



Faculty of Food Science

**APPLICATION OF ISOTOPE RATIO
MEASUREMENTS IN THE FIELD OF SPECIATION
ANALYSIS AND IN THE DETERMINATION OF FOOD
PROVENANCE**

THESIS OF THE PHD DISSERTATION OF
MÁRTA ÜVEGES

Supervisors: Dr. Péter FODOR and Dr. Mihály DERNOVICS

Corvinus University of Budapest
Department of Applied Chemistry
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Name : Doctoral School of Food Science

Field : Food Science

Head : Prof. Dr. Péter FODOR, DSc
Department of Applied Chemistry
Faculty of Food Science
Corvinus University of Budapest

Supervisors: Prof. Dr. Péter FODOR, DSc
Department of Applied Chemistry
Faculty of Food Science
Corvinus University of Budapest

Dr. Mihály DERNOVICS, PhD
Department of Applied Chemistry
Faculty of Food Science
Corvinus University of Budapest

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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Signature of Head of School

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Signature of the supervisor

.....
Signature of the supervisor

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1. INTRODUCTION

Mankind has been always concerned about the surrounding nature and the components it is made of. The theory of indivisible atoms changed when the Curie couple discovered the radioactivity and Thomson proved the existence of the electron. Then, it became clear that the atoms consisted of isotopes that possessed the same number of protons but different number of neutrons. After that Urey discovered deuterium that has been applied as tracer in biochemical analysis. Hungarian György Hevesy associated the above mentioned novelties and first developed the technique of radioactive tracers, wherefore he was awarded the Nobel Prize. **Isotopes** have been used in several fields of science and industry since their existence is known.

The so-called ‘organic isotope dilution’ is a prevalent analytical technique in which the molecule is labelled with stable light ‘bioelements’ (such as ^{13}C , D, etc.) and is used for tracing biochemical processes.

In the last years we have seen the application of **isotope dilution** methodologies in some new analytical fields. One of those new fields is **elemental speciation** where the aim is the determination of the individual chemical species in which an element is distributed in a given sample. This technique provides control of the degradation processes during the sample preparation procedure and gives qualitative and quantitative information of the demanded elemental species in the sample. Since isotope dilution analysis (IDA) is an absolute method there is no need to resort to a calibration procedure in order to obtain quantitative results. Once the complete isotope equilibration between the sample and the spike has been achieved, it is only necessary to determine precisely the isotope ratio in the spiked sample. In this manner more accurate and precise results can be obtained and the limit of detection can be improved.

In the case when the concentration of the demanded elemental species in the sample is low the IDA can provide good solution. It is also the case when **butyltin compounds** have to be determined in environmental samples. Tributyltin (TBT) and its degradation products (monobutyltin, MBT and dibutyltin, DBT) are known as major agents in antifouling paints of ship hulls and serve toxic effects on aquatic organisms in very low concentration (few *ppt*). Thus the European Union included these compounds in the list of priority substances in the field of water policy (EU Decision No. 2455/2001/EC). IDA provides accurate and precise results even if the concentration of these compounds is in the low *ppt* range in the sample.

Besides the IDA, there are other analytical techniques that are based on stable isotope ratio measurements such as the method of isotope ratio variation in nature. With the help of this technique global natural processes can be tracked (e.g. precipitation processes, greenhouse effect or migration of animals).

Nowadays, the application fields of this technique are getting wider. Agriculture and food industry are the fields where the number of applications is increasing. It is due to the fact that applying stable isotope ratio measurements the provenance of food, raw material or feed can be determined and the possible adulterations could be revealed. The good quality and the knowledge of the geographical origin of food and agricultural products are getting to be more and more important for the consumer and for other members of the market.

Besides the light bioelements, the heavy geoelements (e.g. Sr and Pb) can also be applied for the determination of geographical origin. The technique based on strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotope ratio measurements exploits the fact that the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio varies from field to field and from continent to continent depending on the geological circumstances. There are studies in the literature investigating the geographical origin of among others wine, rice, meat or dairy products.

Such a diverse application of isotopes can be assigned to the rapidly improving analytical techniques like the Inductively Coupled Plasma Mass Spectrometry (ICPMS). **ICPMS** provides sensitive and surpassingly precise and accurate determination of elements and isotopes. Due to the complex nature of the above mentioned analysis (butyltin and $^{87}\text{Sr}/^{86}\text{Sr}$ measurements) ICPMS has to be hyphenated to other analytical techniques in order to be able to get over some limitations and gain advantages (matrix effects; preconcentration and selective separation of the species). Hyphenated techniques provide among others better limit of detection, selective determination of the species and more reliable isotope ratio results.

2. OBJECTIVES

- After studying the literature I was led to the conclusion that in the GC-ICPMS technique used for the determination of butyltin compounds dry plasma generates, thus the optimization of the working parameters is complicated. My first objective was to optimize the working parameters of the GC-ICPMS (first used in Hungary) and to investigate the followings:
 - Which parameters have the greatest effect on the signal in dry plasma conditions,
 - Could the xenon (Xe) be applied as a standard for the optimization procedure of dry plasma conditions,
 - Is the xenon a good indicator of the tin signal and could be applied as an internal standard during the determination of tin compounds?

- As to my knowledge there are no method developed in Hungary for the determination of butyltin compounds. Thus my aim was to investigate the suitability of the above mentioned GC-ICPMS system for the determination of butyltin compounds in freshwater environmental samples, derive from Hungary, using isotope dilution technique for quantification.
- Moreover, my aim was also to estimate the butyltin contamination in the Castropol bay (North Spain, Europe) and that to find out whether the GC-ID-ICPMS technique can be applied as a routine analytical method for the determination of butyltin compounds in seawater, sediment and biological sample.
- The quality control of the analytical measurements is an indispensable task, thus my aim was to prepare a test solution stored in different kind of conditions and to investigate the stability of butyltin compounds.
- Since the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio can be applied for the determination of geographical origin of food products my aim was to improve the performance of the manually operated hyphenated FI-Sr/matrix separation-MC-ICPMS system. The objectives were the followings:
 - Automation of the hyphenated system,
 - Reduction the amount of sample and solvent used,
 - Improvement the performance the flow injection (FI) system.
- In order to investigate the suitability of the improved hyphenated system to measure precise and accurate $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio in real samples, I intended to study the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio in asparagus samples collected from different crop lands such as Hungary and Austria (Marchfeld).

3. MATERIALS AND METHODS

3.1. Materials and methods applied in speciation analysis

3.1.1. Samples

The seawater samples (seawater, sediment and biota) were collected in the Castropol bay (North Spain, Europe) the estuarine of the river Eo and the Cantabrian sea, while the freshwater samples (sediment and biota) derived from the river Danube between Dunaföldvár and Paks.

3.1.2. Reagents

All solvents and reagents were of analytical-reagent grade. Deionised water ($R > 18 \text{ M}\Omega$), glacial acetic acid and methanol was used in the extraction procedure. The ethylation of the butyltin species was performed using 2 % (w/v) sodium tetraethyl borate dissolved in 0.2 M NaOH aqueous solution. The derivatized analytes were extracted into hexane.

The following standards were used during the work: MBT (monobutyltin-trichloride, 95 %), DBT (dibutyltin-dichloride, 96 %), TBT (tributyltin-chloride, 96 %), TeEtT (tetraethyltin, 97 %), spike solution containing a mixture of MBT, DBT and TBT enriched in ^{119}Sn , certified reference materials: BCR CRM 646 and BCR CRM477.

For the optimization of system parameters 1 % xenon (Xe) in argon (Ar) gas was used.

3.1.3. Sample preparation

A 100 ml of acidified, non-filtered seawater was measured in all-glass volumetric flask and mixed with 100 μl ^{119}Sn enriched spike solution. It was shaken for 15 min manually, then the pH was set to 5.4 with acetate buffer and 200 μl , 2 % w/v sodium tetraethyl borate was added for the ethylation of the organotin compounds. Immediately 1 ml of hexane was added and the flask was shaken manually for 10 min. Finally, the organic layer were transferred to a 2-ml chromatographic vial and the hexane phase was evaporated under a gentle stream of nitrogen until a few microliters and about 1 μl of this final volume was injected into the GC-ICP-MS system.

A weight of 0.25 g of the freeze-dried sediment sample was measured in a microwave vessel and spiked with 100 μl spike solution. Then, 4 ml of an acetic acid and methanol 3:1 mixture was added as extraction solution. The final slurry was exposed to microwaves at 90 W for 4 min. 250 μl of the liquid phase of the digested sample was ethylated in a 7-ml vial using 4 ml of buffer and 0.5 ml of NaBEt_4 . Immediately 1 ml of hexane was added and the vial was shaken manually for 10 min. The organic layer was transferred to a 2-ml vial and stored at $-18 \text{ }^\circ\text{C}$ in the dark until measurement. Finally, about 1 μl of the final volume was injected into the GC-ICP-MS system.

The lyophilized biological samples were spiked with 100 μl spike solution and after adding 5 ml of a mixture of acetic acid and methanol the vials were introduced in a thermostatic bath at $37 \text{ }^\circ\text{C}$ for at least 2 h under mechanical shaking. Then, 300 μl of the extractant was ethylated as described above and after derivatisation the organic layer was transferred to a 2-ml vial and stored at $-18 \text{ }^\circ\text{C}$ until measurement. The hexane phase was preconcentrated under a gentle stream of nitrogen, 1 μl of the final volume was injected into the GC-ICP-MS system.

3.1.4. Instrumentation

(1) GC-ICPMS, where GC type: Varian Model 3400, while ICPMS type: HP-4500, (2) GC-ICPMS, where GC type: Agilent 6890, while ICPMS type: Agilent 7500ce; Liolab freeze-dryer, Model Digiterm 100 thermal bath, Model 1200 microwave oven.

3.2. Materials and methods applied in strontium isotope ratio measurement

3.2.1. Samples

Five Hungarian and five Austrian asparagus (*Asparagus officinalis*) samples were investigated. The Austrian asparagus samples derived from the Marchfeld region while the exact crop fields of Hungarian samples were unknown.

3.2.2. Reagents

All solvents and reagents were of analytical-reagent grade. Deionised water ($R > 18 \text{ M}\Omega$) and double sub-boiled cc. HNO_3 was used for the standard preparation. Strontium-specific resin with a particle size ranging from 100 to 150 μm was used for all separation procedures. The following standards were used during the work: NIST SRM 987 SrCO_3 and 1000 mg/g Rb stock solution.

3.2.3. Sample preparation

The digestion of asparagus samples is described in detail elsewhere (Swoboda et al, 2008. Analytical and Bioanalytical Chemistry, 390 487-494.)

3.2.4. Instrumentation

Automated FI-Sr/matrix separation-MC-ICPMS hyphenated system.

4. RESULTS

4.1 Optimization the working parameters of GC-ICPMS

I fulfilled the optimization of the hyphenated Agilent 6890 GC and 7500ce ICPMS. 1 % xenon (Xe) containing argon gas served as tuning gas during the investigation. It was found that the ion generation and ion transmitting parameters have the greatest effect on the Xe signal, and that Xe

is a good indicator for tin during tin speciation measurement, thus can be applied as internal standard for tin.

4.2. Determination of butyltin compounds in environmental samples

I estimated the level of contamination in the Castropol bay (North Spain, Europe). Seawater, sediment and biota samples were investigated regarding to the butyltin content determined by GC-ID-ICPMS. Due to the mass discrimination phenomenon rises in ICPMS, the tin isotope ratios measured in the spiked samples were corrected mathematically. The analytical performance was also investigated and the quality control of the method was fulfilled by means of certified reference materials.

Besides the existing contamination sources the water of the Castropol bay represented low butyltin concentration. The butyltin content of the sediment samples showed possible local contaminations mainly in the area of old marina. It was also proved that the oyster samples presented the highest bioaccumulation among the biological samples investigated.

Since to the best of my knowledge the butyltin concentration of Hungarian freshwater samples (sediment and biota) was only investigated by the International Commission for the Protection of the Danube River (ICPDR) in 2001 and 2007, my aim was to use the GC-ICPMS (4.1.) operating with the already optimized working parameters in order to determine the butyltin content of freshwater sediment and mussel samples collected from the river Danube. Due to the mass discrimination phenomenon rises in ICPMS, the tin isotope ratios measured in the spiked samples were corrected mathematically. The analytical performance was also investigated and the quality control of the method was fulfilled by means of certified reference materials.

It was found that the butyltin content measured overlapped with the concentrations published in the literature, although taking into account the pattern of the three species in the samples it can be seen that the level of the MBT exceeds the level of the other two components in each type of sample.

4.3. Investigation the stability of the butyltin compounds

In order to investigate the stability of butyltin compounds I prepared a mix test solution from butyltin standards. I divided the test solution into the following sub-samples:

(1) three samples measured in the first day (initial measurements), (2) samples stored in the fridge at 4 °C, and (3) samples stored in room temperature. During the 24-day-storage the samples were measure 6 times.

In the light of the results it can be concluded that both types of storage are convenient to preserve the stability of the components. No increasing or decreasing tendency could be observed in the butyltin concentrations during the analysis. Thus the average concentrations of the species in the test solution are the followings [ng(Sn)/g]:

$$\text{MBT: } 55,1 \pm 1,7; \quad \text{DBT: } 81,2 \pm 1,9; \quad \text{TBT: } 118,8 \pm 3,7;$$

The test solution is suitable for being a laboratory reference material or can serve as a test sample in an interlaboratory exercise.

4.4. Improvement of the manually operated FI-Sr/matrix separation-MC-ICPMS hyphenated system for the determination of strontium isotope ration in asparagus samples

My aim was to improve the performance of the manually operated hyphenated FI-Sr/matrix separation-MC-ICPMS system assembled by VIRIS Laboratory (BOKU, Vienna, Austria), in order to decrease time, amount of sample and solvent needed for the analysis of asparagus samples. As a result of the experiment I first attained the automation of the hyphenated system; harmonized operation of the units was achieved. The performance of the matrix separation was also increased by changing the parameters of the matrix separation column. In this way the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio could be accurately measured even in a sample containing as high Rb/Sr ratio as 100/1.

Next, those parameters that can affect the precision and accuracy of the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio, such as the spectral and matrix effects, were determined by means of an isotope reference material. The performance of the analytical technique was checked and also it was investigated whether the mathematical corrections on the isotope ratio have any effect on the result.

In the light of the results it is proved that the spectral and matrix interferences and the phenomenon of mass discrimination have to be taken into account in order to obtain accurate and precise $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio.

Finally the improved, automated FI-Sr/matrix separation-MC-ICPMS hyphenated system was used to measure the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio in the Hungarian and Austrian asparagus samples. The results show that the samples can be distinguished based on the measured (and corrected) $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio and that, the Austrian asparagus samples might grow on geographically different soil than the Hungarian asparagus samples. This statement is also true for the samples collected in Hungary since the values of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio between Hungarian asparagus samples differ greatly and in some cases show significant deviation.

5. NEW SCIENTIFIC ACHIEVEMENTS (THESES)

1. I investigated the reliability of 1 % xenon containing argon gas, used as chromatographic carrier gas, as an indicator of tin signal in dry plasma conditions. In the light of the results it can be concluded that among the ICPMS parameters, the ion generating and ion transmitting parameters affected the most the Xe signal in dry plasma conditions. Xenon is suitable for the optimization of ICPMS working parameters and can be used as an internal standard for tin during tin speciation analysis. Although, it was observed that mixed carrier gas (1 % xenon containing argon gas) caused 60 % signal suppression during gas chromatographic determination of the tin species, thus its application should be avoided or limited only to the interval of optimization.
2. I found out that the optimized GC-ICPMS method, combined with isotope dilution (ID) analysis for the quantification, is suitable for tin speciation in freshwater environmental samples derived from the Danube, Hungary. It was proved that the GC-ID-ICPMS method provided suitable limit of detection for all three butyltin species (MBT, DBT and TBT) and accurate and precise isotope ratio results. Moreover, the butyltin concentrations determined in certified reference materials met with the requirements. The butyltin concentration measured in the freshwater sediment and mussel samples showed good agreement with the concentrations published in the literature, although taking into account the pattern of the three species in the samples it can be seen that the level of the MBT exceeds the level of the other two components in each types of sample.
3. Levels of butyltin compounds were evaluated for the first time in the estuary of the river Eo (Northwest Spain). It was found that the seawater of the Castropol bay shows low butyltin concentration, besides the contamination sources existing in the bay. Although, 20 % of the seawater samples exceeded the 3 ng(Sn)/kg TBT limit recommended by the USEPA. In most of the seawater samples I measured $R_{TBT/DBT}$ ratio from 2 to 6, which gives information about the degradation status of TBT to DBT, while in those samples, taken from the vicinity of the military boat, this value increased to the range of 7-14, that suggest novel contamination in that area. It was also proved that the butyltin content of the sediment samples were in the *ng/kg* range, but one of the samples pointed out possible local contaminations mainly in the area of the old marina. The levels of butyltin species measured in the biological samples reflect to the fact that oyster samples show the highest bioaccumulation among the other biota samples.

4. The suitability of a method for the determination of butyltin compounds may be proved with interlaboratory tests. For this reason I prepared mixed test solution made of butyltin standards and stored in different temperatures. It was found that both storage conditions are convenient to preserve the stability of the components during 24-day-storage. No increasing or decreasing tendency was observed in the butyltin concentrations during the experiment. It was statistically estimated that there is no difference between the concentrations measured for each compounds in the measurement days. Based on these results it can be stated that the test solution is suitable for being a laboratory reference material or can serve as a test sample in an interlaboratory exercise.

5. The automation of the manually operated hyphenated FI-Sr/matrix separation-MC-ICPMS system assembled by VIRIS Laboratory was first attained. Harmonized operation of the units was achieved integrating appropriate external devices. The performance of the matrix separation was increased by changing the parameters of the home-made column. Among the columns designed, the one contained 40 μl volume size provided the best matrix separation properties in order to get sufficient $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio results. With the application of this column the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio could be accurately measured even in a sample containing as high Rb/Sr ratio as 100/1, applying longer wash out time (from 4 min to 7 min). After these achievements the amount of sample and solvent used during the measurement could be decreased.

6. In the light of the results of my research it was proved that the automated FI-Sr/matrix separation-MC-ICPMS system provided accurate and precise $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio, thus the Hungarian and Austrian asparagus samples could be distinguished from each other based on their $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio. By means of the measured $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios it was expected that the Austrian asparagus samples supposedly grew on geographically different soil than the Hungarian asparagus samples, thus these samples could be statistically discriminated. This statement is also true for the samples collected in Hungary since the values of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio between Hungarian asparagus samples differ greatly and in some cases show significant deviation. Finally, it was observed that the significant decrease in the strontium signal intensity with time is due to the degradation of the matrix separation column.

6. LIST OF PUBLICATION RELATED TO THE DISSERTATION

Articles published in journals with impact factor

2007 **Márta Üveges**, Pablo Rodríguez-González, J. Ignacio García Alonso, Alfredo Sanz-Medel, Péter Fodor, Isotope dilution analysis mass spectrometry for the routine measurement of butyltin compounds in marine environmental and biological samples, *Microchemical Journal*, 85 (2007) 115-121 (2008 ISI-impact factor: 1.800); **Number of citations (June 2009): 3**

Márta Üveges, László Abrankó and Péter Fodor, Optimization of GC-ICPMS system parameters for the determination of butyltin compounds in Hungarian freshwater origin sediment and mussel samples, *Talanta*, 73 (2007) 490–497. (2008 ISI-impact factor: 3,374); **Number of citations (June 2009): 3**

2008 Patrick Galler, Andreas Limbeck, **Márta Üveges**, Thomas Prohaska, Automation and miniaturization of an on-line flow injection Sr/matrix separation method for accurate, high throughput determination of Sr isotope ratios by MC-ICP-MS, *Journal of Analytical Atomic Spectrometry* 23 (2008), 1388–1391. (2008 ISI-impact factor: 3,269); **Number of citations (June 2009): 1**

Publications in national conference in

Proceedings:

2004 **Üveges Márta**, Investigation the kinetics of Solid Phase Microextraction (SPME), *MÉTE XV. OTDK, Budapest, 2004 (oral presentation)*

Abstract:

2008 Csiki Tímea, Vezmár Albert, Dr. Garay Ferenc, Farkas Gyula, **Üveges Márta**, Fülöp Julianna, Dr. Náráy Miklós, Significance of interlaboratory exercises in the reliability control of the measurement methods, *Magyar Munkahigiénés és Kémiai Biztonsági Tudományos Társaság XXVI. Nemzeti Kongresszusa, Orosháza-Gyopárosfürdő, 2008 (oral presentation)*

Publications in international conference in

Abstract:

2004 Csilla Soeroes, Richard Schäffer, László Abrankó, Zsuzsa Jókai, **Márta Üveges**, Mihály Dernovics, Peter Fodor, The role of As, Hg, Se and Sn speciation in food analysis, 2nd *Central European Congress on Food, Budapest, 2004 (poster)*

2005 **Márta Üveges**, László Abrankó, Péter Fodor, Characterization of a new analytical hyphenated technique: GC-splitting effluent-FID/ICP-MS for the determination of pesticides, *European Winter Conference on Plasma Spectrochemistry, Budapest, 2005 (poster)*

Márta Üveges, Pablo Rodríguez-González, J. Ignacio García Alonso, Alfredo Sanz-Medel, Péter Fodor, Isotope Dilution Analysis Mass Spectrometry for the routine measurement of butyltin compounds in marine environmental and biological samples, *XII Hungarian-Italian Symposium on Spectrochemistry, Pécs, 2005 (oral presentation)*

2006 **Márta Üveges**, László Abrankó, J. Ignacio García Alonso, Péter Fodor, Optimization of GC-ICPMS for the determination of organotin compounds in Hungarian freshwater environmental samples, *13th Biennial National Atomic Spectroscopy Symposium Glasgow, Scotland, 2006 (poster)*

Márta Üveges, Pablo Rodríguez-González, Giuseppe Centineo, J. Ignacio García Alonso, Organización de un ejercicio de intercomparación para la determinación de compuestos organoestannicos, *3th Reunión de la Sociedad Española de Espectrometría de Masas, Oviedo, Spanyolország 2006 (poster)*

2007 **Márta Üveges**, László Abrankó, and Péter Fodor, Investigation of dry plasma conditions in GC-ICPMS coupled system, *European Winter Conference on Plasma Spectrochemistry, Taormina, Italy, February 18-23, 2007 (poster)*