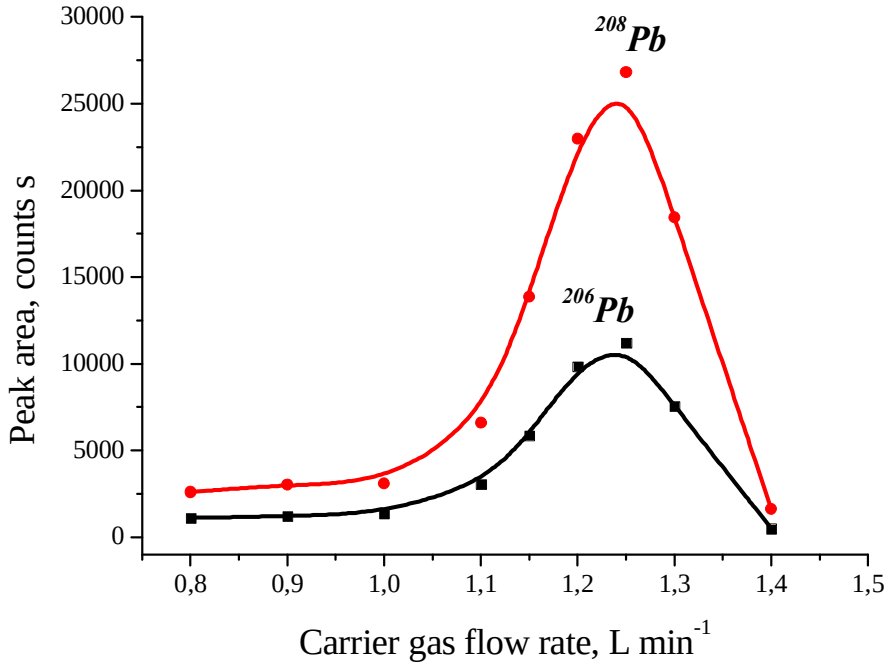

Figure 13: The effect of the argon carrier flow rate on the signal intensity of lead isotopes measured in the trimethyllead peak

Application of organic solvents causes carbon depositions on the ICP-MS nickel cones as a result of uncompleted burning. This can lead to loss of sensitivity for the element signals. In principle, oxygen addition to the plasma gas can be used to oxidize the organic solvent completely in plasma and to avoid carbon depositions on the cones. Therefore, the influence of the oxygen flow rate on the sensitivity of the trimethyllead peak was investigated in detail. Oxygen was introduced through an additional side arm of the glass interface. The flow rate was controlled by a flow meter after pressure reduction. The signal for trimethyllead decreased dramatically, even at an oxygen flow rate of only 15 mL min^{-1} (Figure 14). The addition of oxygen caused also a severe decrease in the sensitivity for mercury species [89]. Therefore addition of oxygen was not applied. All experiments with standard solutions were carried out using the GC injection at high split ratios (up to 100:1) in order to minimize quantity of organic solvent in the plasma and to reduce carbon depositions on the cones. When signal drift was observed, the increase of the RF-power for a few minutes after measurements of samples was also applied to eliminate carbon depositions.

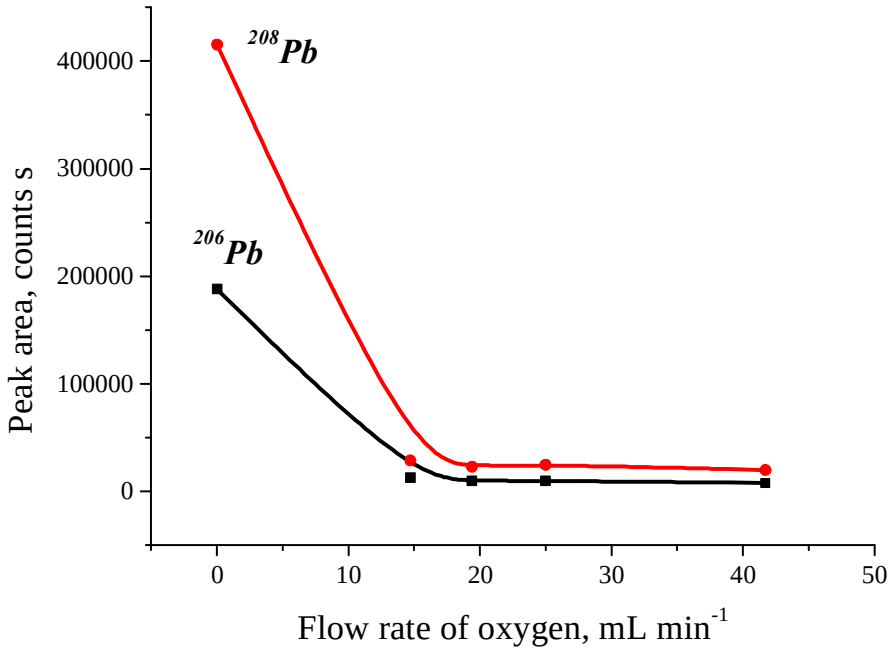

Figure 14: Influence of oxygen as auxiliary gas on the signal intensity of lead isotope measurements in the trimethyllead peak

The optimized operating conditions for the GC-ICP-MS coupling system are summarized in Table 16. These conditions were chosen for all further experiments with trimethyllead.

Table 16: Optimized operating conditions of the GC-ICP-MS coupling system for trimethyllead determination

ICP-MS parameters	
Radio frequency power	1300 W
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	1.20 L min^{-1}
Carrier gas flow rate	1.25 L min^{-1}
Torch position ("Sampling depth")	6 mm
Points per mass	1
Integration time per point	0.1 s
Isotopes	^{206}Pb, ^{208}Pb
GC conditions	
Column	HP-1 (methyl siloxane)
Carrier gas / flow rate	He / 2 mL min^{-1}
Oven program	40°C (2.5min) → 60°C (10°C/min) → 260°C (30°C/min, 5.0 min)
Injection mode	Split (for measurement of standards) Splitless (for measurement of samples)
Splitless time	0.2 min
Injection volume	1 µL
Injection temperature	250°C
Transfer line	Silicosteel tube, 180 cm length, 0.51mm i.d.

3.6.3 Isotope ratio measurements with GC-ICP-MS

The isotope ratio $^{206}Pb/^{208}Pb$ with ^{206}Pb as the spike and ^{208}Pb as the reference isotope was selected for the isotope dilution technique. The isotope ratios of the isotope diluted samples were always determined by an evaluation of the corresponding isotopes in the Me_3Pb^+ peak of the gas chromatogram. Because of better precision of the isotope ratio determination, the peak area and not the peak height was used. Integration of the chromatographic peaks was performed by the ICP-MS software.

Factors such as mass bias, spectral interferences and detector dead time have been taken into account to obtain accurate isotope ratio measurements. There are several approaches for mass bias correction using GC-ICP-MS:

- measurement of an isotope ratio standard at regular intervals between the samples (e.g. ethylated isotopic lead standard NIST 981 [90] or sample of natural abundances for elements which do not show natural variations [91]);
- continuous introduction of a volatile compound with well-known isotope composition from a diffusion cell into the mass spectrometer under the same conditions as for the GC separation (e.g. dimethyl selenide for selenium speciation [92]);
- simultaneous introduction of an element solution with known isotope abundances at the same mass range during the GC measurements (e.g. thallium solution for mass bias corrections of lead and mercury isotopes [93]);
- using the measured isotope abundances or isotope ratios of the spike, the sample, and the isotope diluted sample for calculations by the IDMS equation which compensates mass discrimination effects [79].

The last approach was used in this work for trimethyllead measurements. In the case of lead, natural isotopic variations can occur by radiogenic production. The isotope abundances of all different samples were therefore determined independently and the corresponding values were used for IDMS calculations instead of the IUPAC data.

Spectral interferences by GC-ICP-MS are less severe than by using conventional nebulization. Isobaric interferences (e.g. ^{204}Pb – ^{204}Hg) are normally separated in the chromatographic column as the elution time of different lead and mercury compounds will be different. Polyatomic interferences derived from the plasma gas will only increase the chromatographic baseline by a constant value which will be compensated by the integration of the GC peak.

The dead time of the detector system can significantly affect the measured intensities at high counting rates (more than 10^6 cps). In this case, the dead time of the detector must be calculated and the intensities corrected. No dead time correction is necessary if the solutions of low concentrations are measured [94].

3.6.4 Sample preparation for trimethyllead determination by liquid-liquid extraction GC-ICP-IDMS

The sample treatment procedure for the determination of trimethyllead in road dust was adapted from the work of Witte et al. [95], which was optimized in this work for the isotope dilution technique. The recommended minimum sample amount of 1 g was weighed together with 0.3-0.4 g of a diluted stock spike solution of trimethyllead (usually in the range of about 10 ng g^{-1}, exactly determined) into a 7 mL glass vial with screw cap. A 3 mL aliquot of an extraction reagent, which consists of a citrate buffer (pH 7-8), EDTA and sodium diethyldithiocarbamate (NaDDTC), were added. The mixture was shaken for about 2 h. Trimethyllead has to be derivatized in order to obtain a volatile species for GC separation. After addition of 500 µL of hexane and 500 µL of a 2 % aqueous $NaBEt_4$ solution, the mixture was shaken again for 10 min and then centrifuged (4000 min^{-1}) to facilitate phase separation. The yellow hexane phase was collected with a micropipette and transferred to a 2 mL glass vial. After injection of 1 µL of this extract into the GC (split ratio 10:1 or 20:1), the GC chromatograms of ^{206}Pb and ^{208}Pb were detected by ICP-MS.

In the case of biological samples 0.2-1 g of the sample and 0.1-0.3 g of the diluted stock spike solution were also weighed into a 7 mL vial and mixed with 3-4 mL of TMAH (25 % in water). The mixture was shaken for about 2 h at room temperature in order to dissolve the organic matrix. Sample decomposition can also be carried out by TMAH in a few minutes using microwave-assisted treatment [96]. However, in this case, the single-use 7 mL vials cannot be applied. 0.6-0.8 mL of concentrated HNO_3 and 0.5 mL of 4 mol L^{-1} acetate buffer were then added to obtain pH 5-6. Ethylation and the GC-ICP-MS measurement were similar to the dust sample treatment except that a split ratio of 1:1 or a splitless mode was applied due to the lower

Me_3Pb^+ concentrations in these samples. The schematic sample preparation procedure for the determination of trimethyllead in environmental as well as in biological samples – or more general, in inorganic and organic matrices – by species-specific GC-ICP-IDMS is presented in Figure 15.

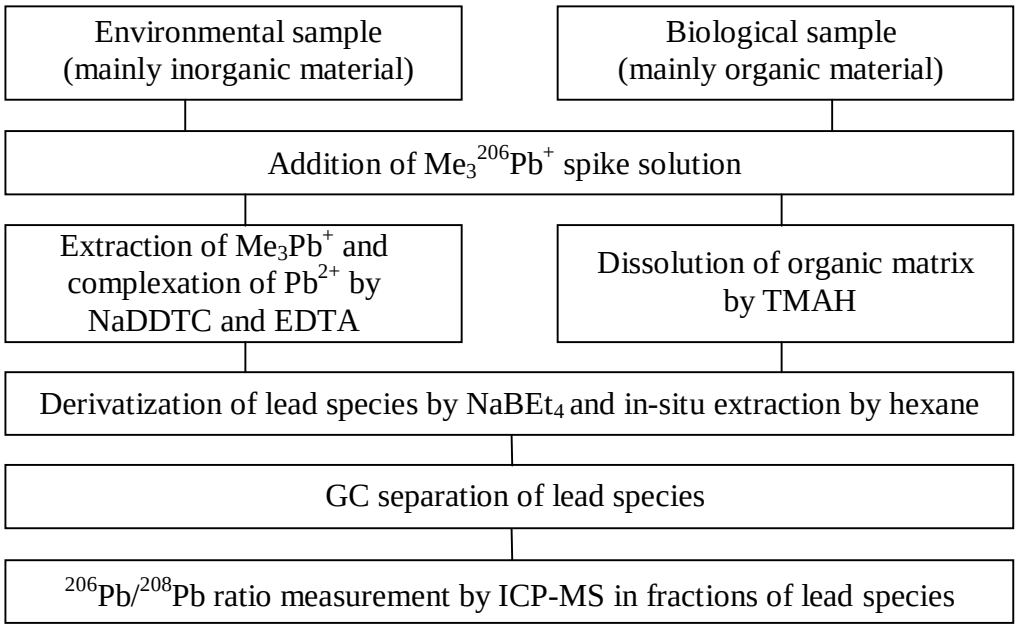

Figure 15: Schematic diagram of the sample preparation procedure of environmental and biological samples for trimethyllead determination by species-specific GC-ICP-IDMS

3.6.5 Investigation of possible trimethyllead transformations

Species-specific ICP-IDMS can also be used for the investigation of possible species transformations during a sample preparation procedure [97]. Previous GC-ICP-IDMS investigations have shown that methylmercury can be transformed to elementary mercury during ethylation by $NaBEt_4$ in the presence of halide ions [98]. Information about the influence of halide ions on a possible trimethyllead transformation is not available from the literature. A synthetic seawater was therefore prepared by dissolving 28.0 g NaCl, 7.1 g $MgSO_4 \cdot 7H_2O$, 5.0 g $MgCl_2 \cdot 6H_2O$, 2.4 g $CaCl_2 \cdot 2H_2O$ and 0.2 g $NaHCO_3$ in 985 mL Milli-Q water. A GC-ICP-MS

chromatogram of a Me_3Pb^+ standard solution, after ethylation in 5 mL synthetic seawater with 0.5 mL acetate buffer at pH 4, is presented in Figure 16. Besides the trimethyllead peak a second small peak was observed at 6.0 min in this chromatogram. It was expected to be from Me_2Pb^{2+}. In order to confirm this, Me_2Pb^{2+} was prepared by reaction of a trimethyllead solution and iodine monochloride [99].

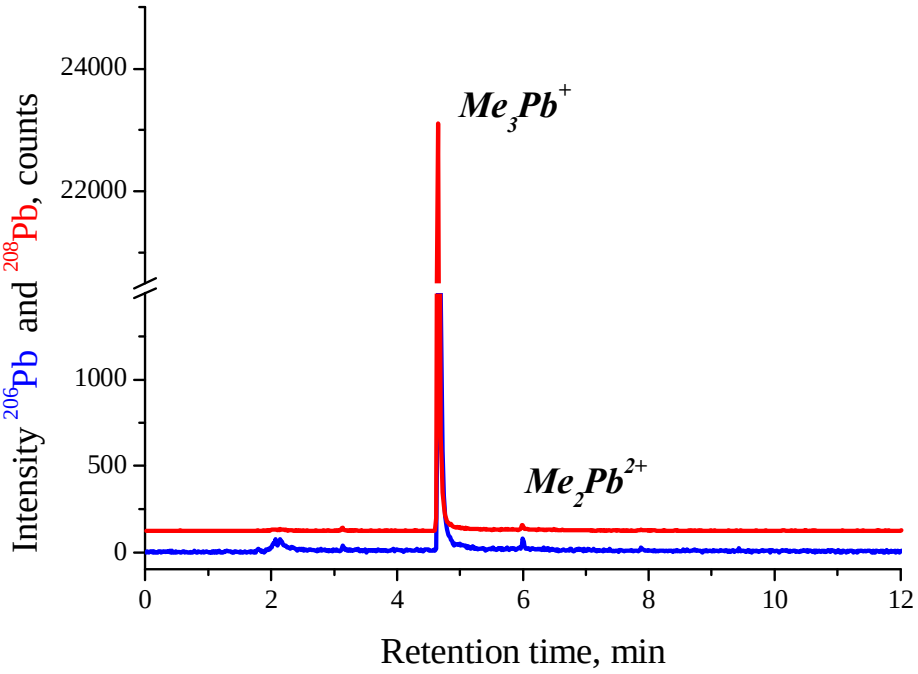

Figure 16: GC-ICP-MS chromatogram of a trimethyllead standard solution after derivatization with $NaBEt_4$ in synthetic seawater

0.15 g iodine monochloride (98 %) were diluted in 4.8 g methanol to obtain a 0.2 mol L^{-1} solution of ICl. 0.07 g of this solution were added to 10.6 g of a 866 ng g^{-1} Me_3Pb^+ standard solution. An aliquot of the obtained solution was analyzed under the same conditions after ethylation. The resulting chromatogram is presented in Figure 17. The main peak at 6.0 min corresponds to dimethyllead and the small peak at 4.7 min – to trimethyllead not transformed into dimethyllead during the reaction with iodine monochloride. The peak at 6.9 min is assumed to be from monomethyllead. Its appearance can be explained by a rearrangement process during or following the ethylation, like the appearance of monoalkyllead species during Grignard derivatization [100].

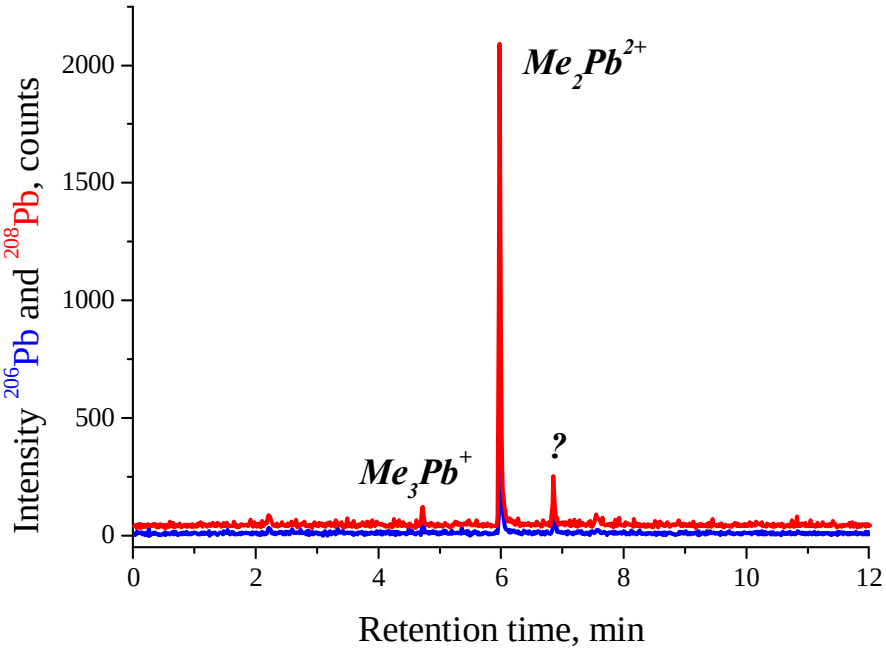

Figure 17: GC-ICP-MS chromatogram of a dimethyllead standard solution after derivatization with $NaBEt_4$

The GC-ICP-MS chromatogram of a Me_3Pb^+ standard solution after ethylation in 5 mL Milli-Q water is similar to that after derivatization in synthetic seawater. The fraction of lead species for these two cases, presented in Table 17, show no influence of halide ions on the behaviour of trimethyllead during derivatization by $NaBEt_4$. The same experiment was carried out with a $Me_3{}^{206}Pb^+$ spike solution and no difference between chromatograms for Milli-Q and seawater was found. Therefore it can be assumed that the Me_2Pb^{2+} peak is not the result of a trimethyllead transformation in the presence of halide ions. The appearance of dimethyllead may be due to a rearrangement process during or following the ethylation, like the appearance of monoalkyllead during Grignard derivatization [100].

Table 17: Determination of the species fraction in a trimethyllead standard solution, ethylated in Milli-Q water and seawater (n=2)

Solvent	Me_3Pb^+, %	Me_2Pb^{2+}, %
Milli-Q water	99.2 ± 0.2	0.8 ± 0.2
Seawater	99.4 ± 0.1	0.6 ± 0.1

The trimethyllead determination in dust and sediment samples was performed using an extraction reagent, which consists of a citrate buffer (pH 7-8), EDTA and NaDDTC (see Chapter 3.6.4). This extraction reagent was found to have no influence on the ethylation of trimethyllead.

In the case of biological samples, TMAH and acetate buffer are mandatory for the sample preparation procedure. Its influence on possible trimethyllead transformations was therefore also studied. The results are listed in Table 18. They show similar portions of dimethyllead at different acetate buffer concentrations.

Table 18: Determination of the species fraction in a trimethyllead standard solution, ethylated at different quantities of acetate buffer (n=2)

Solution	Me_3Pb^+, %	Me_2Pb^{2+}, %
2 mL H_2O / 0 mL acetate buffer	99.5 ± 0.1	0.5 ± 0.1
1.5 mL H_2O / 0.5 mL acetate buffer	99.4 ± 0.1	0.6 ± 0.1
1.0 mL H_2O / 1.0 mL acetate buffer	99.6 ± 0.1	0.4 ± 0.1
0 mL H_2O / 2.0 mL acetate buffer	99.5 ± 0.1	0.5 ± 0.1

The TMAH treatment has no significant influence on the species fraction in trimethyllead spike solution (Table 19). Only the Me_2Pb^{2+} fraction in the presence of TMAH is higher than after a direct derivatization in 0.5 mL acetate buffer without TMAH pretreatment. This can be due to

the influence of different conditions of these two cases (e.g. ionic force) on the derivatization and extraction yield of lead species.

Table 19: Determination of the species fraction in a trimethyllead spike solution, ethylated in the presence or absence of TMAH (n=2)

Treatment	Me_3Pb^+, %	Me_2Pb^{2+}, %	Pb^{2+}, %
Without TMAH	72.1 ± 1.9	0.3 ± 0.1	27.6 ± 1.8
With TMAH	73.2 ± 2.9	1.1 ± 0.1	25.7 ± 2.8

3.6.6 Validation of the GC-ICP-IDMS method for determination of trimethyllead

Certified reference materials are usually applied to validate analytical methods. At the moment only one CRM, urban dust (CRM 605), exists for the quality assurance of trimethyllead. It was prepared and certified by the Standards, Measurement and Testing program (formerly BCR) of the European Union [101]. An attempt was also made to certify the Me_3Pb^+ content in a rainwater sample, another candidate reference material. However, certification was not completed because of doubts about the long term stability of trimethyllead in this artificial rainwater [102].

The urban dust reference material was analyzed according to the sample preparation procedure for trimethyllead determinations in environmental samples by the species-specific GC-ICP-IDMS method described in Chapter 3.6.4. Three independent analyses were performed (Table 20). The corresponding result of 8.01 ± 0.20 µg kg^{-1} (as Me_3Pb^+) was in excellent agreement with the certified value of 7.9 ± 1.2 µg kg^{-1} and shows high precision of the developed method.

Table 20: Determination of trimethyllead in the certified reference material CRM 605 by GC-ICP-IDMS

Analysis №	m(sample), g	m(spike), g	$^{206}Pb/^{208}Pb$	c(Me_3Pb^+), µg kg^{-1}
1	0.9819	0.3962	1.0002	7.88
2	0.9906	0.3996	1.0125	7.91
3	0.9894	0.3982	0.9739	8.24
Average ± SD				8.01 ± 0.20 (2.5%)

Besides the Me_3Pb^+ peak, other peaks were also observed in the GC-ICP-MS chromatogram of the reference material CRM 605 (Figures 18 and 19). The intense peak at 8.3 min corresponds to inorganic lead which could not be completely masked by the added EDTA and therefore derivatized by $NaBEt_4$ to Et_4Pb. Small peaks correspond to dimethyllead and ethylmethyllead species. Triethyl- and diethyllead could also be identified in this reference material after derivatization with $NaBPr_4$.

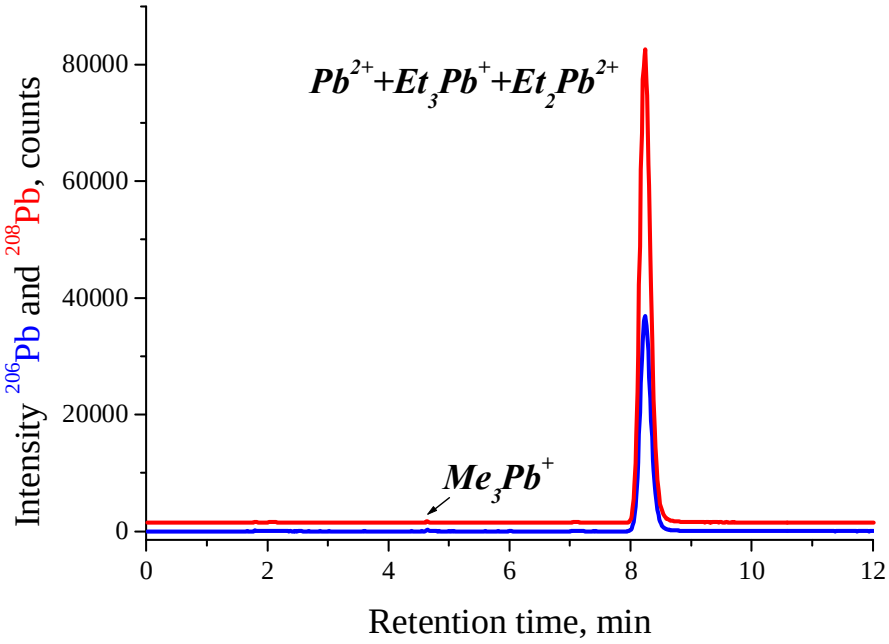

Figure 18: GC-ICP-MS chromatogram of the reference material CRM 605 after addition of $Me_3{}^{206}Pb^+$ spike and derivatization with $NaBEt_4$

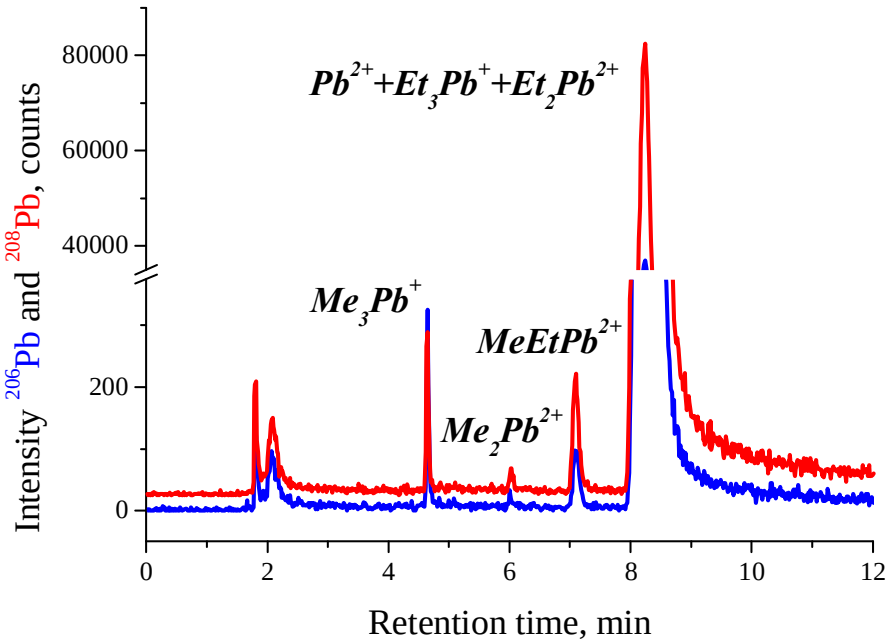

Figure 19: Enlargement of Figure 18

3.7 Application of species-specific GC-ICP-IDMS for determination of Me_3Pb^+

3.7.1 Analysis of reference materials not certified for trimethyllead

The developed GC-ICP-IDMS method was applied for trimethyllead determination in six biological reference materials (seafood) and two reference sediments, which are not certified for this lead species. The trimethyllead concentrations in these reference materials are listed in Table 21 together with the corresponding standard deviations calculated from five independent analyses each. The total lead content (certified value or determined by ICP-IDMS) as well as the fraction of methylated lead in the samples, correlated to total lead, are also presented. Trimethyllead could be determined in all reference materials except in the Antarctic krill (MURST-ISS-A2) and two sediments (CRM 580 and BCR 646), where a concentration of less than the corresponding detection limits was found.

Table 21: Concentrations of trimethyllead and total lead in reference materials

Reference material	Me_3Pb^+ ng g^{-1} as Pb	Total Pb ng g^{-1}	Methylated Pb %
DORM 2 (Dogfish muscle)	6.41 ± 0.29^b	65 ± 7^a	10
CRM 463 (Tuna fish)	4.39 ± 0.16^b	381 ± 39^c	1.2
CRM 422 (Cod muscle)	16.74 ± 0.19^b	85 ± 15^a	20
CRM 477 (Mussel tissue)	0.30 ± 0.02^b	9067 ± 95^c	0.003
CRM 278 (Mussel tissue)	2.72 ± 0.06^b	1910 ± 40^a	0.14
MURST-ISS-A2 (Antarctic krill)	$< 0.06^b$	1110 ± 110^a	<0.01
CRM 580 (Sediment)	$< 0.04^b$	n.d.	-
BCR 646 (Sediment)	$< 0.04^b$	147920 ± 1032^c	<0.00003

[a] certified value, [b] determined by GC-ICP-IDMS, [c] determined by ICP-IDMS,
n.d. not determined

The results for trimethyllead in five commercially available reference materials, determined by species-specific GC-ICP-IDMS, can be used as accurate indicative values to validate other analytical methods for this important elemental species.

GC-ICP-MS chromatogram of the reference material CRM 422 (cod muscle) is presented exemplary in Figure 20. It shows different peak ratios of trimethyllead and inorganic lead than already described in urban dust (Figure 18).

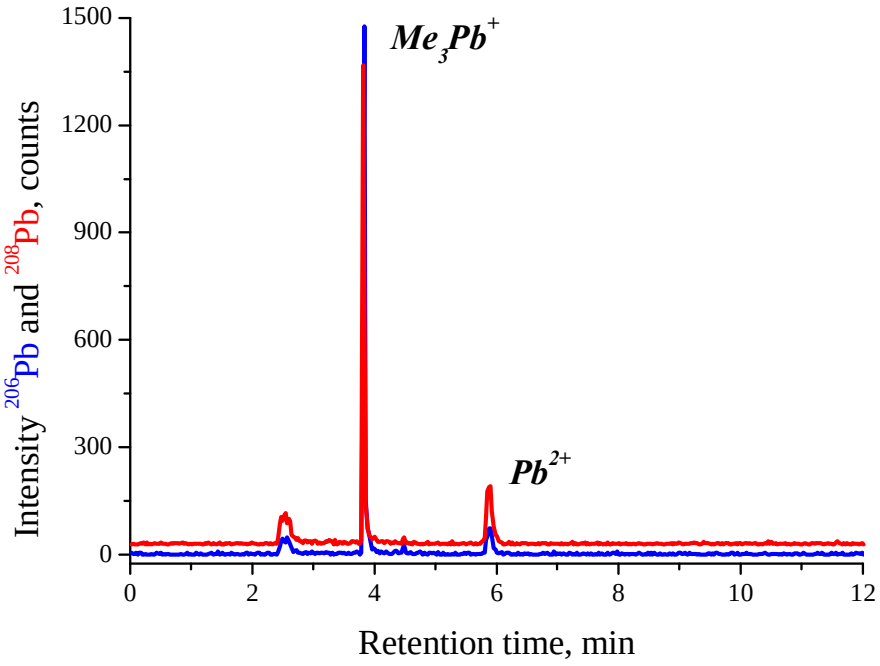

Figure 20: GC-ICP-MS chromatogram of reference material CRM 422, after addition of $Me_3{}^{206}Pb^+$ spike and derivatization with $NaBEt_4$

3.7.2 Analysis of seafood samples from the supermarket

Trimethyllead was also determined in mussels, large peeled prawns, fillets of a tuna fish, a plaice and a pollock bought in supermarkets of Mainz. All samples were freeze-dried directly after purchase, then milled and stored in polyethylene bottles in the dark. The water content of these samples, determined as mass difference before and after the freeze-drying procedure, was in the

range of 75-84 %. All analyses were performed on freeze-dried material and analytical results were calculated on a dry mass basis.

The results of trimethyllead determinations by species-specific GC-ICP-IDMS for these samples are summarized in Table 22. The total lead content determined by ICP-IDMS as well as the fraction of methylated lead in these samples are also presented. Trimethyllead could be determined in all seafood samples. Its concentration varies within a very small range from only 0.3 ng g^{-1} to 0.7 ng g^{-1}.

Table 22: Concentrations of trimethyllead and total lead in seafood samples purchased from a supermarket (n=4)

Sample	Me_3Pb^+ ng g^{-1} as Pb	Total Pb ng g^{-1}	Methylated Pb %
Mussels	0.39 ± 0.02	2634 ± 138	0.02
Prawns	0.48 ± 0.03	115.0 ± 4.2	0.4
Tuna fish	0.40 ± 0.02	5.3 ± 0.9	7.5
Plaice	0.29 ± 0.02	39.8 ± 3.9	0.7
Pollock	0.69 ± 0.06	19.8 ± 2.6	3.5

Figure 21 shows the GC-ICP-MS chromatogram of prawns after addition of $Me_3{}^{206}Pb^+$ spike and derivatization with $NaBPr_4$.

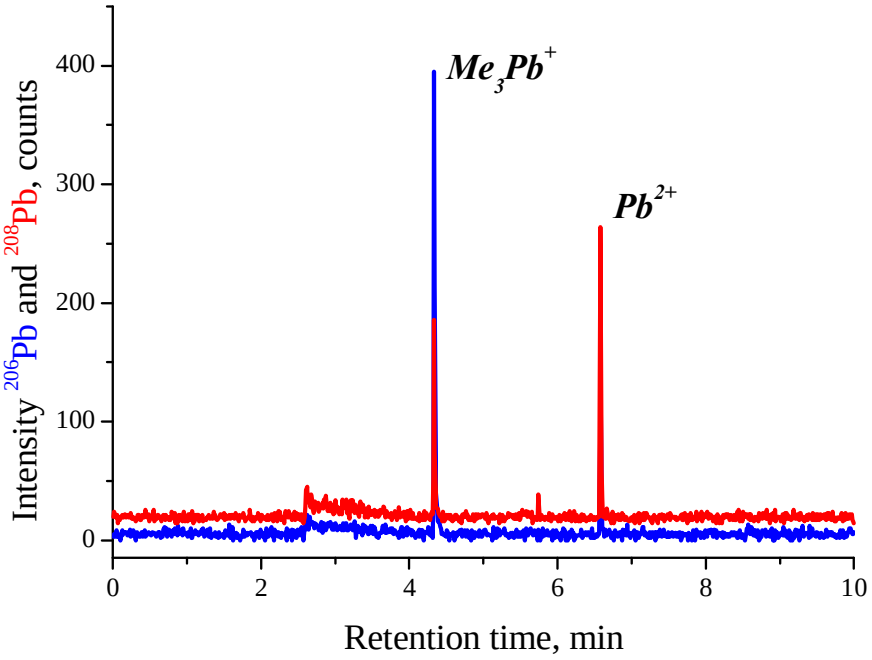

Figure 21: GC-ICP-MS chromatogram of prawns after addition of $Me_3{}^{206}Pb^+$ spike and derivatization with $NaBPr_4$

3.7.3 Analysis of soil samples from Ukraine

Introduction of sophisticated gasoline refinery technology and alternative additives, such as methyl tertiary-butyl ether, made tetraalkylleads an unnecessary option as octane enhancer. That leads to launch a leaded-gasoline phaseout in the USA and Japan in 1975. Leaded-gasoline phaseout was also launched in many countries of Europe in the 1980s and 1990s. In Ukraine the corresponding law of 15th November 2001 bans the import and realization of leaded gasoline and leaded additives to gasoline only from 1st of January 2003. Still in 1998, 182 900 tons of leaded gasoline were consumed in Ukraine [103].

Car emissions led to an ubiquitous distribution of low concentrations of organolead compounds but of large amounts of inorganic lead in the upper layers of soil. However evaporative loss from carburetors and gasoline tanks as well as spillages of leaded gasoline and highly toxic tetraalkyllead compounds during production, transportation or blending at oil refineries and

petrol stations caused more severe soil and groundwater contaminations by organolead compounds. Trialkylspecies, as decomposition products of tetraalkylleads, are relatively stable in soil. Chakraborti et al. observed no decrease of trimethyllead concentration in soil within 100 days [104]. Therefore it was interesting to analyze several soil samples from Ukraine.

Samples were taken in close distance to one petrol station in the town Khmelnytsky. Four samples were taken from different places in a distance of 10 m (two samples), 50 m and 100 m from an underground petrol tank. Samples were prepared for GC-ICP-MS analysis according to the sample pretreatment procedure described in Chapter 3.6.4. No trimethyllead was detected in any of the investigated samples. Chromatograms show only high peaks of inorganic lead (Figure 22). If sodium tetraethylborate is used as derivatization reagent, identification of ethylspecies is impossible. Therefore $NaBPr_4$ was applied to control presence of other lead species in these soil samples. Again only one peak for inorganic lead was observed, which confirmed the absence of ethyl- and mixed ethyl-methyl species in the investigated samples.

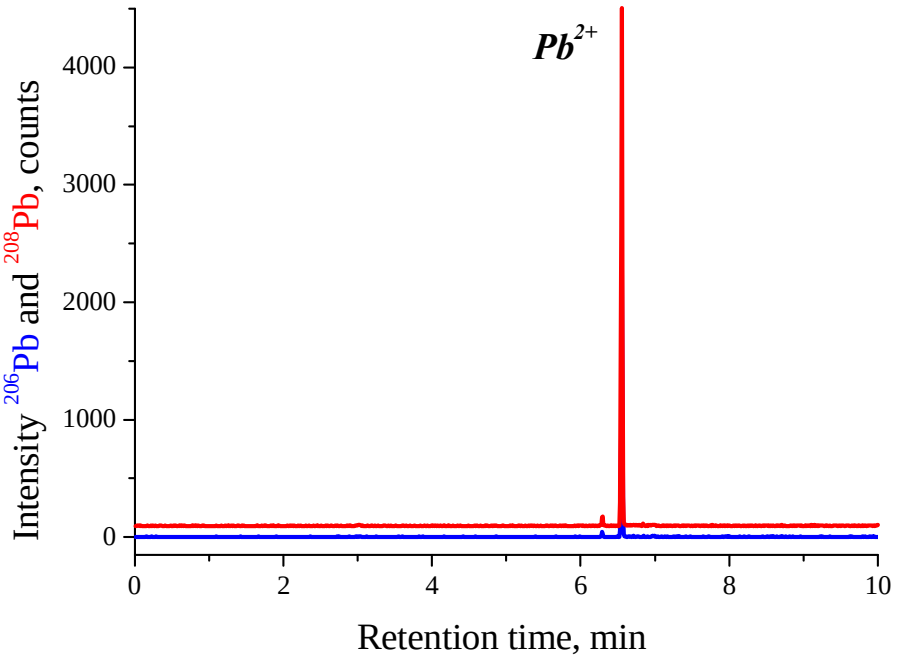

Figure 22: GC-ICP-MS chromatogram of the soil taken in a distance of 10 m from an underground petrol tank after derivatization with $NaBPr_4$

3.8 Development and application of species-specific GC-ICP-IDMS method for simultaneous determination of trimethyllead, methylmercury and butyltins

3.8.1 Optimization of GC-ICP-MS coupling for multi-species determination

The same GC-ICP-MS coupling system described for trimethyllead determination (Chapter 3.6.1) was used for simultaneous measurements of trimethyllead, methylmercury, mono-, di- and tributyltin. Under the conditions used for the determination of Me_3Pb^+ (Table 16, Chapter 3.6.2) peaks of trimethyllead and methylmercury were sharp and suitable for sensitive quantification. However, only one broadened peak was observed for the tin species under these conditions. Addition of argon make-up gas for more effective analyte transport from the GC into the ICP-MS has improved the detection of tin species. For this purpose a 1.5 m-stainless steel tube was adjusted at the end of the GC column to the transfer line via a T-shaped connecting piece. At the injection of standard with three butyltin compounds only two peaks were observed at make-up gas flow rates of 40 mL min^{-1} whereas three peaks could be detected at flows higher than 85 mL min^{-1}. However, peaks of di- and tributyltin were much broadened and not suitable for sensitive and precise measurements. The end of the transfer line on the ICP-MS side, not heated by aid of an electrical transformer (Figure 10, Chapter 3.6.1), was supposed to be responsible for peak broading of less volatile tin species. Therefore the corresponding commercially available GC-ICP-MS injector system with a special torch from Agilent Technologies was applied (Figure 23). The torch consists of two glass tubes with a relatively large spherical ground joint. The temperature of this injector (250˚C) was controlled directly by the GC system.

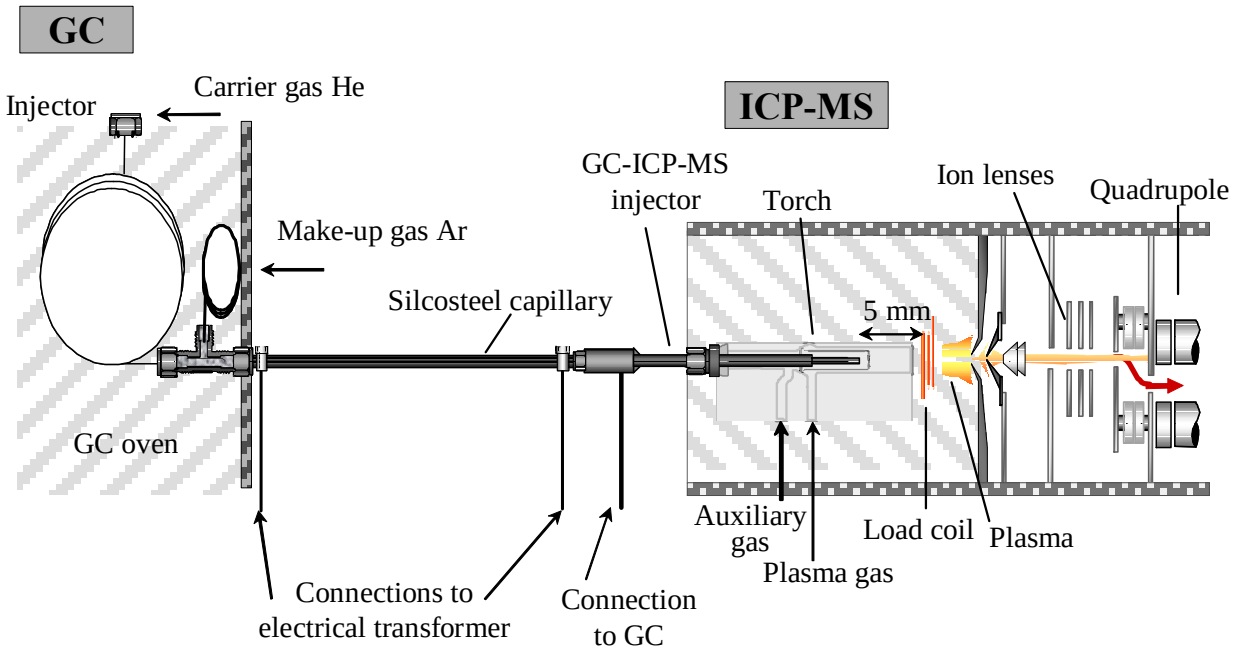

Figure 23: Schematic configuration of the GC-ICP-MS coupling system used for multi-species determination (modified from [87])

Chromatograms for a multi-species standard, measured at optimal flow rates of the make-up argon gas without (a) and with (b) heated injector, are shown in Figure 24. As can be seen the butyltin peaks are much higher and narrower in the second case. The heated injector avoids cold spots and supports an effective transport of compounds directly into the plasma, which is especially important for the less volatile butyltin species. No influence was observed on the peak shape of methylmercury and trimethyllead.

GC conditions were chosen in a way that the elution of the species is sufficiently separated from the solvent elution (hexane). The temperature program and gas flow rate were optimized to get high chromatographic resolution and best symmetric peak shapes for all species.

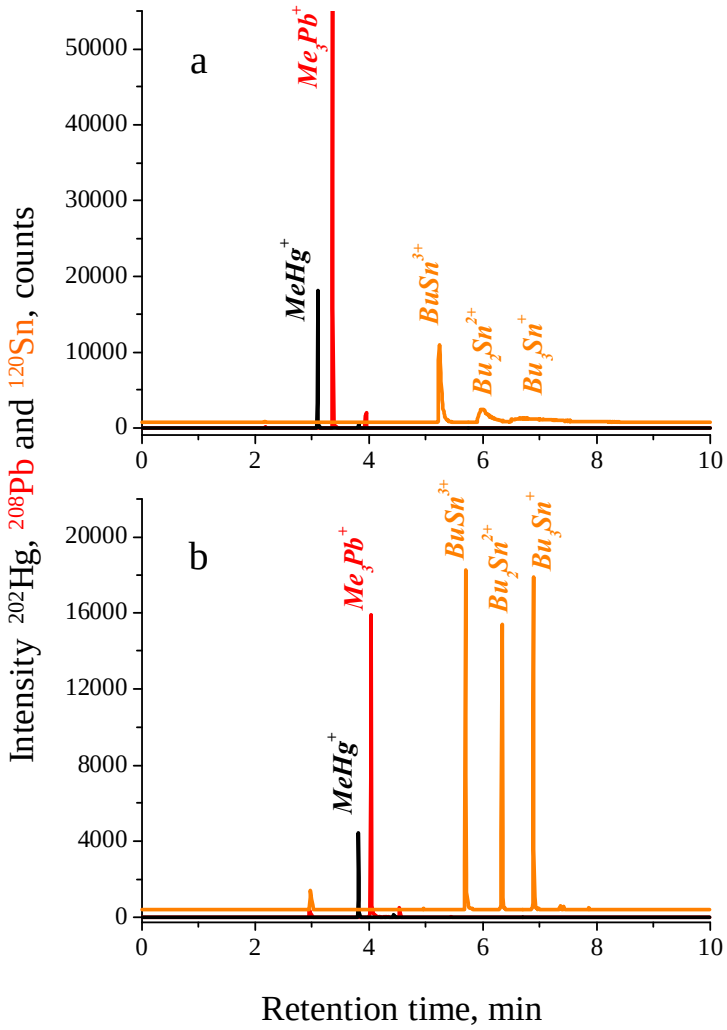

Figure 24: GC-ICP-MS chromatograms of a multi-species standard detected without (a) and with (b) heated injector (derivatization by $NaBEt_4$)

The ICP-MS conditions were also optimized due to maximum sensitivity for lead, mercury and tin isotope ratio measurements under the dry plasma conditions. Parameters like radio frequency power, flow rate of argon make-up gas, and torch position have significant influences on the signal intensity of all species.

A maximum of the signal intensity for trimethyllead, methylmercury and butyltin species was observed at a RF-power of 940-1050 W (Figure 25). The argon make up flow rate has also significant influence on the sensitivity, especially in the case of trimethyllead and butyltins. The optimum range of the argon make up flow rate for all species was found to be 120-135 mL min^{-1} (Figure 26).

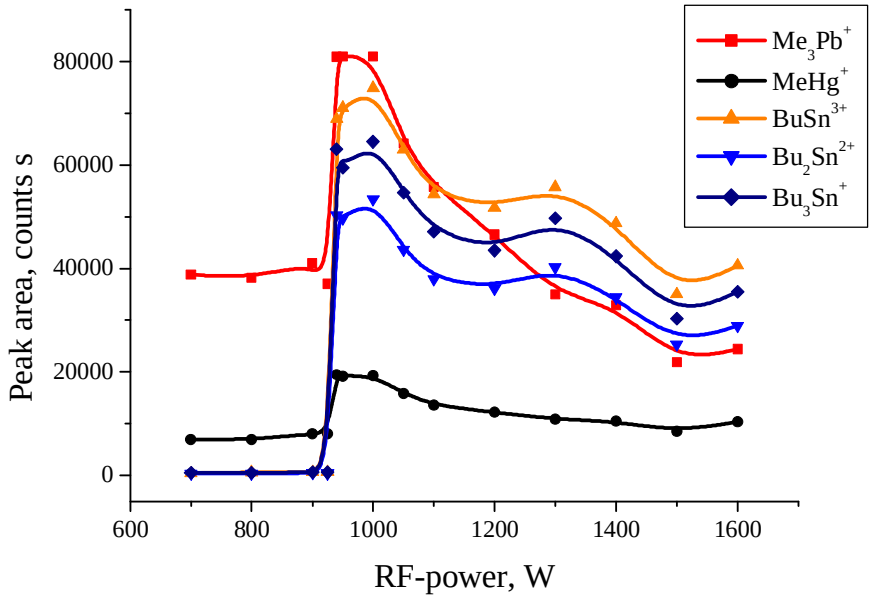

Figure 25: The effect of the RF-power on the signal intensity of lead, mercury and tin isotopes measured in trimethyllead, methylmercury, mono-, di- and tributyltin peaks

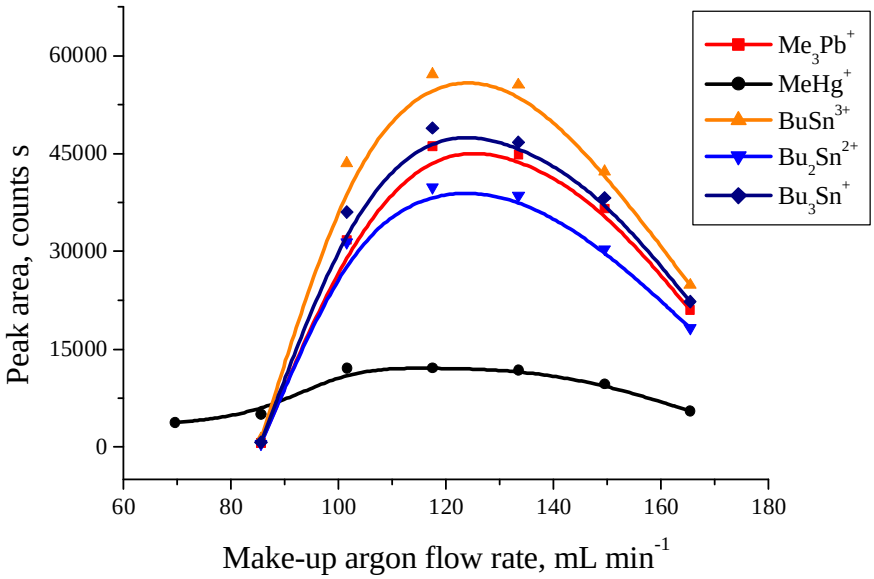

Figure 26: The effect of the argon make up flow rate on the signal intensity of lead, mercury and tin isotopes measured in trimethyllead, methylmercury, mono-, di- and tributyltin peaks

The optimum ion lens settings for ionization of metals obtained for liquids using a nebulizer system differ distinctly from the optimum settings using dry plasma conditions (as for GC-ICP-MS coupling). Maximum sensitivity for the organometallic compounds was always obtained under optimum conditions for m/z=80 ($^{40}Ar^{40}Ar^{+}$) [91,105]. Therefore, optimization of the ion lens of the mass spectrometer and control of the sensitivity under dry plasma conditions was performed by measuring the $^{40}Ar^{40}Ar^{+}$ background signal in the ICP-MS. The optimum conditions together with the other operating parameters for the GC-ICP-MS system are summarized in Table 23.

Table 23: Optimized operating conditions of the GC-ICP-MS system for simultaneous determination of trimethyllead, methylmercury and butyltins

ICP-MS parameters	
Radio frequency power	1000 W
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	1.20 L min^{-1}
Torch position (“Sampling depth”)	6 mm
Points per mass	1
Integration time per point	0.08 s
GC conditions	
Column	SPB-5: poly-diphenyl/dimethylsiloxane(5/95%) 30 m×0.32 mm×0.25 µm
Carrier gas for GC / flow rate	He / 4 mL min^{-1}
Make-up gas for ICP-MS / flow rate	Ar / 125 mL min^{-1}
GC-oven program	50°C (1 min) → 260°C (30°C min^{-1}, 5.0 min)
Injection mode	Split (for measurement of standards) Splitless (for measurement of samples)
Splitless time	0.2 min
Injection volume	1 µL
Injection temperature	250°C
Transfer line GC-ICP-MS	Silicosteel tube connected with heated injector; 180 cm length, 0.51 mm i.d., 1.59 mm o.d.

3.8.2 Isotope ratio measurements

The isotope ratios $^{206}Pb/^{208}Pb$, $^{200}Hg/^{202}Hg$, $^{119}Sn/^{120}Sn$ with ^{206}Pb, ^{202}Hg, and ^{119}Sn as spike isotopes and the others as reference isotopes were selected for the isotope dilution technique because no spectrometric interferences have been observed for these isotopes under the GC-ICP-MS conditions. The isotope ratios of the isotope diluted samples were always determined by an integration of the corresponding isotope peak areas of the gas chromatogram. Mass bias was corrected using methylmercury and butyltin standard solutions of natural isotopic composition, which were measured prior to and after the isotope diluted sample. In the case of determining trimethyllead, the mass bias for the mercury isotope ratio could be used because of the similar mass numbers to be corrected. The mass bias correction factor was calculated using the known (natural) ratio divided by the ratio measured in the standard solution.

Errors in the measurement of isotope ratios may arise at monitoring a rapidly changing transient signal with a scanning instrument. They derive from the non-simultaneous measurement of the intensities at the different masses. This effect is called “spectral skew” and is especially important when fast transient peak profiles (as for GC) of many isotopes must be followed. Therefore the integration time per isotope mass, the number of points per mass unit and the number of monitored isotopes need to be carefully optimized for precise isotope ratios by GC-ICP-MS. For that purpose, a multi-species standard containing methylmercury, trimethyllead, mono-, di- and tributyltin was injected under different data acquisition conditions. Six isotopes (^{119}Sn, ^{120}Sn, ^{200}Hg, ^{202}Hg, ^{206}Pb and ^{208}Pb) were monitored during one chromatogram.

The integration time was varied between 10 ms and 150 ms per isotope using one point per mass. The influence of the integration time on the mean isotope ratios and their standard deviations (from three injections) for all measured compounds are plotted in Figure 27.

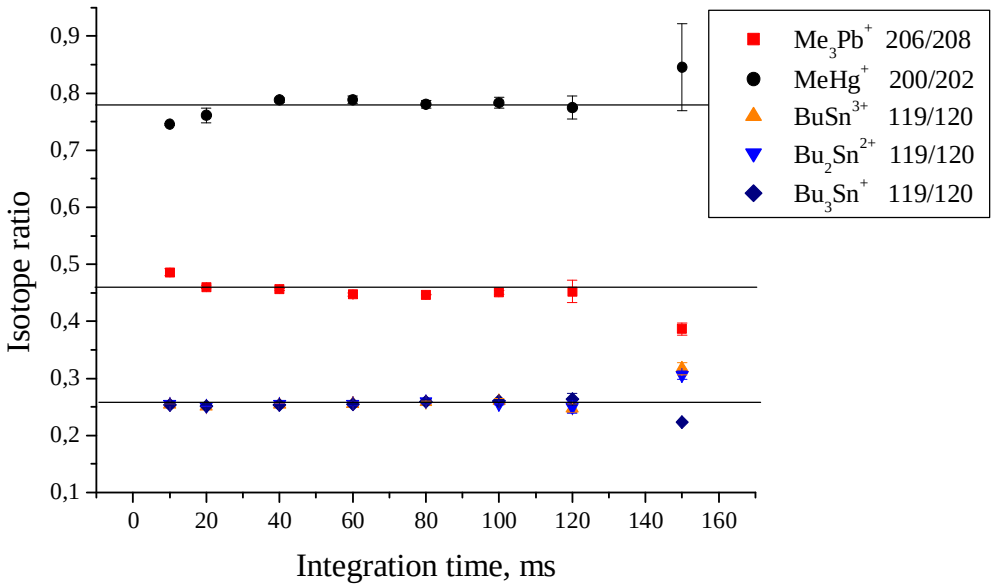

Figure 27: Influence of the integration time on the isotope ratio measurement in trimethyllead, methylmercury, monobutyltin, dibutyltin and tributyltin peaks

As can be seen constant isotope ratios and better precisions (RSD 0.1-1.6 %) were obtained for all elemental species by using integration times in the range of 40–100 ms per mass on peak area mode. At very long integration times, peak areas as well as corresponding isotope ratios result in a poor precision (RSD 2.5-9.0 %) because of worse peak definition. The effect of the integration time on the chromatographic peak profiles obtained for tributyltin at mass numbers 119 and 120 is illustrated in Figure 28.

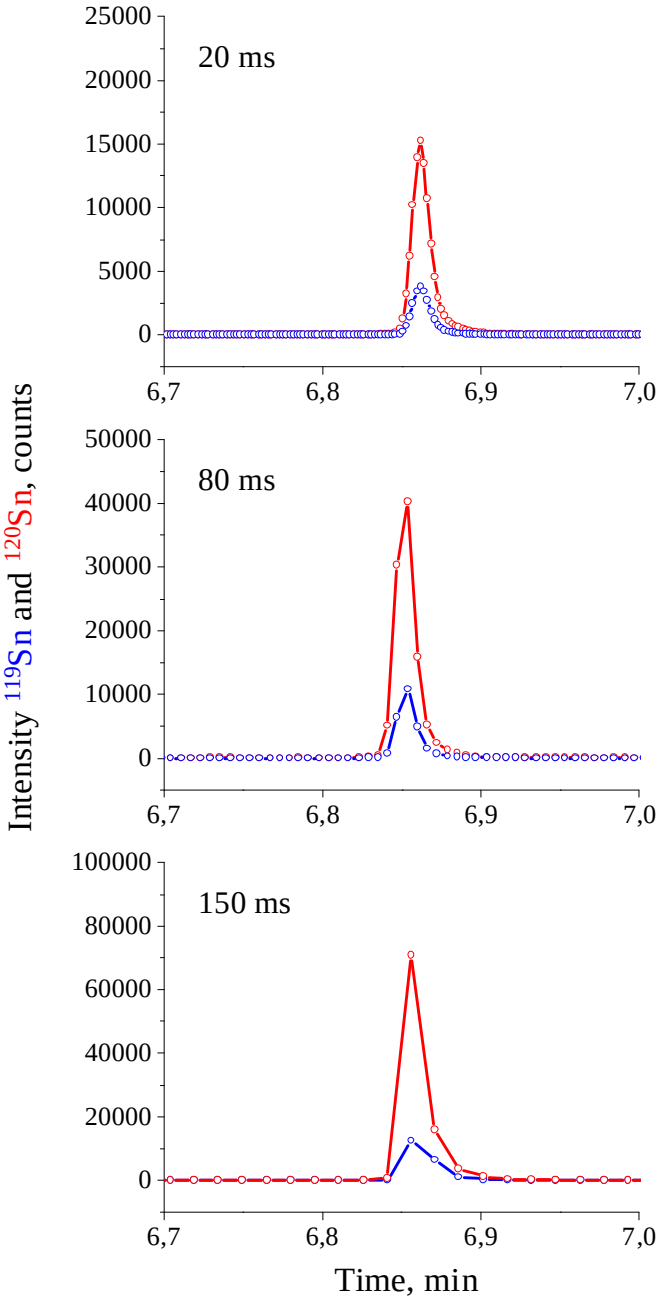

Figure 28: Influence of the integration time on the chromatographic peak profile of tributyltin

Figure 28 shows that at large integration times only a few measurement points define the chromatographic peak. Therefore the peaks are asymmetric and considerable spectral skew for the different masses exists. On the other hand, for low integration times the chromatographic peaks are very well defined and spectral skew is minimized. This effect was also observed for the other compounds monitored. 80 ms was therefore chosen as optimum integration time per isotope mass. The total integration time for six measured isotopes is then 0.48 s. The GC peaks

last about 5-7 s under optimized GC-ICP-MS conditions. Under these circumstances each isotope can be measured at 10 to 14 different points of the GC peak (5 s / 0.48 s ≈ 10; 7 s / 0.48 s ≈ 14) which is enough for precise isotope ratio measurements. It should be noted that spectral skew will affect seriously the isotope ratio precision and accuracy when point-by-point ratios are measured instead of peak area ratios on GC peaks using scanning instruments.

3.8.3 Spike solutions

Species-specific isotope dilution analysis is based on the addition of a known amount of the isotopically enriched species to the sample. Monomethylmercury and butyltin spike compounds are already commercially available. Methylmercury chloride isotopic reference material ERM-AE 670, enriched in ^{202}Hg, and dissolved in 2 %-ethanol/water, was obtained from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). A mixture of the three butyltin compounds, all enriched in ^{119}Sn and dissolved in acetic acid/methanol 3:1, was purchased from Innovative Solutions in Chemistry S.L. (Gijón, Spain). In contrast to methylmercury and the butyltins an isotopically labeled trimethyllead spike is not commercially available so that it was necessary to synthesize it, which was carried out in this work (Chapter 3.5). Table 24 shows isotope abundances and concentrations of the spike solutions used for multi-species determinations in biological and environmental samples.

Table 24: Isotope abundances and concentrations of the spike solutions used for multi-species determination of trimethyllead, methylmercury, mono-, do- and tributyltin

Species	Isotope ratio	Isotope abundance, %		Concentration, $\mu g\ g^{-1}$ as metal
		Spike isotope	Reference isotope	
$Me_3{}^{206}Pb^+$	$^{206}Pb/^{208}Pb$	99.30	0.25	309.4
$Me^{202}Hg^+$	$^{200}Hg/^{202}Hg$	97.685	0.537	35.4
$Bu^{119}Sn^{3+}$	$^{119}Sn/^{120}Sn$	82.40	3.127	0.121
$Bu_2{}^{119}Sn^{2+}$	$^{119}Sn/^{120}Sn$	82.40	3.127	0.748
$Bu_3{}^{119}Sn^+$	$^{119}Sn/^{120}Sn$	82.40	3.127	1.02

GC-ICP-MS chromatograms of the spike and reference isotopes of methylmercury, trimethyllead, and mixed butyltin spike solutions after derivatization by $NaBEt_4$ are presented in Figure 29. All isotopes (^{119}Sn, ^{120}Sn, ^{200}Hg, ^{202}Hg, ^{206}Pb and ^{208}Pb) were monitored simultaneously during the course of the chromatogram. Beside methylmercury, trimethyllead, mono-, di- and tributyltin spike solutions also contain inorganic lead, mercury and tin (Figure 29), which were identified by a comparison of retention times with the corresponding standard solutions after derivatization by $NaBEt_4$ (Chapter 3.8.7). All elemental species are only marked in the chromatogram of the spike isotopes. Corresponding peaks at the same retention time in the reference chromatogram are from identical elemental species.

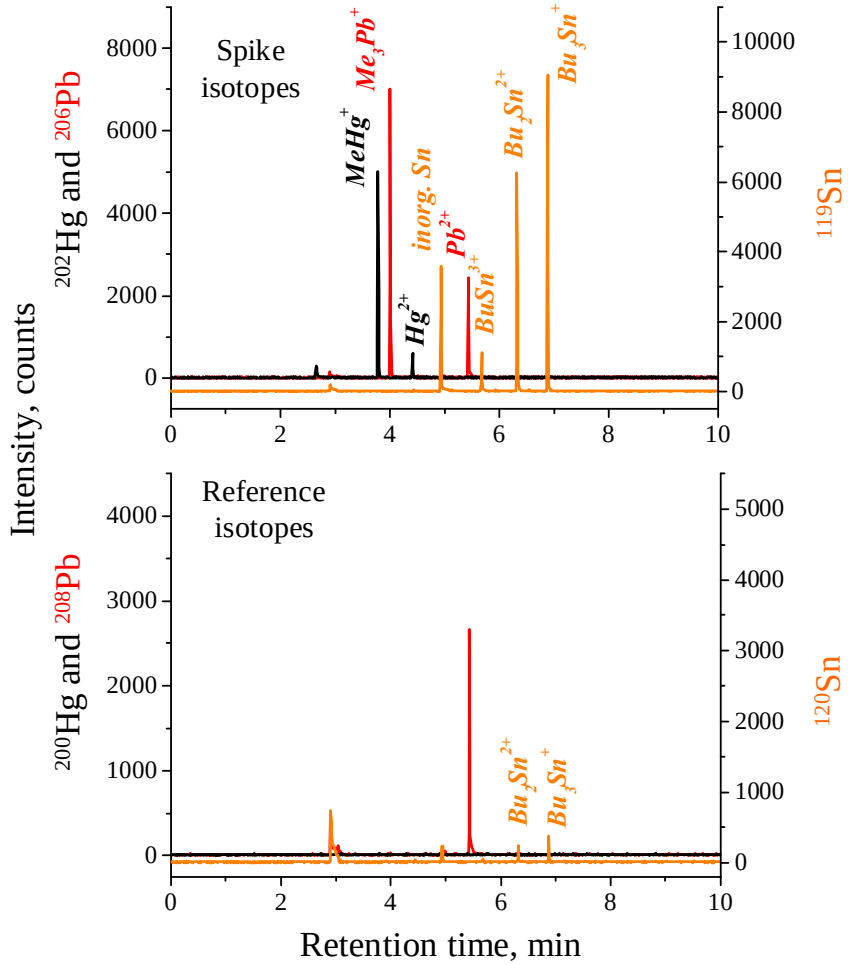

Figure 29: GC-ICP-MS chromatograms of the spike and reference isotopes of methylmercury, trimethyllead, and mixed butyltin spike solutions (derivatization by $NaBEt_4$)

3.8.4 Sample preparation for multi-species determination by liquid-liquid extraction GC-ICP-IDMS

The sample treatment procedure was adapted from the determination of trimethyllead in biological and environmental samples (Chapter 3.6.4), which was now optimized for the multi-species determination.

Depending on the expected species concentration in the samples, the stock spike solutions were diluted (trimethyllead and methylmercury by water and the butyltin mixture by acetic

acid/methanol 3:1) prior to analyses so that the corresponding concentrations fit the optimum range of spike addition for the isotope dilution technique [68]. 0.2-1 g of the biological sample and 0.1-0.5 g of the different diluted spike solutions were exactly weighed into a 7 mL amber glass vial and mixed with 3 mL of TMAH (25 % in water). After closing of the vial by a screw cap the mixture was shaken for about 2 h at room temperature in order to dissolve the organic matrix. About 0.6 mL concentrated HNO_3 and 0.5 mL 4 mol L^{-1} acetate buffer were then added to adjust pH 5. For alkylation of the ionic species 400 µL hexane and 500 µL of an aqueous solution (2 %) of $NaBEt_4$ or $NaBPr_4$ were added. The mixture was then shaken for 10 min and centrifuged (4000 min^{-1}) to facilitate phase separation. There was no difference in the results for the methylated and butylated elemental species by using ethylation or propylation. However, in the case where the samples were checked for possible ethyl compounds, propylation was necessary for identification. The hexane phase was collected with a micropipette and transferred to a 2 mL glass vial and stored at -25˚C until GC-ICP-MS analysis. Prior to the analysis a gentle nitrogen gas flow was passed through the hexane extract in order to concentrate the extract (by a factor of about ten) and to increase the sensitivity and precision of the isotope ratio measurement. After injection of 1 µL concentrated extract into the GC (splitless) the chromatograms of ^{206}Pb, ^{208}Pb, ^{200}Hg, ^{202}Hg, ^{119}Sn, and ^{120}Sn were measured by GC-ICP-MS.

In the case of sediments 0.25-0.6 g of the sample were weighed together with 0.1-0.5 g of the diluted stock spike solutions into a 7 mL glass vial. Then 3 mL of a mixture, which consists of 0.16 mol L^{-1} EDTA and 0.27 mol L^{-1} sodium diethyldithiocarbamate (NaDDTC), was added. The mixture was shaken for about 1 h and then adjusted to pH 5 by 0.5 mL 4 mol L^{-1} acetate buffer. The rest of the sample treatment procedure was identical to biological samples.

Control of possible species transformation during the above described sample preparation procedure was carried out in this work. TMAH solution was used for digestion of biological samples in many speciation studies but usually at higher temperatures (37-60ºC). Therefore, the influence of the temperature of the TMAH pretreatment on the species transformation was studied. For this purpose, $Me^{202}Hg^{+}$ and $Me_3{}^{206}Pb^{+}$ spike solutions and individual standard solutions of mono-, di- and tributyltin with natural isotopic composition were used. A butyltin mixed spike solution, where all species are enriched in ^{119}Sn, cannot be used for this study. Individual standard solutions of mono-, di- and tributyltin (0.5 mL of 10 µg g^{-1}), spike solutions of trimethyllead (0.3 mL of 3 µg g^{-1}) or of methylmercury (0.3 mL of 3 µg g^{-1}) were added to 3 mL of TMAH (25 % in water) and treated for 4 h at room temperature, 37ºC and 60ºC,

respectively. After cooling to room temperature about 0.6 mL concentrated HNO_3 and 0.5 mL 4 mol L^{-1} acetate buffer were added to adjust pH 5 (identical as for biological samples). For alkylation of the ionic species 500 µL of hexane and 300 µL of 2% $NaBEt_4$ solution were then added. The mixtures were shaken for 10 min. After phase separation the hexane was collected with a micropipette and transferred to a 2 mL glass vial. GC-ICP-MS measurements were performed under the same conditions as described in Table 23, Chapter 3.8.1.

Individual standard solutions of mono-, di- and tributyltin as well as $Me^{202}Hg^+$ and $Me_3{}^{206}Pb^+$ spike solutions were also measured without TMAH pretreatment. For that purpose, same quantity of standard or spike solution (see above) was added to 0.5 mL 4 mol L^{-1} acetate buffer. Derivatization, extraction and measurements were then performed in the same way as for the corresponding solutions after TMAH pretreatment.

3.8.5 Validation of the multi-species-specific GC-ICP-IDMS method

3.8.5.1 Analysis of biological reference materials

Three biological reference materials (CRM 477, mussel tissue, certified for the butyltin species; CRM 463, tuna fish, certified for total and methylmercury; DORM 2, dogfish muscle, certified for trace metals and methylmercury) were analyzed to validate the developed species-specific GC-ICP-IDMS method for multi-species determination but also to receive some representative data for homogenized samples of typical seafood.

The results of multi-species determination in these reference materials by species-specific GC-ICP-IDMS are summarized in Table 25. For CRM 463 and DORM 2 the concentrations of the three butyltins were found below the detection limit (0.3 ng g^{-1} for $BuSn^{3+}$ and Bu_3Sn^+, 1.2 ng g^{-1} for Bu_2Sn^{2+}) whereas the Me_3Pb^+ and $MeHg^+$ contents of all CRMs were determined significantly above the detection limit of 0.06 ng g^{-1} and 1.4 ng g^{-1}, respectively.

Table 25: Multi-species determination in biological reference materials by species-specific GC-ICP-IDMS (n=3)

Species (as metal)	Mussel tissue (CRM 477)		Tuna fish (CRM 463)		Dogfish (DORM 2)	
	GC-ICP-IDMS	Certified	GC-ICP-IDMS	Certified	GC-ICP-IDMS	Certified
Me_3Pb^+, ng g^{-1}	0.34 ± 0.03	-	4.10 ± 0.16	-	6.40 ± 0.06	-
$MeHg^+$, µg g^{-1}	0.066±0.002	-	2.95 ± 0.05	2.83 ± 0.15	4.36 ± 0.05	4.47 ± 0.32
$BuSn^{3+}$, µg g^{-1}	0.93 ± 0.04	1.01 ± 0.19	<0.0003	-	<0.0003	-
Bu_2Sn^{2+}, µg g^{-1}	0.82 ± 0.03	0.78 ± 0.06	<0.0012	-	<0.0012	-
Bu_3Sn^+, µg g^{-1}	0.85 ± 0.01	0.90 ± 0.08	<0.0003	-	<0.0003	-

3.8.5.2 Analysis of sediment reference materials

For the validation of the multi-species GC-ICP-IDMS method for environmental samples two sediment reference materials (BCR 646, freshwater sediment, certified for butyltins and phenyltins and CRM 580, estuarine sediment, certified for total mercury and methylmercury) were selected. Results of three independent analyses are presented in Table 26. Methylmercury concentration in BCR 646 was found to be less than the detection limit of 1.0 ng g^{-1}. Trimethyllead was also not detectable in both reference materials.

Table 26: Multi-species determination in the certified reference sediments by species-specific GC-ICP-IDMS (n=3)

Species (as metal)	Freshwater sediment (BCR 646)		Estuarine sediment (CRM 580)	
	GC-ICP-IDMS	Certified	GC-ICP-IDMS	Certified
Me_3Pb^+, ng g^{-1}	<0.04	-	<0.04	-
$MeHg^+$, ng g^{-1}	<1.0	-	261.7 ± 22.3	70.2 ± 3.4
$BuSn^{3+}$, ng g^{-1}	353 ± 14	412 ± 81	65.0 ± 4.7	-
Bu_2Sn^{2+}, ng g^{-1}	401 ± 6	392 ± 46	36.4 ± 1.3	-
Bu_3Sn^+, ng g^{-1}	184 ± 3	196 ± 33	4.3 ± 0.5	-

Chromatograms of the spike and reference isotopes of sediment reference material BCR 646 are shown in Figure 30. Except of butyl- and phenyltin species, monomethyl- and dimethyltin were identified in this sample (Chapter 3.8.7). All identified elemental species are only marked in the chromatogram of the reference isotopes. However, corresponding peaks at the same retention time in the spike chromatogram are from identical elemental species.

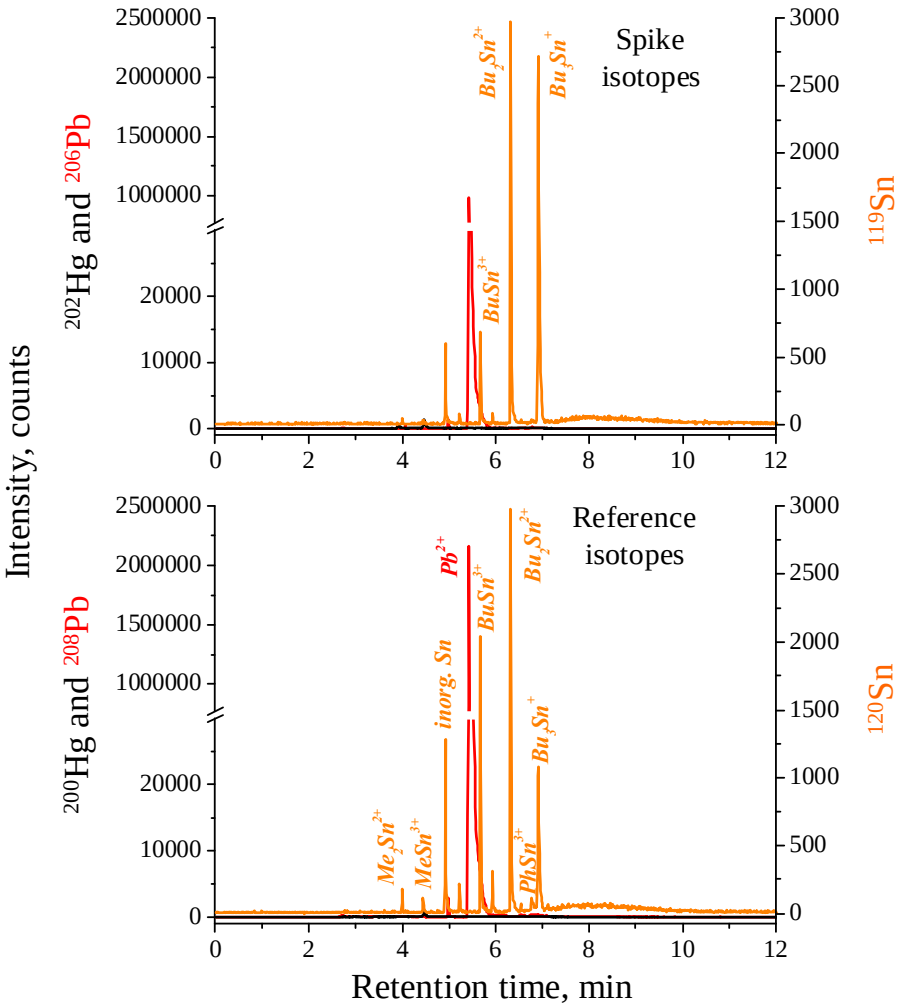

Figure 30: GC-ICP-MS chromatograms of the spike and reference isotopes of mercury, lead, and tin of the species-specific isotope diluted sediment reference material BCR 646 after derivatization with $NaBEt_4$

3.8.6 Application of species-specific GC-ICP-IDMS method for multi-species determination

3.8.6.1 Analysis of seafood samples from supermarket

The developed multi-species GC-ICP-IDMS method was applied to the analysis of representative seafood (mussels, large peeled prawns, filets of a tuna fish, a plaice, a pollock) which were already analyzed for trimethyllead content (Chapter 3.7.2). These samples were purchased in a supermarket in Mainz. The results of the multi-species determination for this seafood are summarized in Table 27.

Methylmercury and trimethyllead were detected in all samples whereas monobutyltin and tributyltin – only in some of them. Concentration of dibutyltin in all investigated samples was found to be less as corresponding detection limit of 1.2 ng g^{-1}.

Table 27: Concentration of methylmercury, trimethyllead and butyltins in seafood samples from a supermarket determined by GC-ICP-IDMS (n=3)

Sample	$MeHg^+$ ng g^{-1} as Hg	Me_3Pb^+ ng g^{-1} as Pb	$BuSn^{3+}$ ng g^{-1} as Sn	Bu_2Sn^{2+} ng g^{-1} as Sn	Bu_3Sn^+ ng g^{-1} as Sn
Tuna fish	1082 ± 60	0.39 ± 0.02	<0.3	<1.2	21.7 ± 0.5
Mussels	85.4 ± 3.1	0.35 ± 0.04	6.4 ± 0.4	<1.2	7.4 ± 0.6
Prawns	40.5 ± 2.7	0.51 ± 0.04	<0.3	<1.2	0.74 ± 0.11
Plaice	170.7 ± 4.8	0.31 ± 0.02	<0.3	<1.2	6.0 ± 0.4
Pollock	704 ± 28	0.66 ± 0.05	<0.3	<1.2	<0.3

GC-ICP-MS isotope chromatograms of the tuna fish sample after addition of the methylmercury, trimethyllead and mixed butyltin spikes and derivatization with $NaBPr_4$ are presented as an example in Figure 31. The chromatogram of the reference isotopes shows presence of

methylmercury, trimethyllead, tributyltin as well as trace amounts of mono-, di- and tetramethyltin in this sample.

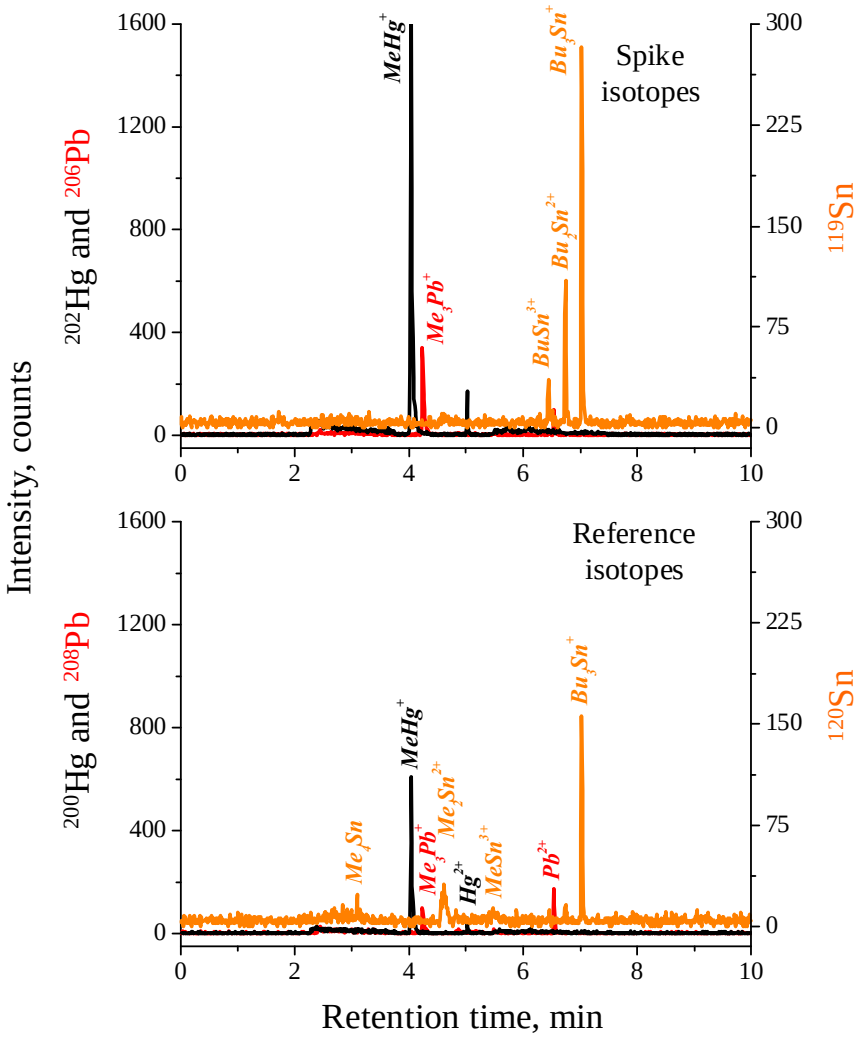

Figure 31: GC-ICP-MS chromatograms of the spike and reference isotopes of mercury, lead, and tin of the species-specific isotope diluted tuna fish sample after derivatization with $NaBPr_4$

3.8.6.2 Analysis of shark samples

The demand for shark products increases worldwide dramatically. A lot of organizations fight for shark protection and conservation and are interested in any information about sharks [106]. Two smoked samples of shark and fresh shark steaks were analyzed in the frame of "Sharkproject". Concentrations of methylmercury and trimethyllead determined by GC-ICP-IDMS for these samples are presented in Table 28. Corresponding results for other fresh shark samples purchased from National Institute of Public Health and Environment (Bilthoven, the Netherlands) are summarized in the same table. Results are calculated for dry weight to make a comparison with results of reference materials and other seafood samples possible but also for wet weight because the maximum permitted concentration of methylmercury in fishery products by legislative regulations is expressed as amount of methylmercury per wet weight.

Table 28: Concentrations of methylmercury and trimethyllead in smoked and fresh shark samples (n=3)

Sample	$MeHg^+$		Me_3Pb^+	
	ng g^{-1} as Hg wet weight	ng g^{-1} as Hg dry weight	ng g^{-1} as Pb wet weight	ng g^{-1} as Pb dry weight
Smoked sea eel	826 ± 21	2191 ± 56	0.42 ± 0.02	1.11 ± 0.05
Smoked dogfish	546 ± 12	1500 ± 33	-	-
Shark steaks	1400 ± 125	8092 ± 723	0.22 ± 0.03	1.27 ± 0.17
Shark 1	841 ± 31	3210 ± 118	0.46 ± 0.02	1.76 ± 0.08
Shark 2	2786 ± 32	10634 ± 122	0.55 ± 0.02	2.10 ± 0.08
Shark 3	765 ± 33	2920 ± 126	0.31 ± 0.01	1.18 ± 0.04
Shark 4	1697 ± 75	6477 ± 286	0.51 ± 0.02	1.95 ± 0.08
Shark 5	3134 ± 35	11962 ± 134	0.36 ± 0.03	1.37 ± 0.11

Concentration of methylmercury was very high in all analyzed shark samples. It was more than 0.5 μg g^{-1} (as Hg wet weight) even in the smoked samples after hot smoking. In contrast to methylmercury concentration of trimethyllead was found to be much lower in the range from 0.22 ng g^{-1} to 0.55 ng g^{-1} as Pb wet weight. Mono-, di- or tributyltin were not detected in any of these samples.

3.8.7 Identification of species

Qualitative identification of unknown peaks in samples was carried out by a comparison of retention times with corresponding standard solutions. Standard solutions of inorganic tin, lead and mercury were prepared by dilution of stock solutions in Milli-Q water. Standard solutions of different tin compounds were prepared and diluted in methanol. Solutions of tetraalkyltin compounds like tetramethyltin, tetraethyltin and tetraisopropyltin were extracted by hexane without any derivatization. All other species (inorganic tin, lead and mercury, mono-, dimethyltin, triethyltin, mono-, di- and triphenyltin) were measured after derivatization by $NaBEt_4$ or $NaBPr_4$ and subsequent in-situ extraction by hexane. Since dimethyllead is not commercially available it was prepared by reaction of a trimethyllead standard solution and iodine monochloride and measured after ethylation or propylation (Chapter 3.6.5). Every standard solution was measured after its individual injection. GC-ICP-MS chromatograms of the mixed tin standard solution derivatized by $NaBEt_4$ or $NaBPr_4$ are presented in Figures 32 and 33. As shown in these figures good separation of all applied tin species was achieved under the chosen experimental conditions.

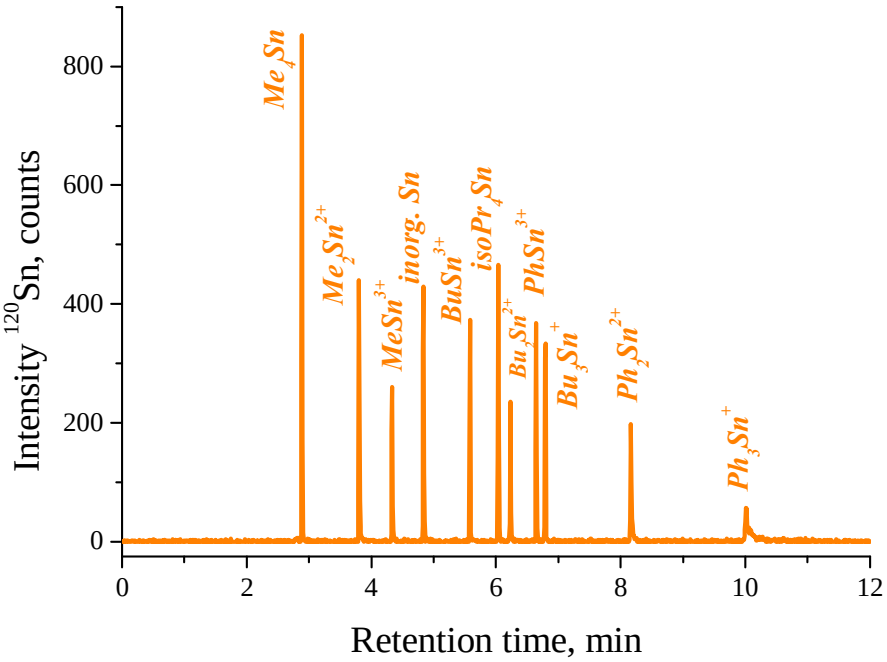

Figure 32: GC-ICP-MS chromatogram of the mixed tin standard solution derivatized by $NaBEt_4$

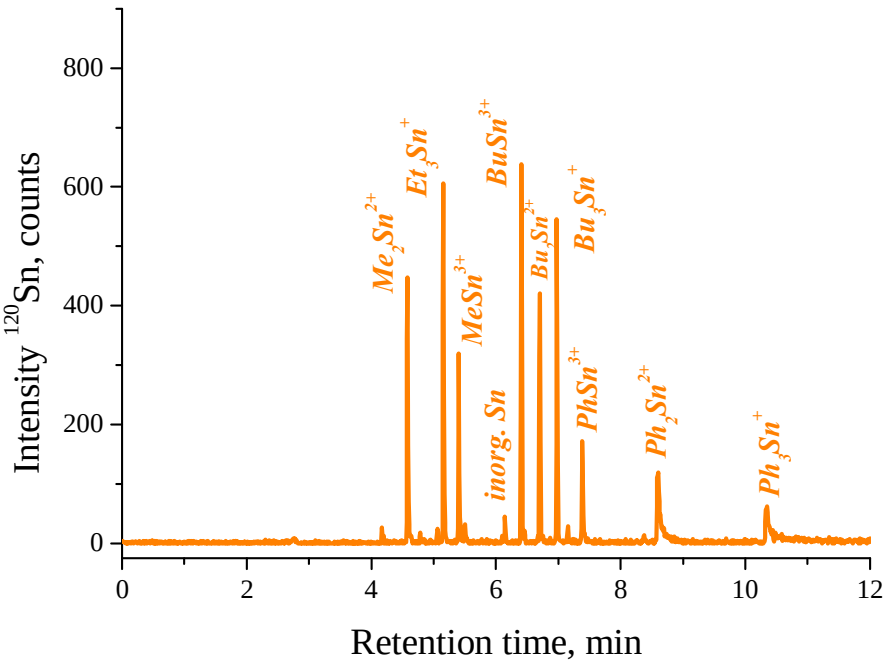

Figure 33: GC-ICP-MS chromatogram of the mixed tin standard solution derivatized by $NaBPr_4$

3.8.8 Stir bar sorptive extraction for multi-species-specific GC-ICP-IDMS determination

3.8.8.1 Basic principles and advantages of SBSE

As an alternative approach to classical liquid-liquid extraction, solid phase micro extraction (SPME) technique was also applied for determination of alkylmetal species [107,108,109,110]. This technique involves the extraction of the volatile or semivolatile analytes directly from aqueous or gaseous samples onto a fused-silica fiber which is coated with a suitable stationary phase. After equilibration the fiber is directly transferred into a GC injector for thermal desorption and analysis. A relatively novel approach is the stir bar sorptive extraction (SBSE). In this case substances are sorbed during stirring into a polydimethylsiloxane (PDMS) layer of a magnetic stirring rod (Figure 34) followed by thermal desorption. PDMS-coated stir bars are commercially available from Gerstel GmbH (Mülheim an der Ruhr, Germany) under the trade name Twister.

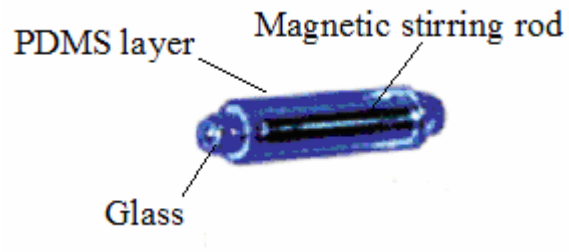

Figure 34: Construction of a PDMS-coated stir bar (Twister) [111]

Due to the increased amount of PDMS in SBSE (55-365 µL) compared to SPME (typically less than 0.5 µL) up to a 500-fold increase in sensitivity can be attained with stirring times between 30 to 60 min [112]. A complete extraction of substances with small octanol-water partitioning coefficients $K_{o/w}$ of ≥ 500 is possible by SBSE in contrast to SPME where poor recoveries for solutes with $K_{o/w}$ values less than 10,000 are observed. SBSE has therefore the following advantages:

- Up to 500 times lower detection limits than SPME;
- Possibility of extraction of substances with small octanol-water partitioning coefficients;
- Simple sample preparation;

- No adsorption of water or solvent;
- Twisters are reusable.

However, SBSE was shown to have bad reproducibilities, e.g for volatile halogenated hydrocarbons [87]. Using isotope dilution technique the extraction yield should have no influence on the result after the isotope dilution step has taken place. Therefore the promising SBSE method was applied in this work for multi-species determination of trimethyllead, methylmercury and butyltins by GC-ICP-IDMS in biological and environmental samples and compared with already optimized liquid-liquid extraction.

3.8.8.2 Sample preparation for multi-species determination by SBSE-GC-ICP-IDMS

Biological and environmental samples were digested according to procedures described in Chapter 3.8.4. Afterwards, 1 mL aliquot of the digestion solution was added to a mixture of 7 mL Milli-Q water and 0.5 mL of 4 mol L^{-1} acetate buffer (pH 5) in a 15 mL amber glass vial closed-cap with Teflon septa from Sigma-Aldrich Chemie GmbH, SUPELCO (Taufkirchen, Germany). In the case of biological samples, an appropriate amount of concentrated nitric acid (about 160-180 µL) was added to neutralize the excess of TMAH. The Twister was added to the solution directly after addition of 500 µL of an aqueous solution (2 %) of $NaBEt_4$. The vial was then tightly closed. The headspace should be reduced to a minimum in order to avoid possible enrichment of the substances in the gas phase. To achieve the best agitation, the maximum stirring rate was used (1250 rotations min^{-1}). Stir bars of 10 mm in length and 0.5 mm of film thickness were used. After several applications stir bars were conditioned at 300˚C for about 1.5 h in the Gerstel tube conditioner.

After extraction (20-60 min) the stir bar was removed from the solution by means of a magnet bar and covered with a clean paper tissue to remove water droplets. The stir bar was then introduced into a glass thermal desorption tube. Compounds were desorbed at 290˚C and trapped at -50˚C in a thermodesorption / cold injection system (TDS 2 / CIS 4, Gerstel GmbH). Subsequently, flash heating (12˚C s^{-1}) up to 290˚C was applied for a rapid transfer of the compounds into the GC column. To make sure that all compounds are removed, temperature was

kept at 290˚C for 4 min. Under these conditions desorption of substances was complete. At lower temperatures (≤ 260°C), the less volatile ethylated dibutyltin and tributyltin compounds were not completely desorbed. A second thermodesorption and measurement of the same twister showed availability of these species in the system. Therefore a temperature of 290˚C was applied for all further experiments. A schematic diagram of the sample preparation procedure of environmental and biological samples for multi-species determination by species-specific SBSE-GC-ICP-IDMS is presented in Figure 35.

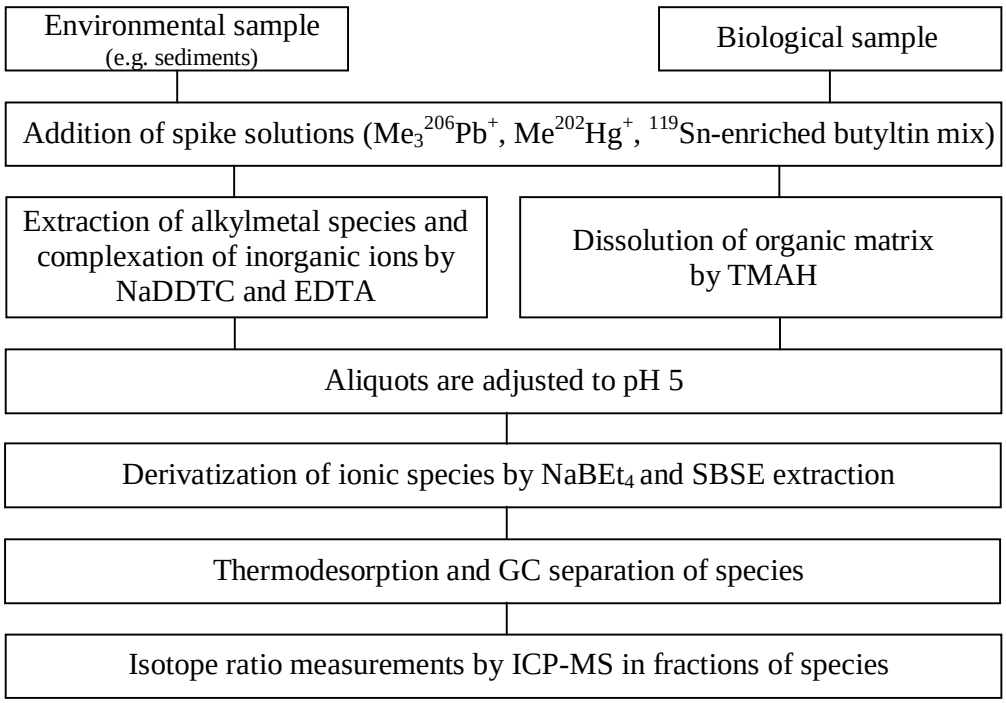

Figure 35: Schematic diagram of the sample preparation procedure of environmental and biological samples for multi-species determination by species-specific SBSE-GC-ICP-IDMS

The optimized operating conditions for the thermodesorption / cold injection system are summarized in Table 29. These conditions were chosen for all further measurements of the trimethyllead, methylmercury and butyltins. GC-ICP-MS operating conditions were the same as described for multi-species measurements after hexane extraction (Table 23, Chapter 3.8.1).

Table 29: Operating conditions of the thermodesorption / cold injection system

TDS parameters	
Initial temperature	30°C
Initial time	1.0 min
Rate	50°C min^{-1}
Final temperature	290°C
Final time	10 min
Transfer line temperature	260°C
Mode	Splitless
CIS parameters	
Initial temperature (during thermal desorption)	-50°C
Rate	12˚C s^{-1}
Final temperature	290˚C
Final time	4.0 min

3.8.8.3 Improvement in the precision of SBSE-GC-ICP-MS measurements

The possibility of an improvement in the measurement precision using SBSE in connection with the isotope dilution technique was studied in this work. In order to obtain optimum sensitivity for precise isotope ratio measurements the influence of the extraction time on the signal intensity was investigated. Standard solutions of methylmercury (2.5 mL of 10 ng g^{-1}), trimethyllead and butyltins (1.0 mL of 10 ng g^{-1}) were added to a mixture of 7 mL water with 0.5 mL acetate buffer (pH 5). After addition of 0.5 mL of 2 % $NaBEt_4$ solution, SBSE was carried out during different times.

Lead, mercury and tin species show different extraction time profiles and an equilibrium could not be reached within 60 min for all species (Figure 36). Long equilibration times can be

explained by the relatively large volume of the PDMS phase and the corresponding slow extraction kinetics.

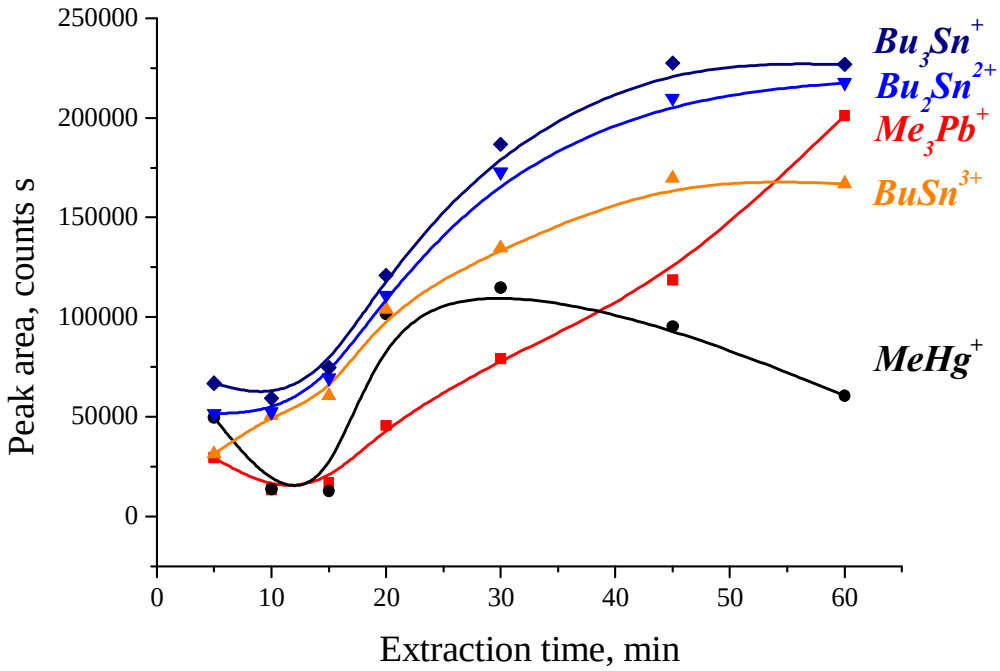

Figure 36: Influence of extraction time on the SBSE extraction efficiency for trimethyllead, methylmercury, monobutyltin, dibutyltin and tributyltin.

A decrease of the extraction capacity was observed for methylmercury at extraction times above 30 min. Most likely a displacement of methylmercury by other species occurred due to the high amounts of analytes used (10-25 ng). An extraction time of 30 min was selected for further measurements. The possibility of complete extraction of alkylmetal species from water solution was tested: the standard solution, where the SBSE has been carried out for 60 min, was extracted again during one hour. All species were detected: butyltin peaks were nearly the same, trimethyllead and methylmercury peaks – by a factor 5-7 less than for first extraction.

Five consecutive extractions of standard solutions containing trimethyllead, methylmercury, mono-, di- and tributyltin were carried out with two twisters for 20 min each. The peak areas in "counts s" and the corresponding isotope ratios, which were calculated from these peak areas, are listed in Tables 30-34. Relative standard deviations of the peak area measurements were in

the range of 32-81 %. In contrast to this result the RSD values for the corresponding isotope ratios only range from 1.3% to 2.1%, demonstrating superior capability for isotope dilution analysis by overcoming the generally poor precision for extraction by SBSE.

Table 30: Precision of peak area and isotope ratio measurements for trimethyllead

Extraction №	Peak area, counts s		Isotope ratio
	^{206}Pb	^{208}Pb	$^{206}Pb/^{208}Pb$
1	51786	113903	0.4547
2	65600	146422	0.4480
3	36922	84333	0.4378
4	67462	148525	0.4542
5	29367	66983	0.4384
Average	50227	112033	0.4466
SD	16941	36451	0.0082
RSD, %	34	33	1.8

Table 31: Precision of peak area and isotope ratio measurements for methylmercury

Extraction №	Peak area, counts s		Isotope ratio
	^{200}Hg	^{202}Hg	$^{200}Hg/^{202}Hg$
1	45868	59041	0.7769
2	49619	62960	0.7881
3	8189	10680	0.7668
4	17803	22376	0.7956
5	6563	8541	0.7684
Average	25608	32720	0.7792
SD	20701	26385	0.0125
RSD, %	81	81	1.6

Table 32: Precision of peak area and isotope ratio measurements for monobutyltin

Extraction №	Peak area, counts s		Isotope ratio
	^{119}Sn	^{120}Sn	$^{119}Sn/^{120}Sn$
1	27426	106562	0.2574
2	38512	146937	0.2621
3	57578	218888	0.2630
4	31837	124779	0.2551
5	28664	111278	0.2576
Average	36803	141689	0.2591
SD	12383	45913	0.0034
RSD, %	34	32	1.3

Table 33: Precision of peak area and isotope ratio measurements for dibutyltin

Extraction №	Peak area, counts s		Isotope ratio
	^{119}Sn	^{120}Sn	$^{119}Sn/^{120}Sn$
1	35119	137674	0.2551
2	46827	181313	0.2583
3	79458	305648	0.2600
4	39128	151076	0.2590
5	34076	135991	0.2506
Average	46922	182340	0.2566
SD	18866	71286	0.0038
RSD, %	40	39	1.5

Table 34: Precision of peak area and isotope ratio measurements for tributyltin

Extraction №	Peak area, counts s		Isotope ratio
	^{119}Sn	^{120}Sn	$^{119}Sn/^{120}Sn$
1	42108	161259	0.2611
2	53070	208180	0.2549
3	84015	332158	0.2529
4	44352	166152	0.2669
5	41639	159807	0.2606
Average	53037	205511	0.2593
SD	17919	73557	0.0055
RSD, %	34	36	2.1

3.8.8.4 Validation of the SBSE-GC-ICP-IDMS method for multi-species determination

No biological certified reference material exists for multi-species determination of trimethyllead, methylmercury, tri-, di- and monobutyltin. Therefore two reference materials, only certified for butyltins (CRM 477, mussel tissue) and certified for total and methylmercury (CRM 463, tuna fish), were chosen for validation of the developed SBSE-GC-ICP-IDMS method. Results of multi-species determination in these reference materials by species-specific SBSE-GC-ICP-IDMS are summarized in Table 35.

The sediment reference material CRM 580 was also analyzed by the SBSE-GC-ICP-IDMS method. Analyses were carried out either of the extract with suspended precipitate particles or as pure extract, where the precipitate was separated by centrifugation. The corresponding results are presented in Table 36. After separation of the precipitate, the butyltin peaks were too small for quantification, which may be due to possible adsorption of ionic butyltin compounds on the solid particles.

Table 35: Multi-species determination in biological reference materials by species-specific SBSE-GC-ICP-IDMS (n=3)

Species (as metal)	Mussel tissue (CRM 477)		Tuna fish (CRM 463)	
	SBSE-GC-ICP-IDMS	Certified	SBSE-GC-ICP-IDMS	Certified
Me_3Pb^+, ng g^{-1}	0.38 ± 0.04	-	4.33 ± 0.25	-
$MeHg^+$, µg g^{-1}	0.069 ± 0.003	-	2.94 ± 0.06	2.83 ± 0.15
$BuSn^{3+}$, µg g^{-1}	0.82 ± 0.02	1.01 ± 0.19	<0.0006	-
Bu_2Sn^{2+}, µg g^{-1}	0.82 ± 0.03	0.78 ± 0.06	<0.0023	-
Bu_3Sn^+, µg g^{-1}	0.94 ± 0.05	0.90 ± 0.08	<0.0002	-

Table 36: Multi-species determination in the certified reference sediment CRM 580 by species-specific SBSE-GC-ICP-IDMS (n=3)

Species (as metal)	SBSE-GC-ICP-IDMS		Certified
	With precipitate	Without precipitate	
Me_3Pb^+, ng g^{-1}	<0.05	<0.05	-
$MeHg^+$, ng g^{-1}	430.7 ± 41.9	109.1 ± 5.3	70.2 ± 3.4
$BuSn^{3+}$, ng g^{-1}	69.4 ± 14.9	- [a]	-
Bu_2Sn^{2+}, ng g^{-1}	38.9 ± 1.4	- [a]	-
Bu_3Sn^+, ng g^{-1}	5.0 ± 1.1	- [a]	-

[a] Peaks were too small for quantitative determination

4 Discussion of results

4.1 Availability of isotope-enriched spikes as main prerequisite of multi-species-specific GC-ICP-IDMS

The application of isotope dilution analysis on trace metal speciation can be carried out by two different modes: the "species-specific" mode, where the species of interest are labeled with an enriched isotope, and the "species-unspecific" mode where the isotope-enriched element is continuously added post-column after a chromatographic separation (Chapter 2.4). In the last few years species-specific isotope dilution analysis has become more advantageous, because it allows correction of some systematic errors which the "species-unspecific" mode is not able to do; e.g. possible species transformation during the sample preparation steps. For the species-specific ICP-IDMS it is essential that the added isotope-labeled spike and the elemental species to be determined are present in the same chemical form. ^{202}Hg enriched methylmercury reference material ERM-AE670 [113] and a mixture of the three butyltin compounds, all enriched in ^{119}Sn, are commercially available (Chapter 3.8.3) but not an isotope-enriched trimethyllead spike. Therefore, it was necessary to synthesize such a spike compound for multi-species determination of trimethyllead, methylmercury and butyltins by GC-ICP-IDMS (Chapter 3.5). Spike availability is the main prerequisite of species-specific isotope dilution analysis and the reason why this technique with many valuable advantages (described in Chapter 2.4) has not become a routine method up to now.

Brown et al. synthesized isotope-enriched trimethyllead and triethyllead in a small scale for species-specific HPLC-ICP-IDMS by the reaction of $PbCl_2$, prepared from ^{206}Pb-enriched metallic lead, with the Grignard reagents MeMgI and EtMgI, respectively [82]:

$$4\ MeMgX + 2\ PbX_2 \rightarrow Me_4Pb + Pb + 4\ MgX_2 \qquad (9)$$

However, with respect to the corresponding stoichiometry of the Grignard reaction, the theoretical yield of synthesis cannot be more than 50% and will probably be in practice even lower (unfortunately, no yields are given in ref. [82]). An alternative synthesis with methyllithium in the presence of excess of iodomethane was therefore selected in this work:

$$3\ MeLi + MeI + {}^{206}PbBr_2 \rightarrow Me_4{}^{206}Pb + LiI + 2\ LiBr \qquad (10)$$

It has the advantage of being a fast reaction at room temperature [84]. The synthesis conditions were optimized for a maximum yield, using at the beginning metallic lead of natural isotopic abundances. These preliminary investigations have shown that application of lead bromide instead of lead chloride increases the yield of tetramethyllead by a factor of about seven. Therefore it was decided to dissolve the ^{206}Pb-enriched metallic lead in HBr solution. Preparation of trimethyllead halide from tetramethyllead is carried out best by halogenation in ethereal solution at -60°C [81]:

$$Me_4{}^{206}Pb + I_2 \rightarrow Me_3{}^{206}PbI + MeI \qquad (11)$$

The GC-ICP-MS chromatogram of lead isotopes from the spike solution did only show some inorganic $^{206}PbI_2$ besides the desired product $Me_3{}^{206}PbI$ (Figure 8, Chapter 3.5.2), but no dimethyllead was found which, in principle, can also be formed by halogenation of tetramethyllead. The obtained total yield of 75% of isotopically enriched $Me_3{}^{206}PbI$ according the reactions (10) and (11) at the milligram scale is sufficient and much higher than using a Grignard reagent, where the theoretical yield is limited by reaction (9) to a maximum yield of 50% with respect to the initial amount of ^{206}Pb. The produced quantity of $Me_3{}^{206}PbI$ (185 mg) is sufficient to perform millions of trimethyllead analyses from real samples, because the spike addition for the isotope dilution technique should be at the same order (usually ng amounts) as the elemental species in the sample. The developed synthesis scheme for the formation of $Me_3{}^{206}Pb^+$ spike can be used for future production of this spike.

Because no GC-ICP-IDMS method was available for the determination of trimethyllead in biological and environmental samples, such a technique was developed in this work (Chapter 3.6). The developed method was successfully applied to Me_3Pb^+ analysis of urban dust (CRM 605), soil and seafood samples, as well as to biological reference materials, not certified for trimethyllead (Chapter 3.7). Then it was extended to multi-species-specific determination of trimethyllead, methylmercury and butyltin compounds (Chapter 3.8).

The results of trimethyllead determination in biological reference materials by mono-species-specific GC-ICP-IDMS and multi-species-specific GC-ICP-IDMS are summarized in Table 37

and show good agreement. This demonstrates that the mono-species GC-ICP-IDMS method could successfully be transferred to a multi-species technique. The relative standard deviations are in the range of 0.9-8.8 % which is quite good at this low concentration level.

Table 37: Trimethyllead determination in biological reference materials by mono-species-specific GC-ICP-IDMS and multi-species-specific GC-ICP-IDMS

Reference material	Me_3Pb^+, ng g^{-1} as Pb	
	Mono-species GC-ICP-IDMS (n=5)	Multi-species GC-ICP-IDMS (n=3)
Dogfish (DORM 2)	6.41 ± 0.29	6.40 ± 0.06
Tuna fish (CRM 463)	4.39 ± 0.16	4.10 ± 0.16
Mussel tissue (CRM 477)	0.30 ± 0.02	0.34 ± 0.03

4.2 Comparison of liquid-liquid extraction with SBSE

Liquid-liquid extraction is still one of the most popular extraction techniques for the analysis of liquid and solid samples. In spite of its simplicity of operation, this technique needs large volumes of organic solvents and is difficult to fully automate for unattended operations. Considering the collective conscience, developed in recent years, about reduction in the use of organic solvents, this technique has therefore been replaced in some cases by solid phase micro extraction (SPME) and stir bar sorptive extraction (SBSE), which eliminate this problem. SBSE is a recently developed technique, aiming to be an extension of SPME. Basic principles and advantages of SBSE in comparison to SPME are described in Chapter 3.8.8.1.

In this work SBSE was applied for the first time in connection with species-specific GC-ICP-IDMS for the determination of trimethyllead, methylmercury and butyltins. The results were compared with liquid-liquid extraction GC-ICP-IDMS. The optimization of operating conditions and the sample preparation for multi-species determination in biological and environmental samples by liquid-liquid extraction GC-ICP-IDMS and SBSE-GC-ICP-IDMS are described in Chapter 3.8.

Detection limits (3s) of multi-species determination in biological and environmental samples by species-specific GC-ICP-IDMS after liquid-liquid extraction (hexane) and SBSE are based on measurements of three spiked sample blanks normalized to a 0.5 g subsample (Table 38).

Table 38: Detection limits of multi-species determination in biological and environmental samples by species-specific GC-ICP-IDMS after liquid-liquid extraction (hexane) and SBSE

Species (as metal)	Biological sample		Environmental sample	
	Hexane	SBSE	Hexane	SBSE
Me_3Pb^+, ng g^{-1}	0.06	0.04	0.04	0.05
$MeHg^+$, ng g^{-1}	1.4	0.9	1.0	1.3
$BuSn^{3+}$, ng g^{-1}	0.3	0.6	0.1	1.3
Bu_2Sn^{2+}, ng g^{-1}	1.2	2.3	0.5	0.5
Bu_3Sn^+, ng g^{-1}	0.3	0.2	0.2	0.5

The best detection limits were obtained for trimethyllead. Detection limits of methylmercury determination are higher than in the case of trimethyllead. This is probably due to the lower extraction and derivatization efficiency (at pH 5) for methylmercury in comparison with organolead and -tin species. Another reason is the difference in the ionization potentials of these elements: mercury is known to have a higher ionization potential and it is only ionized by about 40 % in the argon plasma, whereby lead > 95%. Similar to lead, the ionization of tin is also > 95% in the argon plasma, but the detection limits for butyltin species are higher than for trimethyllead, which can be explained by the presence of tin species in the blank. Table 38 shows that there is no significant difference in detection limits between liquid-liquid extraction and SBSE. Detection limits for both methods are sufficient for the routine determination of methylmercury, trimethyllead and butyltin compounds in most biological and environmental samples.

Using SBSE it was expected to improve the detection limits, because the preconcentration factor is much higher than for a liquid-liquid extraction, where only 1µL from 400 µL hexane extract was injected for GC-ICP-MS analysis. In fact, the obtained detection limits (Table 38) are almost the same. The reason is the influence of matrix on the species recovery, which is much higher for SBSE than for liquid-liquid extraction. A big difference was observed, comparing the signals

from equal amounts of species in different matrices. This effect is mainly caused by the presence of organic material which can also act as a sorbent for the analytes and/or displace the analytes during SBSE, reducing the extracted fraction by the stir bar. There is a correlation between the species recovery (signals from equal amounts of spike species for the isotope diluted sample compared to that for the isotope diluted blank solution) and the amount of organic material present during sample preparation. Using SBSE, a reduced signal intensity by a factor of 50-120 was observed for sediments and of 90-350 for biological samples. Less loss of signal (analyte) was observed applying liquid-liquid extraction: a factor of 5-25 for sediments and of 10-70 for biological samples. It should be noted that this signal suppression has no influence on the accuracy of results, when isotope dilution technique is used for quantification. This was demonstrated by the analysis of different standard reference materials (see below). High precision and accuracy of the obtained results show a great advantage of isotope dilution technique for the analysis of samples with complex matrices.

The developed methods were applied to the determination of two biological reference materials (CRM 477 and CRM 463) and one sediment reference material (CRM 580). The corresponding results are presented in Table 39. They show good agreement and similar precision for both methods (RSD 1.2-8.8 % for liquid-liquid extraction and 2.0-10.5 % for SBSE). Such good precision would be impossible for SBSE-GC-ICP-MS determinations without isotope dilution calibration due to the high influence of the matrix on the species recovery and the poor precision of the peak area measurements (RSD 32-81 %) in comparison with RSD of 1.3-2.1 % for the corresponding isotope ratio measurements (Chapter 3.8.8.3). The same is true for the results of multi-species determination in the sediment reference material CRM 580 (Table 40). The corresponding results for butyltin species in CRM 477 and for methylmercury in CRM 463 are also in good agreement with the certified values (Table 25, Chapter 3.8.5.1).

Table 39: Multi-species determination in biological reference materials by species-specific GC-ICP-IDMS after liquid-liquid extraction (hexane) and SBSE (n=3)

Species (as metal)	Mussel tissue (CRM 477)		Tuna fish (CRM 463)	
	Hexane	SBSE	Hexane	SBSE
Me_3Pb^+, ng g^{-1}	0.34 ± 0.03	0.38 ± 0.04	4.10 ± 0.16	4.33 ± 0.25
$MeHg^+$, µg g^{-1}	0.066±0.002	0.069 ± 0.003	2.95 ± 0.05	2.94 ± 0.06
$BuSn^{3+}$, µg g^{-1}	0.93 ± 0.04	0.82 ± 0.02	<0.0003	<0.0006
Bu_2Sn^{2+}, µg g^{-1}	0.82 ± 0.03	0.82 ± 0.03	<0.0012	<0.0023
Bu_3Sn^+, µg g^{-1}	0.85 ± 0.01	0.94 ± 0.05	<0.0003	<0.0002

Table 40: Multi-species determination in sediment reference material CRM 580 by species-specific GC-ICP-IDMS after liquid-liquid extraction (hexane) and SBSE (n=3)

Species (as metal)	Hexane	SBSE
Me_3Pb^+, ng g^{-1}	<0.04	<0.05
$MeHg^+$, ng g^{-1}	384.5 ± 65.2	430.7 ± 41.9
$BuSn^{3+}$, ng g^{-1}	65.0 ± 4.7	69.4 ± 14.9
Bu_2Sn^{2+}, ng g^{-1}	36.4 ± 1.3	38.9 ± 1.4
Bu_3Sn^+, ng g^{-1}	4.3 ± 0.5	5.0 ± 1.1

High precision and accuracy of the analytical results by the developed methods, liquid-liquid extraction GC-ICP-IDMS and SBSE-GC-ICP-IDMS, are due to the unique advantages of IDMS:

- instrumental instabilities such as long-term signal drifts or matrix effects have no influence on the final value of the species concentration in the sample;
- once complete mixing between the sample and the spike species has been achieved, possible loss of analyte during sample preparation has no influence on the final result;

- derivatization and extraction procedures do not need to be quantitative and no recovery corrections have to be applied.

Both extraction methods can be used for the simultaneous determination of trimethyllead, methylmercury, and butyltins in biological and environmental samples. However, it should be noted that the stir bar sorptive extraction needs additional equipment: thermodesorption / cold injection system, liquid nitrogen for cooling, tube conditioner for Twisters (stirring bars). These supplies are not necessary for liquid-liquid extraction. The applications of SBSE are also restricted by the availability of only one sorbent phase – PDMS. Sample preparation until the extraction is almost the same for both methods. But the extraction steps for both methods are quite different: the hexane extraction takes 10 min time, while SBSE takes 30 min plus additional time for the thermodesorption step (about 20 min). The hexane extract can be used for several GC-ICP-MS measurements, whereas the Twister can only be used for a single measurement. That means for the practical use of SBSE, that e.g. three replicate measurements increase the time consumption of SBSE to 150 min, versus 10 min for hexane extraction. Additionally, the costs for a Twister make this technique less attractive. Therefore, the liquid-liquid extraction GC-ICP-IDMS method was chosen for all further experiments and analyses of different samples.

4.3 Quality assurance of the developed GC-ICP-IDMS method for multi-species determination

4.3.1 Sample preparation and control of possible species transformation

During the last few years species-specific IDMS has been increasingly applied to validate analytical procedures for possible species transformations [71]. TMAH was used for many speciation analyses to digest biological material. Typically a 20-25 % TMAH solution in water was employed at temperatures from 37˚C to 60˚C for 1-4 h. The possibility of species transformation and influence of the temperature on the species behavior during TMAH treatment was investigated in the present work. Individual standard solutions of tri-, di- and monobutyltin with a natural isotopic composition were treated with TMAH for 4 h at room temperature, 37˚C and 60˚C before measurement (Chapter 3.8.4). Besides the major peaks from the species of standard solutions, small peaks from other tin species were observed on the GC-ICP-MS chromatograms. These small peaks could be identified to represent dibutyltin (from the tributyltin standard), monobutyltin (from the dibutyltin standard) and inorganic tin (from the monobutyltin standard). There was no significant increase of these small peaks in dependence on the temperature. It can therefore be assumed that the temperature of the TMAH treatment (up to 60°C) has no influence on the transformation of butyltin species. Table 41 shows these results in comparison with the results after derivatization of standards in a buffer solution (at room temperature, without any treatment by TMAH). As can be seen, in this case additional species in standard solutions are also present which may be due to impurity of standards. Purity of applied standards according to the producer information is only 96-97% (Table 3, Chapter 3.1). The small difference in the species fraction of standards after the TMAH treatment and after derivatization in a buffer solution can be due to the influence of different conditions of these two cases (e.g. ionic force) on the derivatization and extraction yield of tin species.

Table 41: Determination of the species fraction in standard solutions of mono-, di- and tributyltin at different temperatures of the TMAH pretreatment (derivatization by $NaBEt_4$, n=2)

Standard solution	Species, %	20°C (without TMAH)	20°C (with TMAH)	37°C (with TMAH)	60°C (with TMAH)
Bu_3Sn^+	Bu_3Sn^+	99.3 ± 0.1	98.6 ± 0.2	98.6 ± 0.1	98.6 ± 0.1
	Bu_2Sn^{2+}	0.7 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	1.4 ± 0.1
Bu_2Sn^{2+}	Bu_2Sn^{2+}	96.0 ± 0.2	95.4 ± 0.1	95.4 ± 0.1	95.2 ± 0.1
	$BuSn^{3+}$	4.0 ± 0.2	4.6 ± 0.1	4.6 ± 0.1	4.8 ± 0.1
$BuSn^{3+}$	$BuSn^{3+}$	99.9 ± 0.05	99.7 ± 0.1	99.7 ± 0.1	99.5 ± 0.1
	Inorg. Sn	0.1 ± 0.05	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1

Also the species-specific GC-ICP-IDMS determination of butyltins in the reference material CRM 477, after TMAH digestion at the same temperatures as shown above, resulted in no significant difference from the certified values (Table 42). Therefore it can be assumed that the digestion procedure, used in this work, does not cause a significant species transformation of the butyltin compounds. This finding is contrary to the results of Rodríguez-González et al. [114]. They used a triple spike methodology and found that during the TMAH digestion step a transformation of Bu_2Sn^{2+} into $BuSn^{3+}$ (3 % after 0.5 h; 53 % after 4 h) and of Bu_3Sn^+ into Bu_2Sn^{2+} (up to 4.1 % after 4 h) at 60˚C takes place. But the results of this work are in agreement with those of Ceulemans et al. as well as of Chau et al. [115,116], which also confirm no degradation of the organotin compounds during TMAH solubilization.

Table 42: Species-specific GC-ICP-IDMS determination of butyltins in the reference material CRM 477 after TMAH digestion at different temperatures (n=3)

Species	20˚C	37˚C	60˚C	Certified
$BuSn^{3+}$, µg g^{-1} as Sn	0.87 ± 0.06	0.94 ± 0.05	0.85 ± 0.04	1.01 ± 0.19
Bu_2Sn^{2+}, µg g^{-1} as Sn	0.83 ± 0.04	0.81 ± 0.02	0.83 ± 0.01	0.78 ± 0.06
Bu_3Sn^{+}, µg g^{-1} as Sn	0.84 ± 0.05	0.86 ± 0.03	0.86 ± 0.04	0.90 ± 0.08

In contrast to butyltin compounds the pretreatment temperature has significant influence on the species composition in the case of trimethyllead and methylmercury (Table 43). The fraction of inorganic mercury after TMAH treatment of $Me^{202}Hg^{+}$ spike solution is changed from about 17 % at 20°C to 27 % at 60°C. Not only temperature but also TMAH has a tremendous influence on the mercury species composition: methylmercury fraction of about 83 % after TMAH treatment is much less in comparison with 99 % after the simple derivatization in buffer solution (Table 43, Figure 37). Only the results obtained after derivatization of the $Me^{202}Hg^{+}$ spike solution without any TMAH treatment are in agreement with the certificate value of <2 % for inorganic mercury [117]. This indicates on significant demethylation of methylmercury during TMAH treatment which is increased at higher temperatures. Qvarnström and Frech used for the TMAH digestion of biological samples an ultrasonic bath for 1-2 h, where the water bath reached a temperature close to 50°C. Under these conditions they also found a significant demethylation of methylmercury which counteracts the insignificant methylation of Hg^{2+} [118]. It should be noted that degradation of methylmercury during sample preparation has no influence on the final results if the isotope dilution technique is used for quantification [98]. This was confirmed by the analysis of biological reference materials (Table 44, Chapter 4.3.2).

Table 43: Effect of temperature of the TMAH pretreatment (4 h) on the species composition in spike solutions of trimethyllead and methylmercury (derivatization by $NaBEt_4$, n=2)

Spike solution	Species, %	20°C (without TMAH)	20°C (with TMAH)	37°C (with TMAH)	60°C (with TMAH)
$Me_3{}^{206}Pb^+$	Me_3Pb^+	72.1 ± 1.9	73.2 ± 2.9	74.2 ± 2.7	82.0 ± 2.0
	Me_2Pb^{2+}	0.3 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.7 ± 0.1
	Pb^{2+}	27.6 ± 1.8	25.7 ± 2.8	24.8 ± 2.6	17.3 ± 1.9
$Me^{202}Hg^+$	$MeHg^+$	98.8 ± 0.1	83.1 ± 1.7	84.5 ± 2.0	72.8 ± 1.6
	Hg^{2+}	1.2 ± 0.1	16.9 ± 1.7	15.5 ± 2.0	27.2 ± 1.6

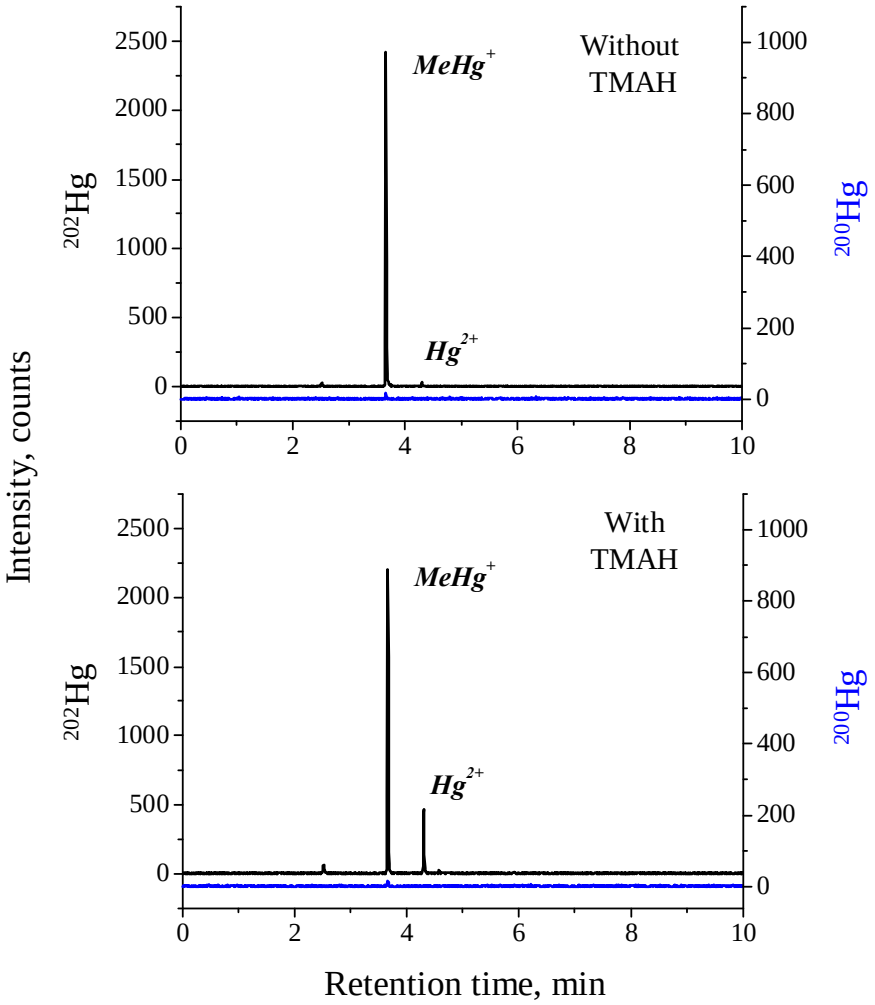

Figure 37: GC-ICP-MS chromatograms of the $Me^{202}Hg^{+}$ spike solution after derivatization by $NaBEt_4$ with and without TMAH pretreatment at 20˚C

In contrast to the observation for mercury species, the fraction of inorganic lead after TMAH treatment of the $Me_3{}^{206}Pb^{+}$ spike solution decreases with increasing temperature: from about 26 % at 20°C to 17 % at 60°C (Table 43). The contribution of methylation at higher temperatures in the trimethyllead spike, which contain inorganic lead, is obviously much higher as the demethylation. It is important that TMAH has no significant influence on the species composition in the trimethyllead spike solution at 20°C: species fractions after TMAH treatment at 20°C are similar to those without any use of TMAH. They are also in good agreement with the results of spike characterization by reverse IDMS (72.3 % of $Me_3{}^{206}Pb^{+}$ and 27.7 % of $^{206}Pb^{2+}$). Therefore the digestion of biological tissues with TMAH for multi-species determination was performed at room temperature by constant shaking.

TMAH treatment of samples was carried out in this work at room temperature in a small glass vials. Alternatively, digestion of the sample can also be performed by microwave-assisted treatment within a few minutes [96,119,120]. However, this prevents exact weighing of the sample and especially of the different small amounts of the spike solutions directly into the relatively heavy microwave vessels. The advantages of the 7 mL glass vial are an exact weighing and prevention of memory effects, because the cheap vials were only single-used.

Methylmercury, trimethyllead, mono-, di- and tributyltin are ionic compounds, which must be transformed into volatile and thermally stable compounds prior to GC separation. Several suitable derivatization reactions have been developed for determination of organometallic compounds: hydride generation, Grignard derivatization and aqueous ethylation or propylation. Aqueous derivatization by sodium tetraethylborate ($NaBEt_4$) or sodium tetra(n-propyl)borate ($NaBPr_4$) offers the best analytical characteristics (Table 2, Chapter 2.3) and it is widely used for determination of organometallic compounds [65, 121 , 122]. The application of these derivatization reagents allow easy handling of the derivatization process and provides the possibility of an in-situ alkylation and extraction. Derivatization conditions described in the literature using $NaBEt_4$ and $NaBPr_4$ for mercury, lead and tin compounds are rather similar. The most important difference between the derivatization procedures is the pH: the efficiency of the derivatization of the ionic alkylmetal species significantly depends on the pH value. The corresponding effect has been already studied and evaluated by others for organolead [95,123], mercury [124] and tin compounds [125]. The optimum condition for ethylation of mercury and tin compounds was found to be at pH 5 [126]. The pH dependence of the optimum trimethyllead recovery shows a relatively broad range of pH 4-10 [95]. Therefore pH 5 was chosen for the common derivatization of trimethyllead, methylmercury, and butyltins in biological samples. For many organometallic species the highest derivatization yield using $NaBPr_4$ was obtained at pH 4 [122]. The optimum pH range for $NaBPr_4$ is somewhat lower than that for $NaBEt_4$ (pH 5).

An excess of derivatization reagent (0.5 mL of 2% solution) was used for multi-species determination, since metals and other matrix components also react with the alkylborates. Investigations on the influence of the reaction time on the derivatization have shown that complete derivatization is achieved after 5 min for most of the organometallic species [122]. A reaction time of 10 min was chosen in this work. A detailed description of the sample

preparation procedure for the determination of trimethyllead and multi-species determination in biological and environmental samples are given in Chapters 3.6.4 and 3.8.4.

4.3.2 Analysis of biological reference materials

Reference materials play a crucial role for the method validation of all types of chemical measurements, including speciation. Commercially available certified reference materials for speciation analysis are generally certified for chemical species of a single element, which makes it difficult to control the quality of a multi-species determination method. The largest group of CRMs exists for methylmercury. However, no reference material is on the market where all important elemental species, relevant for analytical quality assurance of seafood, are certified. A certified reference material for butyltins, methylmercury, and arsenobetaine in oyster tissue (BCR-710) is still in preparation [127]. Thus, only reference materials certified for monomethylmercury or the butyltin species could be used for validation of the multi-species GC-ICP-IDMS method in biological samples.

Selected biological reference materials (dogfish DORM 2, tuna fish CRM 463 and mussel tissue CRM 477) were analyzed by following the procedures of spiking, extraction and derivatization, as described in Chapter 3.8.4. Representative chromatograms of the spike and reference isotopes of the isotope diluted reference material CRM 477 (mussel tissue) are shown in Figure 38. Other identified elemental species than those to be determined in this sample are only marked in the chromatogram of the reference isotopes. Corresponding peaks at the same retention time in the spike chromatogram are from identical elemental species. As can be seen mono-, di- and triphenyltin compounds could be determined by GC-ICP-IDMS under the same conditions if the corresponding spikes would be available.

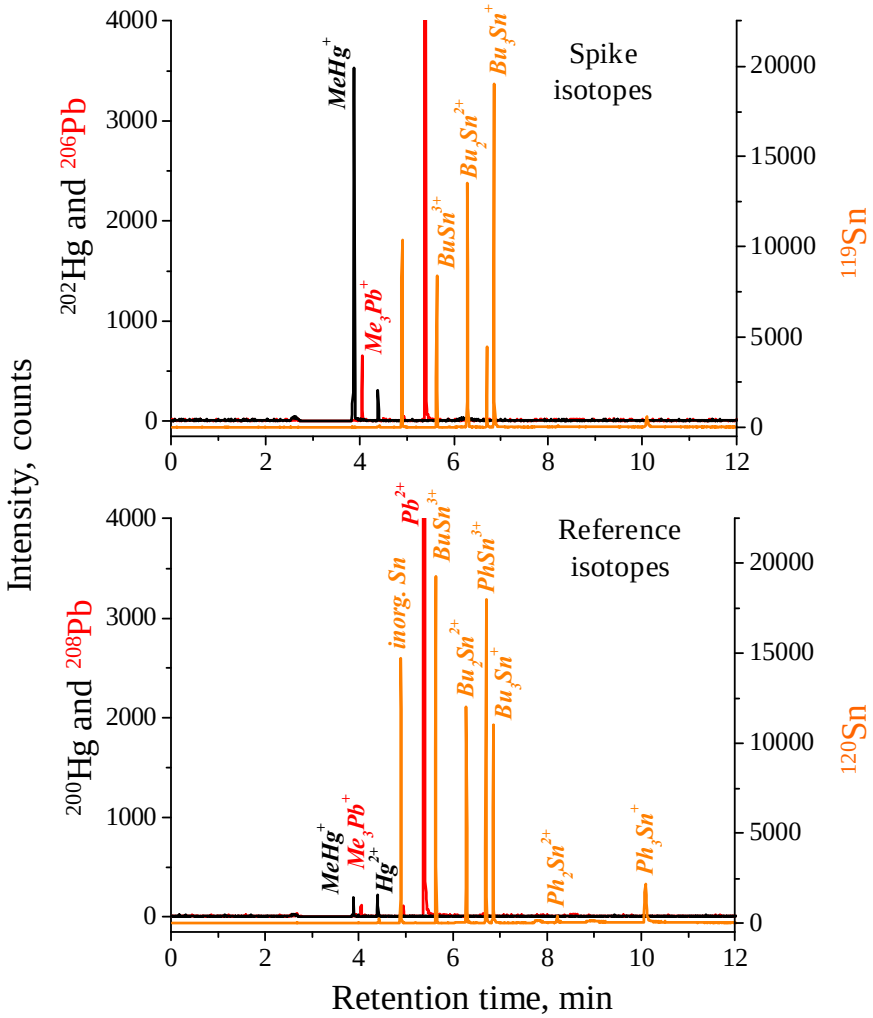

Figure 38: GC-ICP-MS chromatograms of the spike and reference isotopes of mercury, lead, and tin of the species-specific isotope diluted mussel tissue sample CRM 477

The results for methylmercury and mono-, di- and tributyltin determination in biological reference materials are listed in Tables 44 and 45. They show an excellent agreement between the multi-species-specific GC-ICP-IDMS determination and the certified values (mean ± standard deviation of three independent determinations by GC-ICP-IDMS; mean ± expanded uncertainty for certified values). The GC-ICP-IDMS results were also compared with those from the literature obtained by other methods. As can be seen from Tables 44 and 45, the GC-MIP-AES results for methylmercury and those of GC-MS for butyltin compounds agree also well with the certified values within the given uncertainties. However, the relative standard deviation of 1.1-4.3 % by GC-ICP-IDMS is better compared with other methods (RSD 7.1-13.9 %).

Table 44: Results of methylmercury determination in biological reference materials by multi-species GC-ICP-IDMS in comparison with GC-MIP-AES

Reference material	$MeHg^+$, μg g^{-1} as Hg		
	Multi-species GC-ICP-IDMS (n=3)	GC-MIP-AES	Certified
Dogfish (DORM 2)	4.36 ± 0.05	4.34 ± 0.35 [128]	4.47 ± 0.32
Tuna fish (CRM 463)	2.95 ± 0.05	2.87 ± 0.40 [129]	2.83 ± 0.15

Table 45: Results of mono-, di- and tributyltin determination in reference material CRM 477 by multi-species GC-ICP-IDMS in comparison with GC-MS

Species	Mussel tissue (CRM 477)		
	Multi-species GC-ICP-IDMS (n=3)	GC-MS [130]	Certified
$BuSn^{3+}$, μg g^{-1} as Sn	0.93 ± 0.04	1.17 ± 0.09	1.01 ± 0.19
Bu_2Sn^{2+}, μg g^{-1} as Sn	0.82 ± 0.03	0.75 ± 0.10	0.78 ± 0.06
Bu_3Sn^+, μg g^{-1} as Sn	0.85 ± 0.01	0.84 ± 0.06	0.90 ± 0.08

4.3.3 Analysis of urban dust and sediment reference materials

For validation of the multi-species GC-ICP-IDMS method for environmental samples, the urban dust reference material CRM 605 and two sediment reference materials BCR 646 and CRM 580 were selected and analyzed according to the procedure described in Chapter 3.8.4. The urban dust reference material CRM 605, certified for its trimethyllead content, was used to validate the developed species-specific GC-ICP-IDMS method for the analysis of trimethyllead. The corresponding result of 8.01 ± 0.20 μg kg^{-1} (as Me_3Pb^+) is in excellent agreement with the

certified value of 7.9 ± 1.2 μg kg^{-1}. The trimethyllead concentration in this reference material 8.04 ± 0.57 μg kg^{-1} determined by SPME-MCGC-ICP-TOFMS [110] agree also with the certified value. However, the precision of the GC-ICP-IDMS method (RSD 2.5 %) is better than the precision of the SPME-MCGC-ICP-TOFMS method (RSD 7.1 %).

Species-specific isotope dilution GC-ICP-MS method was also applied to multi-species determination in two sediment reference materials (BCR 646 and CRM 580). The GC-ICP-IDMS results of the three butyltin species in the reference freshwater sediment BCR 646 (Table 46) agree well with the certified values within the given uncertainties. The small standard deviations obtained by GC-ICP-IDMS compared with the relatively high uncertainties of the certified values demonstrate that a more precise certification should be possible. Trimethyllead and monomethylmercury in this sample were found below the detection limit of the GC-ICP-IDMS method.

Table 46: Results of the determination of mono-, di- and tributyltin in reference material BCR 646 by multi-species GC-ICP-IDMS in comparison to certified values

Species	Freshwater sediment (BCR 646)	
	Multi-species GC-ICP-IDMS (n=3)	Certified
$BuSn^{3+}$, ng g^{-1} as Sn	353 ± 14	412 ± 81
Bu_2Sn^{2+}, ng g^{-1} as Sn	401 ± 6	392 ± 46
Bu_3Sn^{+}, ng g^{-1} as Sn	184 ± 3	196 ± 33

The reference estuarine sediment CRM 580, certified for monomethylmercury (70.2 ng g^{-1}), which contains a high excess of inorganic mercury (certified value of total mercury 132 ± 3 μg g^{-1}), was analyzed by multi-species-specific GC-ICP-IDMS and SBSE-GC-ICP-IDMS. The corresponding results presented in Table 40 (Chapter 4.2) show good agreement and similar precision for both methods. However, the concentrations of methylmercury were found to be much higher than the corresponding certified value (factor of about six). Additional investigations were carried out to identify the sources of the unintentional methylmercury

formation during analysis of this reference material: ethylation and propylation using liquid-liquid extraction were compared as well as the influence of the presence of undissolved sediment particles during derivatization and SBSE was studied by GC-ICP-IDMS. The results of these investigations are presented in Table 47. Rodríguez Martín-Doimeadios et al. [131] also determined a much higher $MeHg^+$ concentration for CRM 580 by GC-ICP-IDMS than certified (Table 47).

Table 47: Results of methylmercury determination in the sediment reference material CRM 580 by GC-ICP-IDMS after different sample preparation procedures (n=3)

Extraction	Derivatization	Sediment particles	$MeHg^+$, ng g^{-1} as Hg
Hexane (this work)	ethylation	with	384.5 ± 65.2
	propylation	with	261.7 ± 22.3
Isooctane [131]	ethylation	without	290.0 ± 31.3
	propylation	without	125.5 ± 20.9
SBSE (this work)	ethylation	with	430.7 ± 41.9
	ethylation	without	109.1 ± 5.3
Methylene chloride [131]	ethylation	without	97.6 ± 1.2
Certified value			70.2 ± 3.4

As can be seen from Table 47 the methylmercury concentration determined after hexane extraction and derivatization by $NaBEt_4$ was higher than using propylation. This is in agreement with literature data, obtained after isooctane extraction. It should be noted that in the chromatogram of this reference material a high peak of Hg^0 was observed besides that one of Hg^{2+} (Figure 39). Hg^0 can be formed during the sample preparation from the degradation of both, ethylated methylmercury [98] and ethylated inorganic mercury [93]. The contribution from degradation of Et_2Hg (derived from Hg^{2+}) is obviously much higher than that one from low amounts of methylmercury in this material. After ethylation, the Hg^0 peak was especially high in comparison to propylation (factor of about two). This leads to a bad chromatographic separation

of Hg^0 from the methylmercury peak and therefore to a worse reproducibility of the methylmercury determination using $NaBEt_4$ as derivatization reagent (Table 47).

The determination of methylmercury by using SBSE and ethylation was carried out in two different ways. Sediment particles were either separated by centrifugation before derivatization and the SBSE step or they remained in the solution during derivatization and extraction. As can be seen from Table 47 the presence of sediment particles during derivatization and extraction has a tremendous influence on the result. Chromatograms of CRM 580 (Figure 39) show much smaller peaks of Hg^0 and Hg^{2+} after separation of sediment particles. In this case the determined concentration of methylmercury was four times smaller than that one obtained after derivatization and extraction in the presence of sediment particles and the correspondent high amount of inorganic mercury. The SBSE-GC-ICP-IDMS result, after separation of sediment particles, is similar to the literature result, using organic solvent extraction with methylene chloride [131]. This kind of extraction was used to reduce the amount of inorganic mercury present during the derivatization step. In addition, methylmercury concentrations, determined after liquid-liquid extraction and derivatization in the presence of sediment particles, are higher than the corresponding literature results obtained after separation of the particles from digestion solution. In the reference material BCR 646 with a total mercury content of only 1.74 ± 0.08 µg g^{-1} (Table 48, Chapter 4.4.1), no methylmercury was detected.

Artifact formation of methylmercury strongly depends on the amount of inorganic mercury, present in the solution for derivatization. The same effect has also been observed by Horvat et al. during ethylation [132] and by Huang during derivatization using sodium tetra(n-propyl)borate [133]. The methyl and ethyl groups were supposed to be transferred to Hg^{2+} from the derivatization reagent [132], the acetate buffer [134,135] or other organometallic compounds [136,137], e.g. the internal standard, present in the solution. Recent detailed investigations have shown that the amounts of the derivatization reagent and acetate buffer and the presence of other organometallic compounds have little influence on the artifact effect [131,133]. Results of GC-MS investigations lead to the suggestion that $NaBPr_4$ undergoes rather complicated side reactions, such as alkyl cleavage and rearrangement [133]. Artifact methyl- and ethylmercury compounds may serve as side products of the propylation of metals, using $NaBPr_4$ derivatization. Derivatization using $NaBEt_4$ seemed to show a similar phenomenon, but to less extent:

- the total artifact of organomercury compounds caused by $NaBPr_4$ derivatization (up to 3% [133]) is much higher than by $NaBEt_4$ derivatization (up to 1 % [132]);

– the artifact organomercury compounds from $NaBPr_4$ derivatization appeared in various forms ($MeHg^+$, $EtHg^+$, Et_2Hg and some unidentified organomercury compounds) in comparison with the only artifact product, methylmercury, from the $NaBEt_4$ derivatization.

However, if only methylmercury is the species to be determined derivatization by $NaBPr_4$ is advantageous, because in this case the rate of artifact $MeHg^+$ is lower (0.03-0.28 % of Hg^{2+} present) than for $NaBEt_4$ derivatization (up to 1 % of Hg^{2+}). This is confirmed by the results for CRM 580 listed in Table 47.

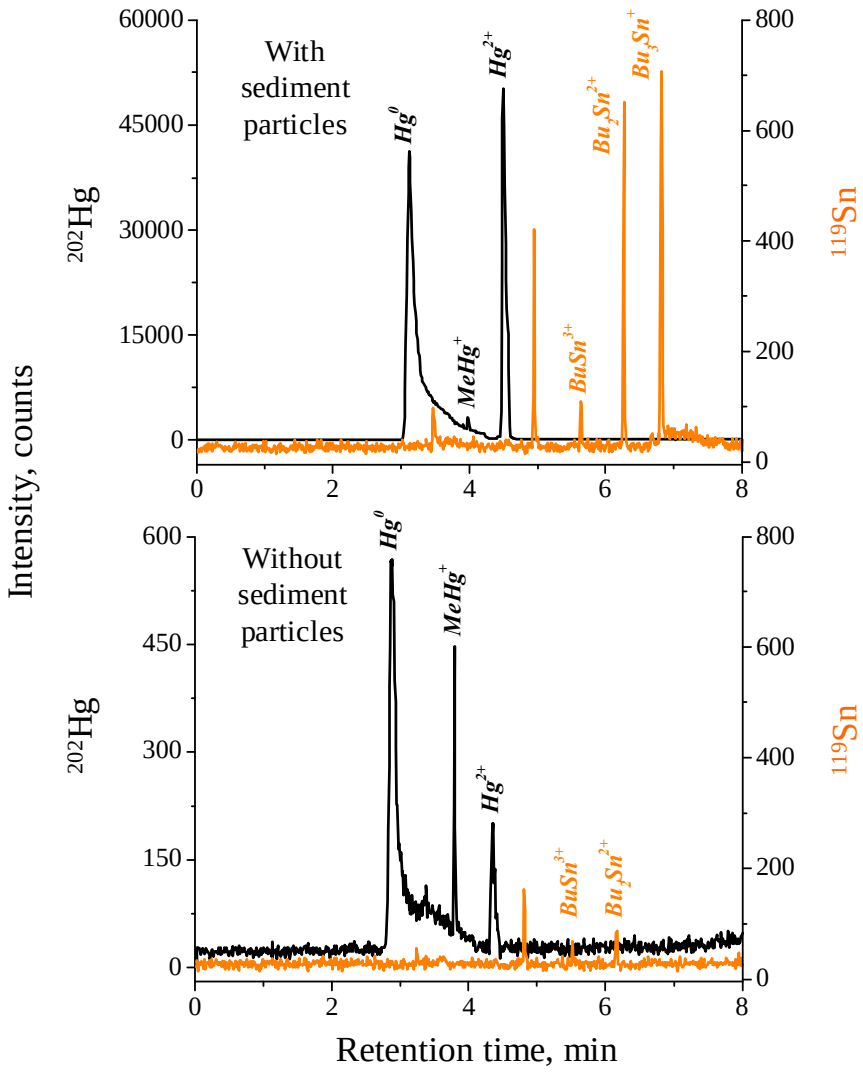

Figure 39: GC-ICP-MS chromatograms of the mercury and tin species from the species-specific isotope diluted sediment reference material CRM 580 after SBSE in presence and absence of sediment particles (derivatization by $NaBEt_4$)

The results clearly demonstrate that species transformation, especially in the case of an extremely high excess of one of the elemental species, has to be checked carefully to obtain accurate results. Possible artifact formation of methylmercury should be taken into account and necessary corrections should be made for samples in which the $MeHg^+$ / Hg^{2+} ratio is extremely small. The corrections are possible by species-specific isotope dilution analysis using $MeHg^+$ and Hg^{2+} spikes, labeled with different isotopes. A multi-isotope labeling approach had been already applied for mercury [118,138,139], tin and other species [114,140,141]. Although this multi-isotope spike technique enables identification and correction of several species transformations simultaneously, the complex system of necessary mathematical equations for evaluation and the lack of availability of isotope-labeled spikes will not favor this method to be a routine application in analytical quality assurance in the near future [96]. A separation of Hg^{2+} from organomercury prior to derivatization may reduce the artifact effect and could be an alternative solution for this problem.

The concentration level of total mercury in most of the sediment reference materials is much higher than in natural sediments, where typically concentrations range from 50 ng g^{-1} to 500 ng g^{-1} dry weight [135]. Total mercury content in reference material IAEA-356 (polluted marine sediment) and CRM 580 (estuarine sediment, analyzed in this work), for example, is certified at 7620 ng g^{-1} and 132000 ng g^{-1} dry weight, respectively. The concentration of artifact $MeHg^+$ correlates with inorganic mercury concentration. Therefore it is not recommended to use reference materials as CRM 580 or IAEA-356 for validation of methylmercury. In the case of biological samples concentrations of methylmercury are relatively high and those of inorganic mercury are low so that the effect of artifact $MeHg^+$ is negligible.

4.4 Simultaneous determination of trimethyllead, methylmercury and butyltins by species-specific GC-ICP-IDMS

4.4.1 Fraction of methylated lead and mercury in reference materials

In Table 48 the trimethyllead concentrations determined by species-specific GC-ICP-IDMS in reference materials, not certified for their Me_3Pb^+ content, are listed together with the corresponding standard deviations calculated from five independent analyses each. The total lead content (certified value or determined by ICP-IDMS) and the fraction of methylated lead from total lead are also presented. For comparison, the corresponding data for monomethylmercury and total mercury are given as far as they are available.

Trimethyllead could be determined in all reference materials, except in Antarctic krill (MURST-ISS-A2) and two sediments (CRM 580 and BCR 646), where concentrations of less than the corresponding detection limits (0.06 ng g^{-1} for biological samples and 0.04 ng g^{-1} for sediments) were found. The trimethyllead concentrations in the other reference materials vary in the range of 0.3–17 ng g^{-1} (as Pb), which is obviously the natural level in seafood as the results in Table 49 (Chapter 4.4.2) indicate. Concentrations of methylmercury vary more considerably (66 ng g^{-1} to 4470 ng g^{-1} as Hg) than those of trimethyllead.

Table 48: Trimethyllead concentrations in reference materials, determined by species-specific GC-ICP-IDMS, in comparison to the concentrations of total lead, methylmercury and total mercury

Reference material	Methylated species		Total metal content		Methylated metal	
	$MeHg^+$ ng g^{-1} as Hg	Me_3Pb^+ ng g^{-1} as Pb	Hg ng g^{-1}	Pb ng g^{-1}	$MeHg^+$ %	Me_3Pb^+ %
DORM 2 (Dogfish muscle)	4470 ± 320[a]	6.41 ± 0.29[c]	4640 ± 260[a]	65 ± 7[a]	96	10
CRM 463 (Tuna fish)	2830 ± 150[a]	4.39 ± 0.16[c]	2850 ± 160[a]	381 ± 39[d]	99	1.2
CRM 422 (Cod muscle)	400 ± 20[b]	16.74 ± 0.19[c]	559 ± 16[a]	85 ± 15[a]	72	20
CRM 477 (Mussel tissue)	66 ± 2[c]	0.30 ± 0.02[c]	191.4 ± 3.4[d]	9067 ± 95[d]	34	0.003
CRM 278 (Mussel tissue)	130 ± 50[b]	2.72 ± 0.06[c]	188 ± 7[a]	1910 ± 40[a]	69	0.14
MURST-ISS-A2 (Antarctic krill)	n.d.	< 0.06[c]	13 ± 3[b]	1110 ± 110[a]	-	<0.01
CRM 580 (Sediment)	70.2 ± 3.4[a]	< 0.04[c]	132000±3000[a]	n.d.	0.05	-
BCR 646 (Sediment)	< 1.0[c]	< 0.04[c]	1735 ± 83[d]	147920±1032[d]	<0.06	$<3\times10^{-5}$

[a] certified value, [b] indicative value, [c] determined by GC-ICP-IDMS,
[d] determined by ICP-IDMS, n.d. not determined

The fraction of methylated lead from total lead varies in a brought range of 0.003-20 %, which is in contrast to methylated mercury in the same samples (Figure 40). Monomethylmercury is always the most abundant mercury species in marine animals and reaches nearly 100 % in some samples (dogfish muscle and tuna fish sample). Whereas a good positive correlation of methylmercury with the total mercury content could be found (correlation coefficient 0.999), no correlation was observed between trimethyllead and total lead in the investigated reference materials (Figures 41 and 42). Also trimethyllead does not correlate with methylmercury in these samples.

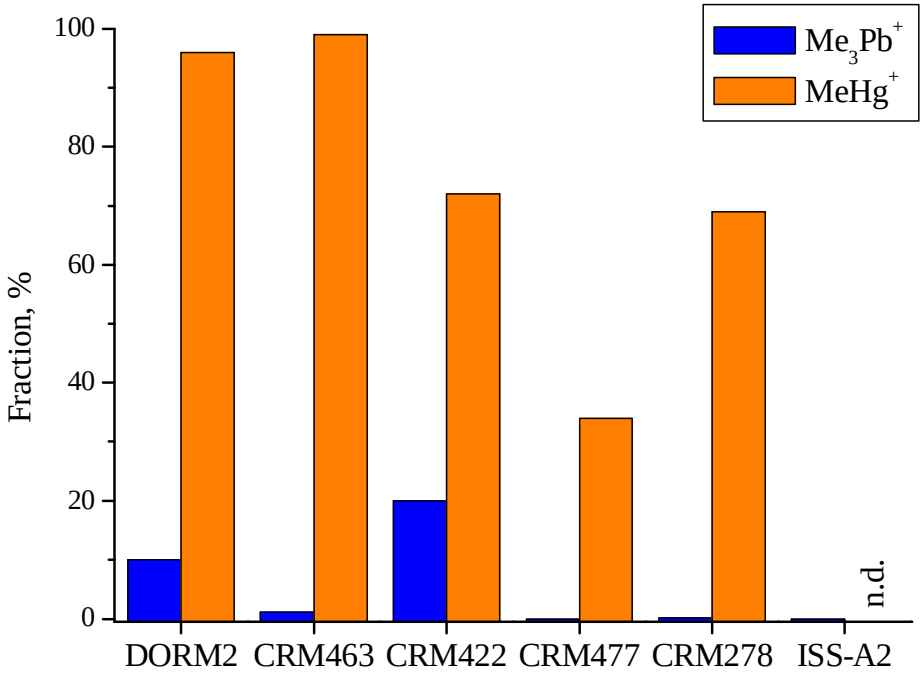

Figure 40: Fraction of trimethyllead and methylmercury in biological reference materials

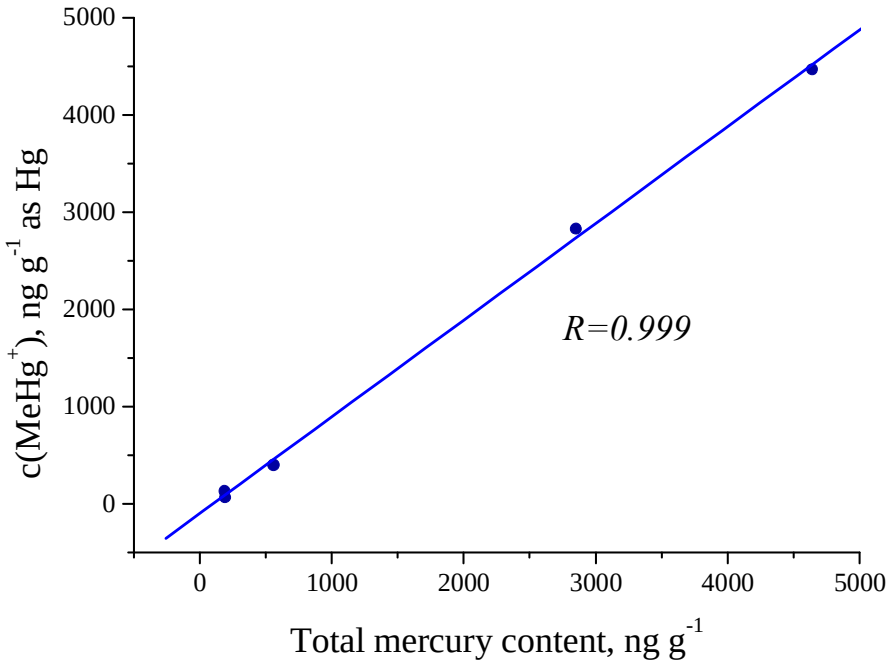

Figure 41: Dependence of methylmercury concentration on the total mercury content in biological reference materials

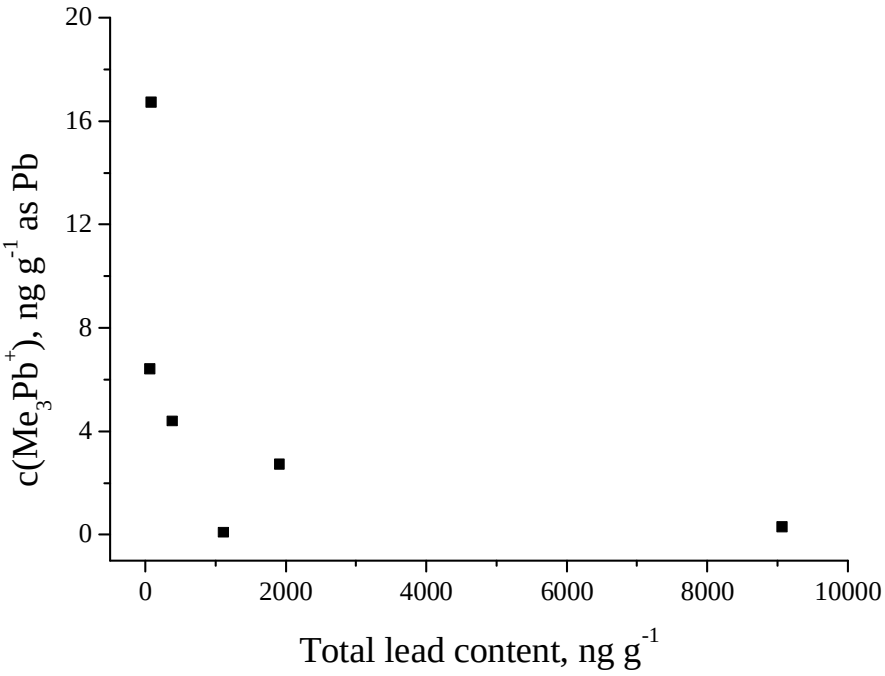

Figure 42: Dependence of trimethyllead concentration on the total lead content in biological reference materials

Relative standard deviations in the range of 1.1-5.4 % were obtained for species-specific GC-ICP-IDMS determinations of trimethyllead in different reference materials (Table 48), which is exactly at the same low level as for the certified reference material urban dust (2.5 %). The trimethyllead concentrations in these five different commercially available reference materials (determined by species-specific GC-ICP-IDMS) can be used as accurate indicative values to validate other analytical methods for this important elemental species.

4.4.2 Fraction of alkylated lead, mercury and tin in seafood samples

The developed GC-ICP-IDMS method was applied to the analysis of representative seafood (mussels, large peeled prawns, filets of tuna fish, plaice, pollock and shark). The results of the multi-species determination of these seafood samples are summarized in Table 49. The corresponding results for the biological reference materials are also listed here for comparison. The columns in the middle of Table 49 include the total metal contents determined by ICP-IDMS and in the last three columns the corresponding fraction of the alkylated species are presented. The water content of seafood samples is also listed to enable the calculation of the wet weight concentrations, which is usually the basis for legislative limits.

Table 49: Concentrations of methylmercury, trimethyllead and butyltins in seafood samples from the supermarket and in biological reference materials and corresponding alkylated fractions with respect to the total metal content (n=3)

Sample	Alkylated species, ng g^{-1} dry weight			Total metal content, ng g^{-1} dry weight			Fraction of alkylated metal, %		
	$MeHg^+$ as Hg	Me_3Pb^+ as Pb	∑ Butyltins as Sn	Hg	Pb	Sn	Hg	Pb	Sn
Tuna fish[b] 75.5% H_2O	1082 ± 60	0.39 ± 0.02	21.7	1184 ± 27	5.1 ± 1.1	43.9 ± 6.5	91	7.6	49
Tuna fish (CRM 463)	2830 ± 150[a]	4.10 ± 0.16	<0.3[c]	2850 ± 160[a]	360 ± 29	1169 ± 58	99	1.1	<0.02
Mussels[b] 82.3% H_2O	85.4 ± 3.1	0.35 ± 0.04	13.8	179.9 ± 3.6	2505 ± 113	71.4 ± 6.7	47	0.01	19
Mussels (CRM 477)	66 ± 2	0.34 ± 0.03	2690[a]	191.4 ± 3.4	9057 ± 229	12254 ± 818	34	0.004	22
Prawns[b] 82.7% H_2O	40.5 ± 2.7	0.51 ± 0.04	0.7	50.8 ± 2.8	116.4 ± 4.7	58.9 ± 6.0	80	0.4	1.2
Plaice[b] 83.7% H_2O	170.7 ± 4.8	0.31 ± 0.02	6.0	186.1 ± 6.3	33.0 ± 4.5	31.2 ± 4.2	92	0.9	19
Pollock[b] 80.1% H_2O	704 ± 28	0.66 ± 0.05	<0.3[c]	784 ± 27	18.1 ± 3.6	16.9 ± 2.3	90	3.6	<1.8
Shark 1[b] 73.8% H_2O	3210 ± 118	1.76 ± 0.08	<0.3[c]	3996 ± 344	85.1 ± 8.8	96.9 ± 5.7	80	2.1	<0.3
Shark 2[b] 73.8% H_2O	10634 ± 122	2.10 ± 0.08	<0.3[c]	10859 ± 351	81.7 ± 11.5	113.7 ± 10.3	98	2.6	<0.3
Dogfish (DORM 2)	4470 ± 320[a]	6.40 ± 0.06	<0.3[c]	4640 ± 260[a]	65 ± 7[a]	91.4 ± 5.6	96	9.8	<0.3

[a] Certified value [b] Seafood from supermarket [c] Detection limit for tributyltin

The concentration of trimethyllead in the different seafood samples varies within a small range from only 0.3 ng g^{-1} to 2.1 ng g^{-1}, almost independent from the total lead content, which varies over three orders of magnitude. The fraction of methylated lead therefore also varies over more than two orders of magnitude from 0.01 to 7.6% (Figure 43).

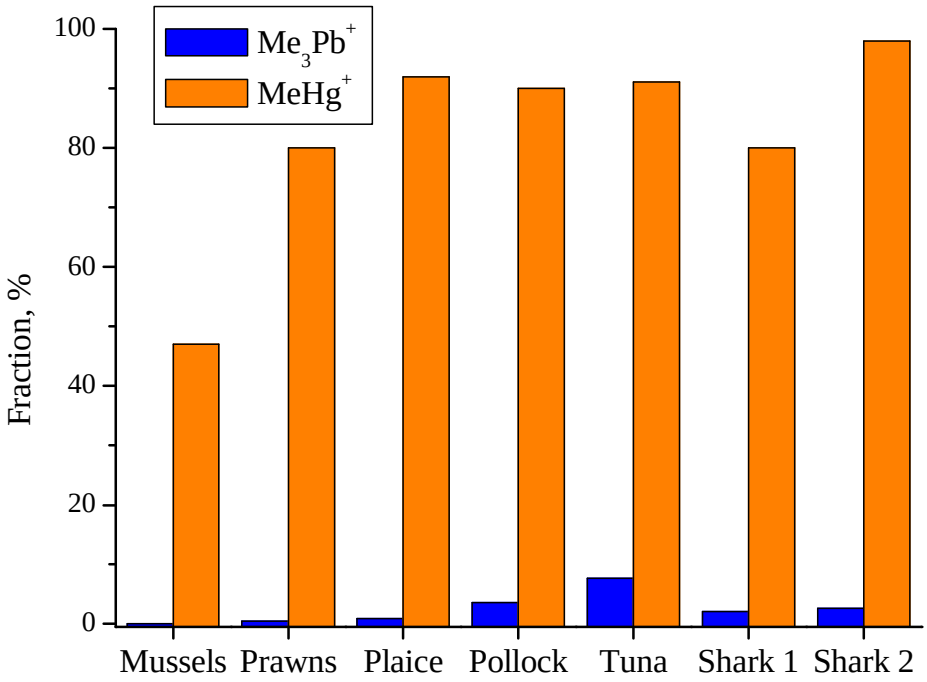

Figure 43: Fraction of trimethyllead and methylmercury in seafood samples

Contrary to trimethyllead, the concentration of methylmercury in the marine samples is much higher and varies considerably from 40.5 ng g^{-1} in prawns to 10634 ng g^{-1} in shark sample. Moreover, the fraction of methylated mercury is also very high and reaches 98% in shark samples (Figure 43). It is of special importance for the quality control of seafood, that the total mercury concentration correlates very good (correlation coefficient 0.998) with the concentration of toxic methylmercury (Figure 44). This means, that in the first approximation the analysis of total mercury would be sufficient to qualify a seafood sample for methylmercury. On the contrary, no correlation was observed between trimethyllead and total lead in the investigated samples (Figure 45). These findings are in excellent agreement with those for biological

reference materials (Figures 40-42, Chapter 4.4.1) and indicate different up-take mechanisms for methylated lead and mercury by marine animals and/or different efficiency of environmental methylation of these metals. They are also in good agreement with literature data, where no positive correlation between organic and total lead in mussels was found [142].

Table 49 shows that the results of methylated lead and mercury in reference materials are in good relation to the obtained results for the different seafood samples from the supermarket. Therefore it can be assumed, that the methylated lead and mercury concentrations in the investigated CRMs are representative for corresponding seafood samples. This increases the usefulness of these reference materials for analytical quality assurance of seafood.

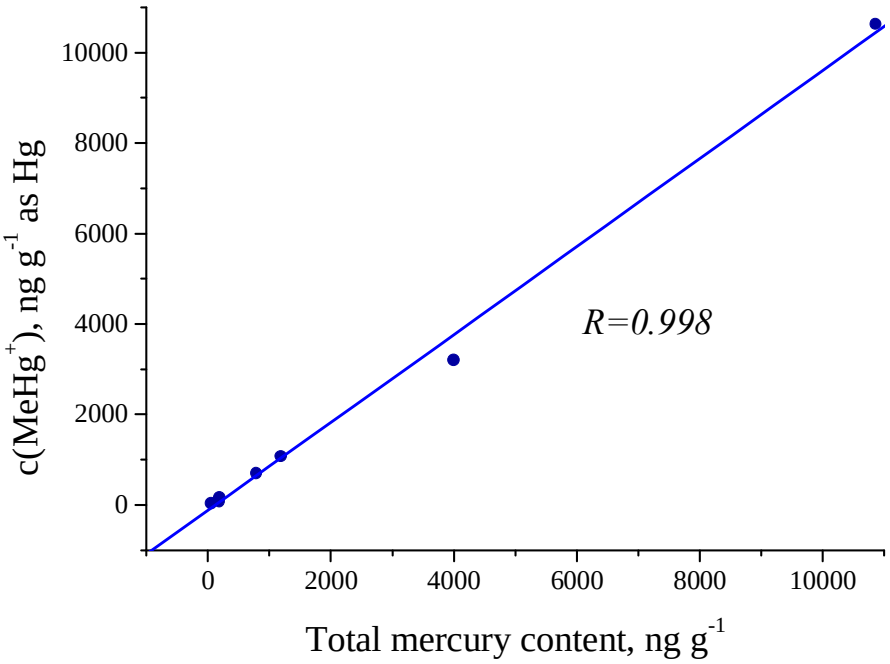

Figure 44: Dependence of methylmercury concentration from the total mercury content in seafood samples

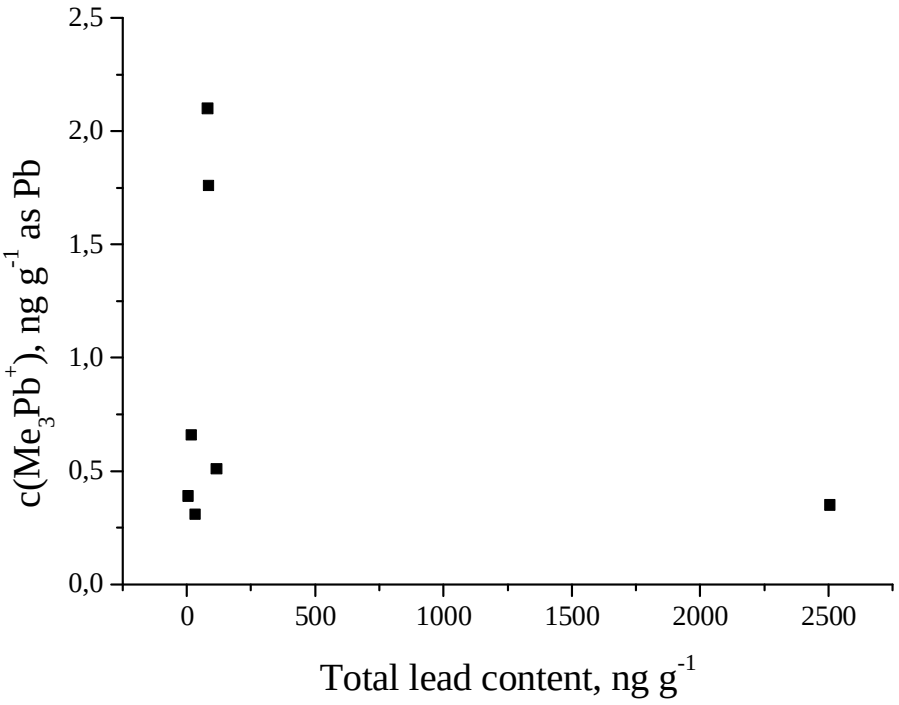

Figure 45: Dependence of trimethyllead concentration from the total lead content in seafood samples

On the basis of the correlation of different alkyllead species, as well as the appearance of trimethyllead in mussels from reference sites (far from maritime activities and gasoline stations), it was suggested that an additional origin of trimethyllead – other than directly from the demethylation of Me_4Pb gasoline additive – could be biomethylation [142,143]. Studies on the alkyllead distribution in marine snails [144] led to the same suggestion. The results of this work, constant levels of trimethyllead in different seafood samples and biological reference materials from various origin, confirm the suggestion as well. According to Pongratz and Heumann [30,31] trimethyllead in the marine environment is formed by marine bacteria and macroalgae.

As can be seen from Table 49 the highest methylmercury contents were found in tuna fish (CRM 463 and sample from supermarket) and in the different shark samples (DORM 2, Shark 1, 2 and all other shark samples, Table 28, Chapter 3.8.6.2), lowest concentrations were found in prawns and mussels (CRM 477 and mussels from supermarket). The level of methylmercury in seafood and fish is related to the level of mercury and methylmercury in the aquatic environment and

place of animal in the food chain. The results are an indication that methylmercury accumulate within the food chain. This is the reason why predatory fish species have higher levels of methylmercury than those at the other end of the food chain. Thus consumption of seafood from marine animals, like sharks and tuna, which are at the top end of the marine food chain, can be risky with respect to toxic methylmercury. The permitted maximum concentration of methylmercury in all species of shark and other predatory fishes (pike, tuna, swordfish etc.) is 1.0 mg kg^{-1} wet weight and in all other fishery products 0.5 mg kg^{-1} wet weight [145,146]. According to this regulation four shark samples investigated in this work (Shark steaks, Sharks 2, 4 and 5, see Table 28, Chapter 3.8.6.2) contain higher levels of methylmercury than allowed. A more than three times higher concentration was determined in Shark 5 (3.13 µg g^{-1}).

In contrast to methylated lead and mercury, butyltin compounds could only be determined in half of the investigated samples. The anthropogenic character of tributyltin and its use for boat paints is the reason that this elemental species and its degradation products are preferably found in marine animals, which live close to a harbor, where corresponding contaminations are probable. Therefore it was not surprising to find high butylated tin fractions of about 20 % in mussels (CRM 477 and mussels from supermarket), which often grow up close to harbor areas. On the other hand sharks, normally living in the open ocean, were not contaminated by butylated tin compounds. Tributyltin was always the most abundant tin species in samples from the supermarket: mussels 7.4 ± 0.6 ng g^{-1}, prawns 0.74 ± 0.11 ng g^{-1}, tuna fish 21.7 ± 0.5 ng g^{-1}, plaice 6.0 ± 0.4 ng g^{-1} (as Sn). Monobutyltin was only found in mussels (6.4 ± 0.4 ng g^{-1}), whereas no dibutyltin was detectable in all these samples (Table 27, Chapter 3.8.6.1). The highest tributyltin fraction of 49 % was observed in a tuna fish sample from the supermarket which shows that this fish was affected by a corresponding contamination.

The precision of multi-species-specific GC-ICP-IDMS determination of alkylated metal compounds in seafood samples is sufficiently good for ng g^{-1} concentration levels (RSD 3.8-11.8% for trimethyllead, 1.1-6.7 % for methylmercury and 2.3-14.9 % for butyltins).

4.5 Comparison of GC-ICP-IDMS results with results of simultaneous multi-species determination from literature

The GC-ICP-IDMS results of this work were compared with results of simultaneous multi-species determination from the literature. Recently published results of the reference materials DORM 2, CRM 477 and CRM 605 by Jitaru et al. [110] - where headspace SPME-MCGC-ICP-TOFMS was used - are fairly different from the results obtained in this work by GC-ICP-IDMS. Table 50 shows that only the results of trimethyllead for CRM 605 and methylmercury for DORM 2 are in good agreement with those of GC-ICP-IDMS and the results of trimethyllead for DORM 2 are in the same concentration range. All other results differ extremely.

Table 50: Comparison of GC-ICP-IDMS results with literature results for reference materials CRM 605, DORM 2 and CRM 477

Sample	Me_3Pb^+, ng g^{-1} as Pb		$MeHg^+$, ng g^{-1} as Hg		Butyltin content, ng g^{-1} as Sn [110]
	GC-ICP-IDMS (This work)	SPME-GC-ICP-TOFMS [110]	GC-ICP-IDMS (This work)	SPME-GC-ICP-TOFMS [110]	
CRM 605 (Urban dust)	6.58 ± 0.16	6.60 ± 0.47	-	<0.002	14
DORM 2 (Dogfish muscle)	6.40 ± 0.06	9.9 ± 1.1	4360 ± 50	4280 ± 210	76
CRM 477 (Mussel tissue)	0.34 ± 0.03	<0.00003	66 ± 2	<0.001	2830

The disagreement in the results for CRM 477 may be due to the high excess of butyltin compounds in this sample, which can cause problems during isolation of minor analytes by the headspace SPME technique. On the contrary, trimethyllead results of both methods for reference material CRM 605, where butyltin content is at the same concentration level as this analyte, are in excellent agreement. Good agreement was also observed for methylmercury in DORM 2, where its concentration is much higher than for other species. The results show that displacement of volatile species during headspace SPME by other compounds, especially in the case of an extremely high excess of one of the elemental species, has to be carefully checked, if accurate results should be obtained. Additionally, it should be noted that precision of GC-ICP-IDMS

results (RSD 0.9-2.4 %) is much better than that of SPME-GC-ICP-TOFMS results from literature (RSD 4.9-11.1 %).

This comparison clearly demonstrates the necessity for the development of accurate and precise multi-species methods using species-specific isotope dilution mass spectrometry which are especially useful for the analysis of samples with complex matrices and extremely different concentrations of species to be analyzed. The GC-ICP-IDMS method developed in this work can easily be extended to multi-species-specific determination of other organometallic compounds (e.g. phenyl- and methyltin species, ethyllead species etc.). Commercial availability of the corresponding isotope-enriched spike solutions with known isotopic abundances and concentrations will favor the routine application of GC-ICP-IDMS for analytical quality control of environmental and biological samples.

5 Abbreviations

AAS	atomic absorption spectrometry
AES	atomic emission spectrometry
AFS	atomic fluorescence spectrometry
ATP	adenosine triphosphate
CE	capillary electrophoresis
CIS	cold injection system
CRM	certified reference material(s)
DNA	deoxyribonucleic acid
ECD	electron capture detector
EDTA	ethylene-diamine-tetraacetic acid
GC	gas chromatography
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-IDMS	inductively coupled plasma isotope dilution mass spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ICP-TOFMS	inductively coupled plasma time-of-flight mass spectrometry
IDMS	isotope dilution mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LC	liquid chromatography
MCGC	multi-capillary gas chromatography
MIP-AES	microwave induced plasma atomic emission spectrometry
MS	mass spectrometry
NaDDTC	sodium diethyldithiocarbamate
PDMS	polydimethylsiloxane
PE	polyethylene
PFA	perfluoroalkoxy, Teflon
PTFE	polytetrafluorethylen, Teflon
PVC	polyvinyl chloride
QAAS	quartz furnace atomic absorption spectrometry
RF	radio frequency
RNA	ribonucleic acid

RSD	relative standard deviation
SBSE	stir bar sorptive extraction
SD	standard deviation
SFC	supercritical fluid chromatography
SPME	solid phase micro extraction
SRB	sulphate reducing bacteria
TDS	thermodesorption system
TIMS	thermal ionization mass spectrometry
TMAH	tetramethylammonium hydroxide

6 References

[1] Lobinski R. *Elemental speciation and coupled techniques.* Appl. Spectrosc. **1997**, 51, 260A-278A.

[2] Eto K. *Minamata disease.* Neuropathology **2000**, 20, S14-S19.

[3] Clarkson T.W. *The three modern faces of mercury.* Environ. Health Persp. **2002**, 110, 11-23.

[4] Yoshinaga J. In *Organometallic Compounds in the Environment;* Craig P.J., Ed.; Wiley & Sons: West Sussex, England, 2003; pp 151-194.

[5] Alzieu C., Sanjuan J., Michel P., Borel M., Dreno J.P. *Monitoring and assessement of butyltins in Atlantic coastal waters.* Mar. Pollut. Bull. **1989**, 20, 22-26.

[6] Cima F., Craig P.J., Harrington C. In *Organometallic Compounds in the Environment;* Craig P.J., Ed.; Wiley & Sons: West Sussex, England, 2003; pp 101-149.

[7] Rodríguez-González P., Marchante-Gayón J.M., García Alonso J.I., Sanz-Medel A. *Isotope dilution analysis for elemental speciation: A tutorial review.* Spectrochim. Acta B **2005**, 60, 151-207.

[8] Craig P.J. In *Organometallic Compounds in the Environment;* Craig P.J., Ed.; Longman: Essex, England, 1986; pp 65-110.

[9] Maguire R.J. *Environmental aspects of tributyltin.* Appl. Organomet. Chem. **1987**, 1, 475-498.

[10] Craig P.J., Eng G., Jenkins R.O. In *Organometallic Compounds in the Environment;* Craig P.J., Ed.; Wiley & Sons: West Sussex, England, 2003; pp 1-55.

[11] Jensen S., Jernelöv A. *Biological methylation of mercury in aquatic organisms.* Nature **1969**, 223, 753-754.

[12] Mason R.P., Benoit J.M. In *Organometallic Compounds in the Environment;* Craig, P.J., Ed.; Wiley & Sons: West Sussex, England, 2003; pp 57-99.

[13] Robinson J.B., Tuovinen O.H. *Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses.* Microbiol. Rev. **1984**, 48, 95-124.

[14] Compeau G., Bartha R. *Sulfate-reducing bacteria: Principle methylators of mercury in anoxic estuarine sediment.* Appl. Environ. Microbiol. **1985**, 50, 498-502.

[15] Choi S., Bartha R. *Cobalamin-mediated mercury methylation by Desulfovibrio desulfuricans LS.* Appl. Environ. Microbiol. **1993**, 59, 290-295.

[16] Choi S., Chase T., Bartha R. *Enzymatic catalysis of mercury methylation by Desulfovibrio desulfuricans LS.* Appl. Environ. Microbiol. **1994**, 60, 1342-1346.

[17] Choi S., Chase T., Bartha R. *Metabolic pathways leading to mercury methylation in Desulfovibrio desulfuricans LS.* Appl. Environ. Microbiol. **1994**, 60, 4072-4077.

[18] Ekstrom E.B., Morel F.M.M., Benoit J.M. *Mercury methylation independent of the acetyl-coenzyme a pathway in sulfate-reducing bacteria.* Appl. Environ. Microbiol. **2003**, 69, 5414-5422.

[19] King J.K., Saunders F.M., Lee R.F., Jahnke R.A. *Coupling mercury methylation rates to sulfate reduction rates in marine sediments.* Environ. Toxicol. Chem. **1999**, 18, 1362-1369.

[20] King J.K., Kostka J.E., Frischer M.E., Saunders F.M. *Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments.* Appl. Environ. Microbiol. **2000**, 66, 2430-2437.

[21] King J.K., Kostka J.E., Frischer M.E., Saunders F.M., Jahnke R.A. *A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria.* Environ. Sci. Technol. **2001**, 35, 2491-2496.

[22] Wood J.M. *Biological cycles for toxic elements in the environment*. Science **1974**, 183, 1049-1052.

[23] Reisinger K., Stoeppler M., Nürnberg H.W. *Evidence for the absence of biological methylation of lead in the environment.* Nature **1981**, 291, 228-230.

[24] Thayer J.S. *Biological methylation of less-studied elements.* Appl. Organomet. Chem. **2002**, 16, 677-691.

[25] Rapsomankis S., Donard O.F.X., Weber J.H. *Methylation of tin(II) and lead(II) in sediment by carbanion donors*. Appl. Organomet. Chem. **1987**, 1, 115-118.

[26] Craig P.J., Rapsomanikis S. *Methylation of tin and lead in the environment: oxidative methyl transfer as a model for environmental reactions.* Environ. Sci. Technol. **1985**, 19, 726-730.

[27] Ahmad I., Chau Y.K., Wong P.T.S., Carty A.J., Taylor L. *Chemical alkylation of lead (II) salts to tetraalkyllead (IV) in aqueous solution*. Nature **1980**, 287, 716-717.

[28] Wong P.T.S, Chau Y.K., Luxon P.L. *Methylation of lead in the environment*. Nature **1975**, 253, 263-264.

[29] Walton A.P., Ebdon L., Millward G.E. *Methylation of inorganic lead by Tamar estuary (UK) sediments*. Appl. Organomet. Chem. **1988**, 2, 87-90.

[30] Pongratz R., Heumann K.G. *Production of methylated mercury, lead, and cadmium by marine bacteria as a significant natural source for atmospheric heavy metals in Polar Regions*. Chemosphere **1999**, 39, 89-102.

[31] Pongratz R., Heumann K.G. *Production of methylated mercury and lead by polar macroalgae – a significant natural source for atmospheric heavy metals in clean room compartments*. Chemosphere **1998**, 36, 1935-1946.

[32] Jackson J.A., Blair W.R., Brinckman F.E., Iverson W.P. *Gas-chromatographic speciation of methylstannanes in the Chesapeake Bay using purge and trap sampling with a tin-selective detector*. Environ. Sci. Technol. **1982**, 16, 110-119.

[33] Donard O.F.X., Quevauviller Ph., Bruchet A. *Tin and organotin speciation during wastewater and sludge treatment processes*. Water Res. **1993**, 27, 1085-1089.

[34] Pécheyran C., Quetel C.R., Martin Lecuyer F.M., Donard O.F.X. *Simultaneous determination of volatile metal (Pb, Hg, Sn, In, Ga) and nonmetal species (Se, P, As) in different atmospheres by cryofocusing and detection by ICPMS*. Anal. Chem. **1998**, 70, 2639-2645.

[35] Feldmann J., Koch I., Cullen W.R. *Complementary use of capillary gas chromatography–mass spectrometry (ion trap) and gas chromatography–inductively coupled plasma mass spectrometry for the speciation of volatile antimony, tin and bismuth compounds in landfill and fermentation gases*. Analyst **1998**, 123, 815-820.

[36] Quevauviller P., Donard O.F.X., Wasserman J.C., Martin F.M., Schneider J. *Occurrence of methylated tin and dimethyl mercury compounds in a mangrove core from Sepetiba Bay, Brazil.* Appl. Organomet. Chem. **1992**, 6, 221-228.

[37] Tiigrul S., Balkas T.I., Goldberg E.D. *Methyltins in the marine environment.* Mar. Pollut. Bull. **1983**, 14, 297-303.

[38] Amouroux D., Tessier E., Donard O.F.X. *Volatilization of organotin compounds from estuarine and coastal environments*. Environ. Sci. Technol. **2000**, 34, 988-995.

[39] Yonezawa Y., Fukui M., Yoshida T., Ochi A., Tanaka T., Noguti Y., Kowata T., Sato Y., Masunaga S., Urushigawa Y. *Degradation of tri-n-butyltin in Ise Bay sediment*. Chemosphere **1994**, 29, 1349-1356.

[40] Craig P.J. *Organometallic Compounds in the Environment.* Wiley & Sons: West Sussex, England, 2003.

[41] Craig P.J. *Organometallic Compounds in the Environment.* Longman: Essex, England, 1986.

[42] Hempel M., Kuballa J., Jantzen E. *Discovery of a transalkylation mechanism – identification of ethylmercury^{+} at a tetraethyllead-contaminated site using sodiumtetrapropylborate, GC-AED and HPLC-AFS.* Fresenius J. Anal. Chem. **2000**, 366, 470-475.

[43] Merian E. *Metalle in der Umwelt. Verteilung, Analytik und biologische Relevanz.* Chemie: Weinheim, Deerfield Beach, Florida, Basel, 1984.

[44] Hamasaki T., Sato T., Nagase H., Kito H. *The mutagenicity of organotin compounds as environmental pollutants.* Mutat. Res. **1993**, 300, 265-271.

[45] Food standards Australia New Zealand. *Mercury in fish.* **2004**.

[46] Canadian food inspection agency. Fact sheet. *Mercury and fish consumption.* **2002**.

[47] *Opinion of the scientific panel of the European Food Safety Authority on contaminants in the food chain on a request from the Commission related to mercury and methylmercury in food.* The European Food Safety Authority J. **2004**, 34, 1-14.

[48] Thompson J.J., Houk R. S. *Inductively coupled plasma mass spectrometric detection for multielement flow injection analysis and elemental speciation by reversed-phase liquid chromatography*. Anal. Chem. **1986**, 58, 2541-2548.

[49] Vela N.P., Caruso J.A. *Potential of liquid chromatography – inductively coupled plasma mass spectrometry for trace metal speciation. Invited lecture.* J. Anal. At. Spectrom. **1993**, 8, 787-794.

[50] Liu Y., Lopez-Avila V., Alcaraz M., Beckert W.F. *Simultaneous determination of organotin, organolead and organomercury compounds in environmental samples using capillary gas chromatography with atomic-emission detection.* J. High Resolut. Chromatogr. **1994**, 17, 527-536.

[51] Uden P.C. *Element-specific chromatographic detection by atomic absorption, plasma atomic emission and plasma mass spectrometry.* J. Chromatogr. A **1995**, 703, 393-416.

[52] Liang L., Horvat M., Bloom N.S. *An improved speciation method for mercury by GC/CVAFS after aqueous phase ethylation and room temperature precollection.* Talanta **1994**, 41, 371-379.

[53] Blake E., Raynor M.W., Cornell D. *On-line capillary supercritical fluid chromatography – inductively coupled plasma mass spectrometry for the analysis of organometallic compounds.* J. High. Res. Chromatogr. **1995**, 18, 33-37.

[54] Kumar U.T., Vela N.P, Caruso J.A. *Multi-element detection of organometals by supercritical fluid chromatography with inductively coupled plasma mass spectrometric detection.* J. Chromatogr. Sci. **1995**, 33, 606-610.

[55] Olesik J.W., Kinzer J.A., Olesik S.V. *Capillary electrophoresis inductively coupled plasma spectrometry for rapid elemental speciation.* Anal. Chem. **1995**, 67, 1-12.

[56] Dabek-Zlotorzynska E., Lai E.P.C., Timerbaev A.R. *Capillary electrophoresis: the state-of-the-art in metal speciation studies.* Anal. Chim. Acta **1998**, 359, 1-26.

[57] Lobinski R., Adams F.C. *Speciation analysis by gas chromatography with plasma source spectrometric detection.* Spectrochim. Acta B **1997**, 52, 1865-1903.

[58] Ebdon L., Hill S., Ward R.W. *Directly coupled chromatography - atomic spectroscopy. Part 1. Directly coupled gas chromatography-atomic spectroscopy. A review.* Analyst **1986**, 111, 1113-1138.

[59] Ebdon L., Hill S., Ward R.W. *Directly coupled chromatography-atomic spectroscopy. Part 2. Directly coupled liquid chromatography-atomic spectroscopy. A review.* Analyst **1987**, 112, 1-16.

[60] Waddell R., Lewis C., Hang W., Hassell C., Majidi V. *Inductively coupled plasma mass spectrometry for elemental speciation: Applications in the new millennium.* Appl. Spectrosc. Reviews **2005**, 40, 33-69.

[61] Peters G.R., Beauchemin D. *Versatile interface for gas chromatographic detection or solution nebulization analysis by inductively coupled plasma mass spectrometry: preliminary results.* J. Anal. At. Spectrom. **1992**, 7, 965-969.

[62] De Smaele T., Moens L., Dams R., Sandra P. *Capillary gas chromatography – ICP mass spectrometry: a powerful hyphenated technique for the determination of organometallic compounds.* Fresenius J. Anal. Chem. **1996**, 355, 778-782.

[63] Heisterkamp M., De Smaele T., Candelone J.P., Moens L., Dams R., Adams F.C. *Inductively coupled plasma mass spectrometry hyphenated to capillary gas chromatography as a detection system for the speciation analysis of organolead compounds in environmental waters.* J. Anal. At. Spectrom. **1997**, 12, 1077-1081.

[64] Bouyssiere B., Szpunar J., Lobinski R. *Gas chromatography with inductively coupled plasma mass spectrometric detection in speciation analysis.* Spectrochim. Acta B **2002**, 57, 805-828.

[65] García Alonso J.I., Encinar J.R. In *Handbook of elemental speciation. Techniques and methodology;* Cornelis R., Caruso J., Crews H., Heumann K., Eds.; Wiley & Sons: West Sussex, England, 2003; pp 163-200.

[66] Heumann K.G. *Isotope dilution mass spectrometry of inorganic and organic substances.* Fresenius Z. Anal. Chem. **1986**, 325, 661-666.

[67] De Bièvre P. *Isotope dilution mass spectrometry: what can it contribute to accuracy in trace analysis?* Fresenius Z. Anal. Chem. **1990**, 337, 766-771.

[68] Heumann K.G. In *Inorganic Mass Spectrometry;* Adams F., Gijbels R., van Grieken R., Eds.; Wiley: London, 1988; pp 301-376.

[69] Heumann K.G. In *Metal speciation in the environment;* Broekaert J.A.C., Güçer Ş., Adams A., Eds.; NATO ASI Series, Vol. G23, Springer-Verlag: Berlin, 1990; pp 153-168.

[70] Encinar J.R. *Isotope dilution analysis for speciation.* Anal. Bioanal. Chem. **2003**, 375, 41-43.

[71] Heumann K.G. In *Handbook of elemental speciation. Techniques and methodology;* Cornelis R., Caruso J., Crews H., Heumann K., Eds.; Wiley & Sons: West Sussex, England, 2003; pp 547-562.

[72] Encinar J.R., Rodríguez-González P., García Alonso J.I., Sanz-Medel A. *Isotopically labelled compounds for validating organometallics speciation analysis.* Trends Anal. Chem. **2003**, 22, 108-114.

[73] Rottmann L., Heumann K.G. *Development of an on-line isotope dilution technique with HPLC/ICP-MS for the accurate determination of elemental species.* Fresenius J. Anal. Chem. **1994**, 350, 221-227.

[74] Yang L., Sturgeon R.E. *Blank correction considerations for isotope dilution and reverse isotope dilution calibration: determination of methylmercury in fish tissue.* J. Anal. At. Spectrom. **2005**, 20, 724-729.

[75] Sargent M., Harrington C., Harte R. *Guidelines for achieving high accuracy in isotope dilution mass spectrometry (IDMS).* The Royal Society of Chemistry: Cambridge, 2002.

[76] Quevauviller P., Donard O.F.X., Bruchet A. *Leaching of organotin compounds from poly(vinyl chloride) (PVC) material.* Appl. Organomet. Chem. **1991**, 5, 125-129.

[77] http://icpms.geology.ucdavis.edu/Instrumentation.html

[78] Krachler M., Radner H., Irgolic K.J. *Microwave digestion methods for the determination of trace elements in brain and liver samples by inductively coupled plasma mass spectrometry.* Fresenius J. Anal. Chem. **1996**, 355, 120-128.

[79] Heumann K.G., Gallus S.M., Rädlinger G., Vogl J. *Precision and accuracy in isotope ratio measurements by plasma source mass spectrometry.* J. Anal. Atom. Spectrom. **1998**, 13, 1001-1008.

[80] Rosal C.G., Momplaisir G.M., Heithmar E.M. *National park aquatic samples: multielement analysis by inductively coupled plasma mass spectrometry.* Winter Conference on Plasma Spectrochemistry, Fort Lauderdale, FL, January 14, **2000**.

[81] Willemsens L.C., van der Kerk G.J.M. *Investigations in the Field of Organolead Chemistry*; Schotanus & Jens: Utrecht, The Netherlands, 1965; pp 59-82.

[82] Brown A.A., Ebdon L., Hill S.J. *Development of a coupled liquid chromatography-isotope dilution inductively coupled plasma mass spectrometry method for lead speciation.* Anal. Chim. Acta **1994**, 286, 391-399.

[83] Richards J.R. *Isotopic composition of Australian leads. I. Preparation of tetramethyllead samples*. Mikrochim. Acta **1962**, 4, 620-627.

[84] Gilman H., Jones R.G. *Reactions of metallic thallium and metallic lead with organic halides.* J. Amer. Chem. Soc. **1950**, 72, 1760-1761.

[85] Van Cleuvenbergen R., Dirkx W., Quevauviller P., Adams F. *Preliminary study on degradation of ionic alkyllead species in water.* Intern. J. Environ. Anal. Chem. **1992**, 47, 21-32.

[86] Quevauviller P., Beatriz de la Calle-Guntiñas M., Maier E.A., Cámara C. *A survey on stability of chemical species in solution during storage: the BCR experience.* Mikrochim. Acta **1995**, 118, 131-141.

[87] Schwarz A. *Entwicklung einer online Kopplungsmethode von GC mit ECD- und ICPMS-Detektion zur Bestimmung biogener leichtflüchtiger bromierter und iodierter Kohlenwasserstoffe in Meerwasser und der Atmosphäre.* PhD thesis, University Mainz, **2003**.

[88] Schwarz A., Heumann K.G. *Two-dimensional on-line detection of brominated and iodinated volatile organic compounds by ECD and ICP-MS after GC separation.* Anal. Bioanal. Chem. **2002**, 374, 212-219.

[89] Slaets S., Adams F., Rodriguez Pereiro I., Lobinski R. *Optimization of the coupling of multicapillary GC with ICP-MS for mercury speciation analysis in biological materials.* J. Anal. At. Spectrom. **1999**, 14, 851-857.

[90] Ruiz Encinar J., Granadillo I.L., García Alonso J.I., Sanz-Medel A. *Isotope ratio measurements using gas chromatography inductively coupled plasma mass spectrometry for the assessment of organolead sources.* J. Anal. Atom. Spectrom. **2001**, 16, 475-480.

[91] Ruiz Encinar J., García Alonso J.I., Sanz-Medel A. *Synthesis and application of isotopically labelled dibutyltin for isotope dilution analysis using gas chromatography-ICP-MS.* J. Anal. At. Spectrom. **2000**, 15, 1233-1239.

[92] Gallus S.M., Heumann K.G. *Development of a gas chromatography inductively coupled plasma isotope dilution mass spectrometry system for accurate determination of volatile element species. Part 1. Selenium speciation.* J. Anal. Atom. Spectrom. **1996**, 11, 887-892.

[93] Rodríguez Martín-Doimeadios R.C., Krupp E., Amouroux D., Donard O.F.X. *Application of isotopically labeled methylmercury for isotope dilution analysis of biological samples using gas chromatography/ICPMS.* Anal. Chem. **2002**, 74, 2505-2512.

[94] Valles Mota J.P., Ruiz Encinar J., Fernández de la Campa M.R., García Alonso J.I., Sanz-Medel A. *Determination of cadmium in environmental and biological reference materials using isotope dilution analysis with a double focusing ICP-MS: a comparison with quadrupole ICP-MS.* J. Anal. Atom. Spectrom. **1999**, 14, 1467-1473.

[95] Witte C., Szpunar-Lobinska J., Lobinski R., Adams F.C. *Determination of trimethyl-lead in rainwater and road dust by capillary GC MIP-AE spectrometry after in situ ethylation and axtraction.* Appl. Organomet. Chem. **1994**, 8, 621-627.

[96] Heumann K.G. *Isotope-dilution ICP–MS for trace element determination and speciation: from a reference method to a routine method?* Anal. Bioanal. Chem. **2004**, 378, 318-329.

[97] Monperrus M., Krupp E., Amouroux D., Donard O.F.X., Rodríguez Martín-Doimeadios R.C. *Potential and limits of speciated isotope-dilution analysis for metrology and assessing environmental reactivity.* Trends Anal. Chem. **2004**, 23, 261-272.

[98] Demuth N., Heumann K.G. *Validation of methylmercury determinations in aquatic systems by alkyl derivatization methods for GC analysis using ICP-IDMS.* Anal. Chem. **2001**, 73, 4020-4027.

[99] Baena J.R., Cárdenas S., Gallego M., Valcárcel M. *Speciation of inorganic lead and ionic alkyllead compounds by GC/MS in prescreened rainwaters.* Anal.Chem. **2000**, 72, 1510-1517.

[100] Van Cleuvenbergen R.J.A., Chakraborti D., Adams F.C. *Occurrence of monoalkyllead species during the speciation of organolead.* Anal. Chim. Acta **1986**, 182, 239-244.

[101] Quevauviller P., Ebdon L., Harrison R.M., Wang, Y. *Certification of trimethyl-lead in an urban dust reference material (CRM 605).* Appl. Organomet. Chem. **1999**, 13, 1-7.

[102] Quevauviller P., Ebdon L., Harrison R.M., Wang Y. *Certifying the contents of trimethyllead in an artificial rainwater reference material.* Analyst **1998**, 123, 971-976.

[103] *Decision №1825 of 1st October 1999*. Cabinet of Ministers of Ukraine, Kiev, **1999**.

[104] Chakraborti D., Dirkx W., Van Cleuvenbergen R.J.A., Adams F.C. *Speciation of ionic alkyllead in potable water and soil.* Sci. Total Environ. **1989**, 84, 249-257.

[105] Leal Granadillo I.A., García Alonso J.I., Sanz-Medel A. *Determination of the speciation of organolead compounds in airborne particulate matter by gas chromatography–inductively coupled plasma mass spectrometry*. Anal. Chim. Acta **2000**, 423, 21-29.

[106] http://www.sharkproject.org/index.php

[107] Moens L., De Smaele T., Dams R., Broeck P., Sandra P. *Sensitive, simultaneous determination of organomercury, -lead, and -tin compounds with headspace solid phase microextraction capillary gas chromatography combined with inductively coupled plasma mass spectrometry.* Anal. Chem. **1997**, 69, 1604-1611.

[108] Centineo G., González E.B., Sanz-Medel A. *Multielemental speciation analysis of organometallic compounds of mercury, lead and tin in natural water samples by headspace-solid phase microextraction followed by gas chromatography-mass spectrometry.* J. Chromatogr. A **2004**, 1034, 191-197.

[109] Parkinson D., Bruheim I., Christ I., Pawliszyn J. *Full automation of derivatization – solid phase microextraction – gas chromatography – mass spectrometry with a dual-arm system for the determination of organometallic compounds in aqueous samples.* J. Chromatogr. A **2004**, 1025, 77-84.

[110] Jitaru P., Infante H.G., Adams F.C. *Simultaneous multi-elemental speciation analysis of organometallic compounds by solid-phase microextraction and multicapillary gas chromatography hyphenated to inductively coupled plasma – time-of-flight – mass spectrometry.* J. Anal. At. Spectrom. **2004**, 19, 867-875.

[111] http://www.gerstel.com/supplies_twister_d.htm

[112] Baltussen E., Sandra P., David F., Cramers C. *Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles.* J. Microcolumn Separations **1999**, 11, 737-747.

[113] Snell J.P., Quétel C.R., Lambertsson L., Qvarnström J. *Preparation and certification of ERM-AE670, a ^{202}Hg enriched methylmercury isotopic reference material.* J. Anal. At. Spectrom. **2004**, 19, 1315-1324.

[114] Rodríguez-González P., García Alonso J.I., Sanz-Medel A. *Development of a triple spike methodology for validation of butyltin compounds speciation analysis by isotope dilution mass spectrometry. Part 2. Study of different extraction procedures for the determination of butyltin compounds in mussel tissue CRM 477.* J. Anal. At. Spectrom. **2004**, 19, 767-772.

[115] Ceulemans M., Witte C., Lobinski R., Adams F.C. *Simplified sample preparation for GC speciation analysis of organotin in marine biomaterials.* Appl. Organomet. Chem. **1994**, 8, 451-461.

[116] Chau Y.K., Yang F., Brown M. *Evaluation of derivatization techniques for the analysis of organotin compounds in biological tissue.* Anal. Chim. Acta **1997**, 338, 51-55.

[117] Taylor P., Emons H. *Certificate of analysis, ERM-AE670.* European Commission - Joint Research Centre, Institute for Reference Materials and Measurements, **2004**.

[118] Qvarnström J., Frech W. *Mercury species transformations during sample pre-treatment of biological tissues studied by HPLC-ICP-MS.* J. Anal. At. Spectrom. **2002**, 17, 1486-1491.

[119] Szpunar J., Schmitt V.O., Lobinski R., Monod J.L. *Rapid speciation of butyltin compounds in sediments and biomaterials by capillary gas chromatography-microwave-induced plasma atomic emission spectrometry after microwave-assisted leaching / digestion.* J. Anal. At. Spectrom. **1996**, 11, 193-199.

[120] Tseng C.M., de Diego A., Martin F.M., Donard O.F.X. *Rapid and quantitative microwave-assisted recovery of methylmercury from standard reference sediments.* J. Anal. At. Spectrom. **1997**, 12, 629-635.

[121] Rapsomanikis S. *Derivatization by ethylation with sodium tetraethylborate for the speciation of metals and organometallics in environmental samples. A review.* Analyst **1994**, 119, 1429-1439.

[122] De Smaele T., Moens L., Dams R., Sandra P., Van der Eycken J., Vandyck J. *Sodium tetra(n-propyl)borate: a novel aqueous in situ derivatization reagent for the simultaneous determination of organomercury, -lead and -tin compounds with capillary gas chromatography – inductively coupled plasma mass spectrometry.* J. Chromatogr. A **1998**, 793, 99-106.

[123] Zufiaurre R., Pons B., Nerín C. *Speciation of trimethyllead in rainwater by gas chromatography – mass spectrometry after ethylation with sodium tetraethylborate. Comparison with other alkylation methods.* J. Chromatogr. A **1997**, 779, 299-306.

[124] García Fernández R., Montes Bayón M., García Alonso J.I., Sanz-Medel A. *Comparison of different derivatization approaches for mercury speciation in biological tissues by gas chromatography / inductively coupled plasma mass spectrometry.* J. Mass Spectrom. **2000**, 35, 639-646.

[125] Morabito R., Massanisso P., Quevauviller P. *Derivatization methods for the determination of organotin compounds in environmental sample.* Trends Anal. Chem. **2000**, 19, 113-119.

[126] Monperrus M., Rodríguez Martín-Doimeadios R.C., Scancar J., Amouroux D., Donard O.F.X. *Simultaneous sample preparation and species-specific isotope dilution mass spectrometry analysis of monomethylmercury and tributyltin in a certified oyster tissue.* Anal. Chem. **2003**, 75, 4095-4102.

[127] Morabito R., Massanisso P., Camara C., Larsson T., Frech W., Kramer K.J.M., Bianchi M., Muntau H., Donard O.F.X., Lobinski R., McSheehy S., Pannier F., Potin-Gautier M., Gawlik B.M., Bowadt S., Quevauviller P. *Towards a new certified reference material for butyltins, methylmercury and arsenobetaine in oyster tissue.* Trends Anal. Chem. **2004**, 23, 664-676.

[128] Donais M.K., Uden P.C., Schantz M.M., Wise S.A. *Development, validation, and application of a method for quantification of methylmercury in biological marine materials using gas chromatography atomic emission detection.* Anal. Chem. **1996**, 68, 3859-3866.

[129] Landaluze J.S., de Diego A., Raposo J.C., Madariaga J.M. *Methylmercury determination in sediments and fish tissues from the Nerbioi-Ibaizabal estuary (Basque Country, Spain).* Anal. Chim. Acta **2004**, 508, 107-117.

[130] Magi E., Di Carro M., Rivaro P. *Analysis of butyltin compounds by gas chromatography - mass spectrometry: an application to the Antarctic bivalve Adamussium colbecki.* Appl. Organometal. Chem. **2004**, 18, 646-652.

[131] Rodríguez Martín-Doimeadios R.C., Monperrus M., Krupp E., Amouroux D., Donard O.F.X. *Using speciated isotope dilution with GC-inductively coupled plasma MS to determine and unravel the artificial formation of monomethylmercury in certified reference sediments.* Anal. Chem. **2003**, 75, 3202-3211.

[132] Horvat M., Bloom N.S., Liang L. *Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Part 1. Sediments*. Anal. Chim. Acta **1993**, 281, 135-152.

[133] Huang J.H. *Artifact formation of methyl- and ethyl-mercury compounds from inorganic mercury during derivatization using sodium tetra(n-propyl)borate.* Anal. Chim. Acta **2005**, 532, 113-120.

[134] Craig P.J., Moreton P.A. *The role of speciation in mercury methylation in sediments and water.* Environ. Pollut. B **1985**, 10, 141-158.

[135] Hammerschmidt C.R., Fitzgerald W.F. *Formation of artifact methylmercury during extraction from a sediment reference material.* Anal. Chem. **2001**, 73, 5930-5936.

[136] Rosenkranz B., Bettmer J., Buscher W., Breer C., Cammann K. *The behaviour of different organometallic compounds in the presence of inorganic mercury (II): transalkylation of mercury species and their analysis by the GC-MIP-PED system.* Appl. Organomet. Chem. **1997**, 11, 721-725.

[137] Weber J.H. *Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment.* Chemosphere **1993**, 26, 2063-2077.

[138] Qvarnström J., Lambertsson L., Havarinasab S., Hultman P., Frech W. *Determination of methylmercury, ethylmercury, and inorganic mercury in mouse tissues, following administration of thimerosal, by species-specific isotope dilution GC - inductively coupled plasma-MS.* Anal. Chem. **2003**, 75, 4120-4124.

[139] Hintelmann H., Falter R., Ilgen G., Evans R.D. *Determination of artifactual formation of monomethylmercury (CH_3Hg^+) in environmental samples using stable Hg^{2+} isotopes with ICP-MS detection: calculation of contents applying species specific isotope addition.* Fresenius J. Anal. Chem. **1997**, 358, 363-370.

[140] Rodríguez-González P., Ruiz Encinar J., García Alonso J.I., Sanz-Medel A. *Development of a triple spike methodology for validation of butyltin compounds speciation analysis by isotope dilution mass spectrometry. Part 1. Synthesis of the spike, characterisation and development of the mathematical equations.* J. Anal. At. Spectrom. **2004**, 19, 685-691.

[141] Huo D., "Skip" Kingston H.M. *Correction of species transformations in the analysis of Cr(VI) in solid environmental samples using speciated isotope dilution mass spectrometry.* Anal. Chem. **2000**, 72, 5047-5054.

[142] Mikac N., Branica M., Wang Y., Harrison R.M. *Organolead compounds in mussels (Mytilus galloprovincialis) from the Eastern Adriatic coast.* Environ. Sci. Technol. **1996**, 30, 499-508.

[143] Mikac N., Branica M., Harrison R.M. *Total and organic lead distribution in water, sediment and organisms from the Eastern Adriatic coast.* Chem. Speciat. Bioavail. **2001**, 13, 121-128.

[144] Krishnan K., Marshall W.D., Hatch I. *Ionic alkylleads in salt marsh periwinkles (Littorina irrorata).* Environ. Sci. Technol. **1988**, 22, 806-811.

[145] Codex alimentarius commission. *Joint FAO/WHO food standards programme. Codex committee on food additives and contaminants. General standard for contaminants and toxins in foods.* CX/FAC 05/37/19, the Hague, **2005**.

[146] *Guideline levels for methylmercury in fish.* CAC/GL 7, **1991**.