

GC/ICP-MS

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Speciation of Organotin Compounds in Biological Tissues by GC/ICP-MS Using the NexION 300D/350D

Introduction

The environmental concerns regarding organotin compounds are of great importance due to their extensive use

for agricultural, industrial and domestic applications.¹ Butyl- and phenyl-tins have been widely used as active biocides in applications such as antifouling paints, PVC stabilizers, timber treatment, and others.² For example, the use of antifouling paints resulted in extensive damage to non-target organisms at ultratrace concentration levels (ppt) and accumulation in sediments and biota.³⁻⁴ As a result, the European Union (EU) added tributyl tin (TBT) and its degradation products to the list of priority pollutants (Decision 2455/2001/EC amending the Water Framework Directive 2000/60/EC).⁴ The U.S. Environmental Protection Agency (EPA) established the ambient aquatic-life water quality criteria for TBT in which the criterion to protect marine aquatic life from chronic toxic effects is 3 ng Sn/L and from acute toxic effects 172 ng Sn/L.⁴ The different toxicity levels between TBT, triphenyl tin (TPHT) and their degradation products (di- and monosubstituted and inorganic tin,) as well as the monitoring of their environmental impact, makes organotin speciation analysis necessary.⁵

Nowadays, the method of choice for trace organometals analysis is gas chromatography coupled with inductively coupled plasma mass spectrometry (GC/ICP-MS) due to its high sensitivity, selectivity, multielemental and multiisotopic capabilities.³ To carry out organotin speciation via GC/ICP-MS, a derivatization reaction with sodium tetrapropylborate or sodium tetraethylborate is necessary to increase the volatility of the species. In addition, for the analysis of complex matrices, such as biological tissues

and sediments, a soft extraction step is required prior to the derivatization of the organotins in order to preserve the original species of the analytes. Open focused microwave extraction is the most popular extraction procedure because it is fast and efficient.³ The work presented here encompasses the GC/ICP-MS (Clarus® GC and NexION® ICP-MS) speciation analysis of organotins, more specifically, mono-, di-, and tri-butyl-tin species in biological samples via external calibration. Special attention is given to the optimization of the GC transfer line parameters, which allows the coupling of the GC and ICP-MS.

Experimental Conditions

Reagents and Chemicals

Stock solutions of monomethyltin trichloride (MMT, 97%), dimethyltin dichloride (DMT, 97%), trimethyltin chloride (TMT, 97%), monobutyltin trichloride (MBT, 95%), dibutyltin dichloride (DBT, 97%), and tributyltin chloride (TBT, 96%) were purchased from Sigma-Aldrich™ and prepared in glacial acetic acid (HAC) (GFS Chemicals). Sodium tetrapropylborate (NaBPr₄) (Merseburger Spezialchemikalien, Germany) was used for derivatization. The buffer solution (0.1M, pH 4.9) used during the derivatization was prepared by dissolving sodium acetate and glacial acetic acid (HAC) in MQ water. HPLC-grade isooctane and Optima-grade methanol (MeOH) were obtained from Thermo Fisher Scientific™. High-purity ammonium hydroxide (NH₄OH), nitric acid (HNO₃), hydrochloric acid (HCl) and tetramethylammonium hydroxide (TMAH; 25%) were obtained from GFS Chemicals. Reference material CRM 477 Mussel Tissue, obtained from the Institute for Reference Materials and

Measurements (IRMM; Geel, Belgium), and an oyster tissue sample from the Arcachon Bay in France were evaluated in this experiment.

Instrumentation

Chromatographic analysis was performed using a PerkinElmer® Clarus 580 Gas Chromatograph coupled to a NexION 300D ICP Mass Spectrometer detector by means of a PerkinElmer GC/ICP-MS transfer line (Figure 1). The Silcosteel® tube transfer line, equipped with an inner deactivated capillary column, was inserted into the quartz torch and injector. Optimization of the operational parameters (i.e. distance of the inner deactivated capillary column, Ar makeup and oxygen (O₂) flow rates) necessary to work under this dry plasma configuration were evaluated. An SP-D Discover focused microwave system (CEM Corporation, Matthews, North Carolina, USA) was used for the extraction of the organotins from the matrices.

The operating conditions are listed in Table 1. Data collection and analysis was accomplished using NexION ICP-MS and Chromera® CDS software.



Figure 1. Clarus 580 GC (right) connected to NexION 300D ICP-MS (left) via GC transfer line.

Table 1. GC/ICP-MS operating conditions.

GC Parameters (Clarus 580)	Conditions
Column:	Elite-5MS (5%Diphenyl-dimethylpolysiloxane) (30 m, id 0.25 mm, d.f. 0.25 µm)
Injection port:	Splitless
Injection port temperature:	250 °C
Injection volume:	1.0 µL
He carrier gas flow (mL/min):	2.0
Transfer line temperature:	300 °C
Transfer line capillary:	Elite - Siltek deactivated fused-silica (id 0.25 mm)
Transfer line capillary position:	7 cm back from tip of Silcosteel® tube
Oven program:	50 °C → ramp 10 °C/min → 100 °C → ramp 45 °C/min → 290 °C (3 min)
ICP-MS Parameters (NexION 300)	Conditions
RF power:	1600 W
Nebulizer flow rate:	0.90 L/min
Auxiliary gas flow rate:	1.2 L/min
Plasma gas flow rate:	15 L/min
Oxygen gas flow rate:	0.025 L/min
Injector diameter:	1.2 mm i.d.
Isotope/dwell times:	Sn: 118, 119, 120 (30 ms) N: 15 (30 ms)

Sample Preparation

The sample preparation, microwave extraction and derivatization procedures were presented previously in the literature.³⁻⁴ Stock solutions (1000 µg/mL) of the methylated and butylated tin solutions were prepared in concentrated HAc. Working solutions were prepared fresh daily in 1% HCl by appropriate dilution of the stock solutions and stored in the refrigerator. Calibration curves were generated by triplicate injections of the standards solutions. For the derivatization procedure, the acetate buffer solution (0.1 M, pH 4.9) was prepared by weighing approximately 4.4 g of sodium acetate and 1.0 mL of HAc and dissolving with MQ water to make 500 mL. In addition, 2.5% NaBPr₄ solution of the derivatizing agent was prepared in MQ water and stored in the dark.

Microwave Extraction

For the microwave extraction of the organotin compounds from the mussel and oyster tissue, 0.15 g of dry sample was added to separate 10 mL extraction vials. Then, 4 mL of 25% TMAH was added to the biological tissues. A small magnetic stir bar was added to each vial in order to fully homogenize the resulting slurry during the extraction process. After microwave digestion, the samples were centrifuged (if necessary), and the supernatants transferred to clean vials and stored in the refrigerator until analysis. The operational conditions of the microwave program used are listed in Table 2.

Derivatization Procedure

The derivatization step was performed in 20 mL glass vials. First, 5 mL of the acetate buffer solution was added to 20, 50, 100 and 200 µL of a 5 ng/mL working standard solution containing the organotin species; the pH of each solution was then adjusted to 5.0 using HCl or NH₄OH, if necessary. Next, 1 mL isooctane and 250 µL of 2.5% NaBPr₄ was added and the vial capped and manually shaken for 5 minutes. The organic phase was then transferred to a GC vial for analysis. Three blank solutions were prepared in the same manner for detection limit determination and to characterize any contamination likely to occur during the preparation step. In the case of the biological tissue, 5 mL of the buffer solution was added to 30 and 300 µL of the mussel tissue and oyster tissue extract, respectively, and the pH of each solution was adjusted to 5.0 using HCl or NH₄OH. Then, 1 mL isooctane and 500 µL of 2.5% NaBPr₄ were added, the vial capped and manually shaken for 5 minutes. The organic phase was transferred to a GC vial for analysis. Figure 2 shows a summarized flow chart of the sample preparation procedure employed throughout this work.

It is very important to work under clean conditions for successful analysis at low concentrations. The containers were washed with detergent, followed by successive HNO₃ and HCl baths (10% v:v in MQ water) with MQ water rinses between steps. Once the cleaning procedure was finished, the containers were dried under a laminar flow hood and stored until use.

Table 2. Optimized microwave extraction conditions

CEM Microwave (SP-D Discover)	Conditions
Power:	75 W
Ramp time:	1.0 min
Temperature:	70 °C
Hold time:	4.0 min

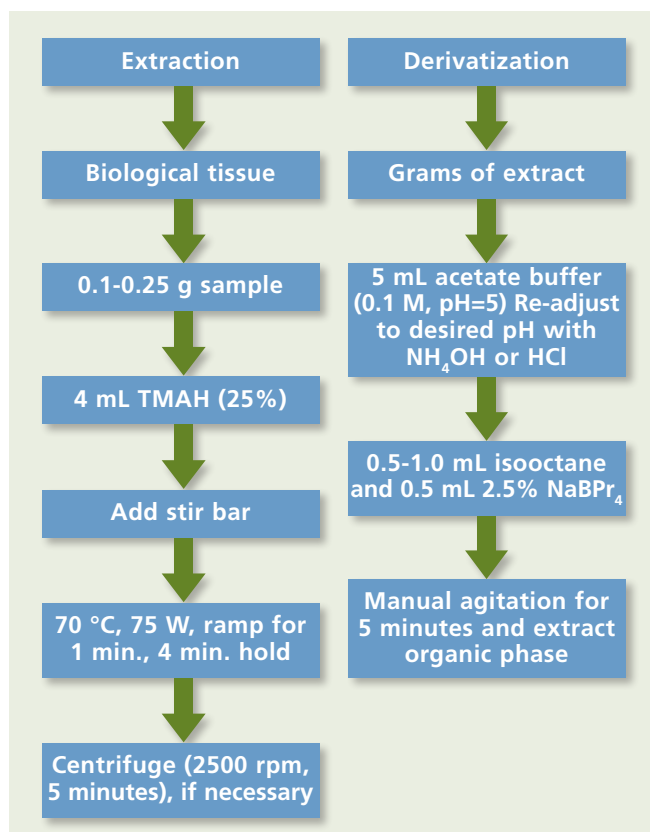


Figure 2. Flow chart of the extraction and derivatization procedures used.

Results and Discussion

GC Transfer Line Optimization

Optimization of the operational parameters related to the GC transfer line and ICP-MS torch, such as the distance of the inner capillary column, the Ar makeup gas flow rate (i.e. the nebulizer gas) and the addition of oxygen gas (O_2), were evaluated using the continuous signal of the nitrogen (N_2) impurities (isotope 15) from the helium (He) carrier gas. The addition of O_2 is necessary to prevent the deposition of carbon on the ICP-MS sampling cone⁵, resulting from the isooctane. Although the $^{15}N^+$ response was used for the optimization here, the addition of xenon/argon gas mixture (50 ppm) has been employed previously.⁶

The GC transfer line provided by PerkinElmer has been designed to be used with the Clarus or AutoSystem™ GCs and can be coupled to either the NexION or ELAN® ICP-MS platforms. The GC transfer line temperature is controlled by the Clarus GC system to maintain the analytes in the gas phase and prevent cold spots in the transfer line.⁷ The Ar makeup gas flow rate is controlled from the ICP-MS nebulizer control and is heated as it enters the transfer line. It is important to achieve sufficient flow to efficiently transfer the analytes into the plasma. The O_2 gas, added through a "T", also passes through the transfer line. Once the setup is complete and the plasma ignited, the $^{15}N^+$ response is measured and parameters optimized. From previous work, the optimum values for the addition of O_2 gas and the position of the inner deactivated capillary column (relative to the end of the Silcosteel® tube in the transfer line) were determined to be 0.025 L/min and 7 cm, respectively.⁶ The Ar makeup gas flow rate (with the O_2 set at 0.025 L/min) was then optimized by varying the flow rate between 0.8-1.0 L/min until a maximum $^{15}N^+$ response was observed. Once the optimum conditions were determined, injection of organic solvent (e.g., isooctane, hexane, acetone etc.) was performed to confirm that the analyte reaches the plasma. Figure 3 shows the typical response of

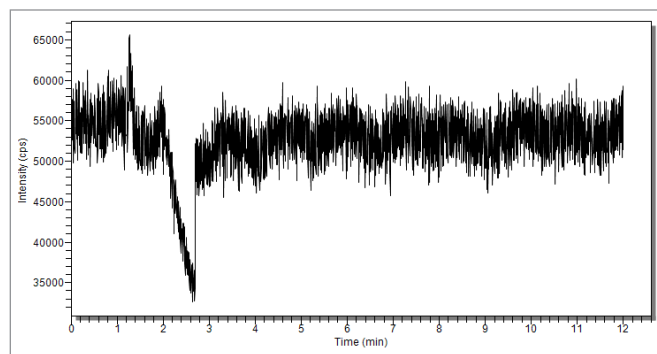


Figure 3. Typical $^{15}N^+$ signal for an isooctane solvent injection.

the $^{15}N^+$ isotope signal for an injection of isooctane. The suppression of the $^{15}N^+$ baseline signal at 2.5 min corresponds to the combustion of the organic solvent in the plasma and thus confirms efficient GC gas flow penetration into the plasma. The optimum conditions for the GC/ICP-MS system are listed in Table 1 (Page 2).

Analytical Performance

The analytical response characteristics were determined for the butylated tin standard solutions. The calibration curves were generated for the Sn isotopes (118, 119 and 120) responses for the butyl-tin compounds through triplicate injections of 1 μ L across a concentration range from 0 (i.e. analytical blank) to 2 ng/mL in isooctane. Good linearity and satisfactory coefficients of correlation (R^2 values) were observed for the ^{120}Sn response functions (Figure 4). The limits of detections ($LODs = 3\sigma_{blank}/m$) were determined from each calibration response function, and absolute LODs of 18, 200 and 7 fg were obtained for MBT, DBT and TBT as ^{120}Sn , respectively. The high absolute LOD value observed for DBT is probably due to the high blanks values which are related to contamination from the containers and reagents used in the chemical preparation procedure. This is usually observed since DBT is used in the industrial synthesis of plastics.

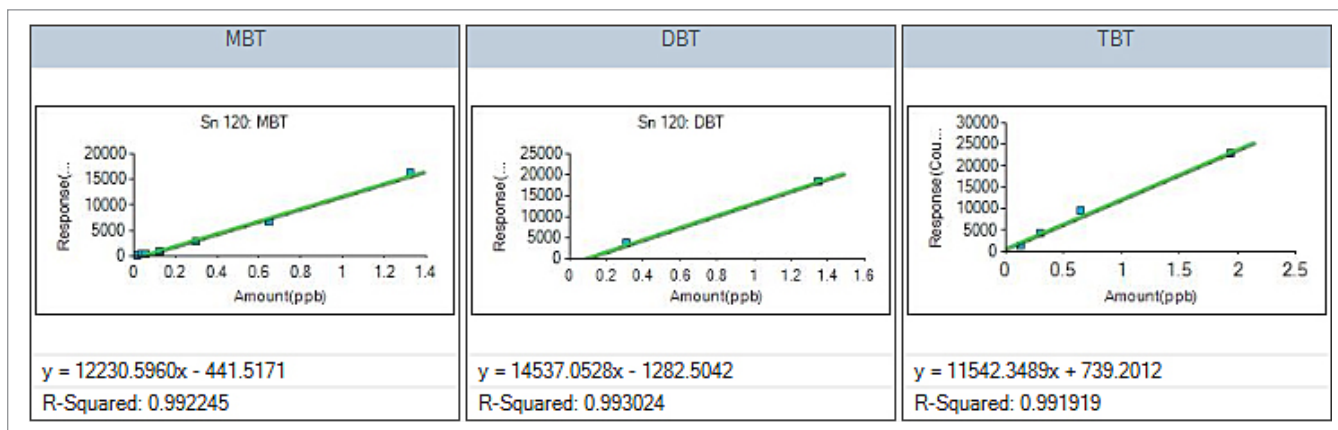
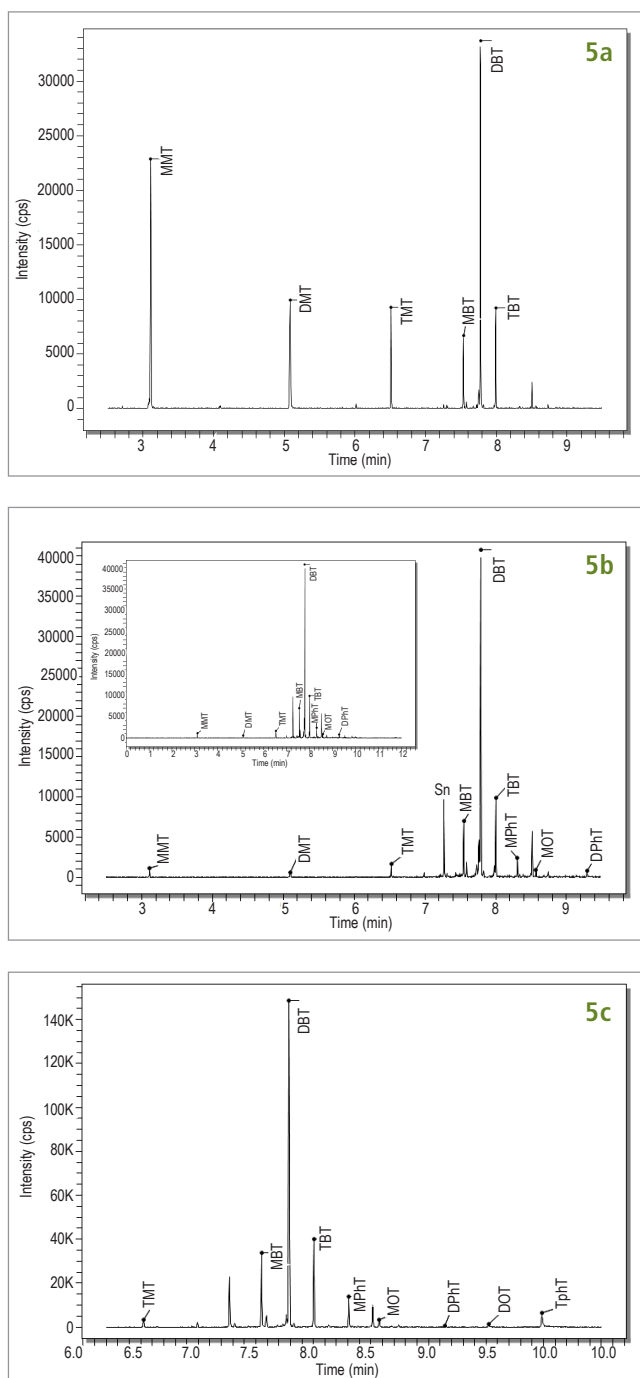


Figure 4. Typical $^{15}N^+$ calibration curves for Sn ($^{120}Sn^+$).



Figures 5a-5c. Chromatographic separation for the organotin species (^{120}Sn isotope) for a 0.25 ng/mL standard in isooctane (5a), oyster tissue (5b), and mussel tissue reference material (5c).

Chromatographic Separation and Method Validation

Figures 5a-c show the chromatographic separation for the organotin species (^{120}Sn isotope) in a 0.25 ng/mL standard in isooctane, oyster tissue and mussel tissue reference material, respectively. Multiple Sn isotopes (118, 119, 120) were monitored during method development, but only results for ^{120}Sn are presented for simplicity. The chromatographic conditions (Table 1) were chosen in a way that elution of the species was away from the zone disturbed by the solvent elution.¹ Quantification results by external calibration were obtained for the butyl-tin compounds present in the biological tissue samples. Triplicate extraction of the mussel tissue reference material was used for the validation of this methodology. The certified values for MBT, DBT and TBT were compared to the experimental values obtained, and the results are shown in Table 3. Table 3 also shows the concentration values obtained for the butyl-tin species present in the oyster tissue sample, although these compounds were not certified. The temperature program listed in Table 1 provided good separation for the organotin compounds in less than 11 minutes with peak widths of 2-3s. Figure 5a shows the chromatogram obtained under the optimum conditions for a 0.25 ng/mL standard in isooctane containing methyl-tin and butyl-tin species at mass 120. As seen in Figures 5b-c, the chromatographic separation of the oyster and mussel tissues also shows the presence of additional organotin species (monooctyl-, dioctyl-, monophenyl-, diphenyl- and triphenyltin) usually observed in environmental samples. All the peaks observed are clearly identified in the chromatograms. The peak around 8.5 minutes is probably due to NaBEt_4 impurities in the NaBPr_4 reagent employed during the derivatization procedure which would form ethylated forms of the organotin species. To corroborate its identity, further work is required.

Table 3. Concentration results for MBT, DBT and TBT (expressed ng/g as Sn) obtained in the analysis of the biological tissues (n=3, triplicate extraction)

	Certified/Indicative Values	Experimental Values	Recovery (%)
CRM 477 - Mussel Tissue			
MBT	1013 ± 189	928 ± 130	92
DBT	785 ± 61	745 ± 90	95
TBT	900 ± 78	953 ± 42	106
Oyster Tissue			
MBT	-	23 ± 3	-
DBT	-	83 ± 7	-
TBT	-	26 ± 1	-

Conclusions

The successful coupling of the GC transfer line between the Clarus GC and the NexION ICP-MS detection system for the analysis of biological tissues was presented here. Response functions for the butyl-tin species with satisfactory linearity were generated, and absolute LODs were determined after optimization of the operational parameters. The GC chromatographic separation for the organotin compounds was applied to the analysis of a mussel tissue reference material and an oyster tissue. The quantification and validation of the butyl-tin species in the biological tissues was carried out by external calibration with recoveries of $\geq 92\%$ and RSDs of $\leq 14\%$.

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