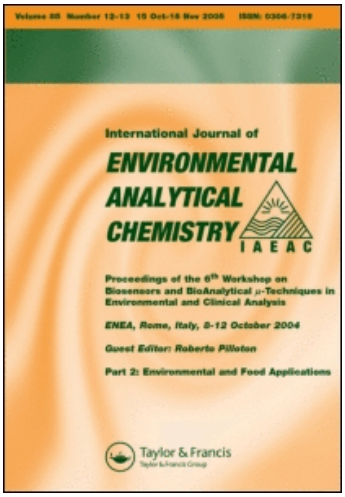

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Schubert, Patricia , Rosenberg, Erwin and Grasserbauer, Manfred(2000) 'Optimization of a Sample Preparation Procedure for the Speciation of Organotin Compounds in Sediment Samples Using GC-AED', International Journal of Environmental Analytical Chemistry, 78: 3, 185 — 204
To link to this Article: DOI: 10.1080/03067310008041341
URL: http://dx.doi.org/10.1080/03067310008041341

Intern. J. Environ. Anal. Chem., Vol. 78(3-4), pp. 185-204
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OPTIMIZATION OF A SAMPLE PREPARATION PROCEDURE FOR THE SPECIATION OF ORGANOTIN COMPOUNDS IN SEDIMENT SAMPLES USING GC-AED

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(Received 15 February 2000; In final form 31 May 2000)

Results of a comprehensive study of all analytical steps involved in the sample preparation procedure for the speciation of butyl- and phenyltin compounds in sediments are presented. The proposed method is based on acid leaching (using aqueous acetic acid) and simultaneous extraction of the ionic species into an organic solvent (n-hexane/ethyl acetate) with the addition of a complexing agent (diethyl dithiocarbamic acid). After evaporation to dryness, the residue is derivatized with sodium tetraethylborate in an aqueous buffer solution (acetate buffer, 0.1 M, pH 5) and extracted into n-hexane. Cleanup is performed over basic alumina and the ethylated organotin species are analyzed with a gas chromatograph coupled to a microwave-induced helium plasma atomic emission detector (GC-AED). The optimized method was validated within an interlaboratory study for the certification of tributyltin, triphenyltin and their degradation products in a freshwater sediment, the BCR candidate reference material 646.

Keywords: Organotin compounds; Speciation; Sediments; GC-AED

INTRODUCTION

Once released into the aquatic environment organotin compounds may undergo a variety of degradation reactions until they finally are adsorbed onto suspended solids and sediments. Sediments are considered to be the ultimate sinks for organotin compounds, thus allowing the evaluation of integrated organotin compound pollution over a larger time scale. In addition to being a record of past contamination with organotin compounds of a specific area, sediments also form

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a potential ecotoxicological risk, arising from the fact that remobilization of adsorbed organotin compounds from the sediment may occur. This remobilization is enhanced e.g. by complexing agents.

Therefore, development of reliable analytical methods for the determination of organotin compounds in sediments is essential. In the past two decades a great number of analytical methods has been introduced for the speciation of organotin compounds which have recently been reviewed [1,2,3].

Commonly used sample preparation procedures for organotin speciation in sediments consist of a variety of successive steps like *(i)* extraction of the analytes from the sample matrix, *(ii)* formation of volatile derivatives (only necessary when using gas chromatographic separation), *(iii)* preconcentration, *(iv)* cleanup and *(v)* analysis. Such multi-step procedures inherently have various sources of errors and, therefore, need to be checked and optimized very carefully by considering each single step.

Respective to point *(i)*, extraction of the analytes from the matrix is considered to be one of the most critical steps in the whole sample preparation procedure due to the limited stability of the analytes and the strong interaction between analytes and matrix. Basic approaches to release organotin compounds from sediments involve acidic leaching in aqueous media or in combination with organic solvents. A great variety of acids and solvents, covering a wide range of polarities, have found their application. In some cases, acidic leaching in aqueous media is followed by a liquid-liquid extraction with a water non-miscible solvent in order to extract the organotin compounds into an aprotic solvent. Addition of complexing agents (e.g. tropolone, diethyl dithiocarbamic acid or pyrrolidine dithiocarbamic acid) or salting out effects increase the solubility of the analytes in the organic solvent. Beside the very time consuming Soxhlet extraction, simple stirring, shaking or sonication are applied as additional mechanic energy input in order to increase extraction efficiencies. Alternative approaches are the use of microwave assisted extraction (MAE)[4,5,6,7], accelerated solvent extraction (ASE)[8]or supercritical fluid extraction (SFE)[1,9]. Abalos *et al.*[1] comprehensively reviewed the procedures applied to speciation analysis of organotin compounds in sediments and biota.

Gas chromatography (GC) seems to be the method of choice for organotin speciation analysis even though other separation techniques like liquid chromatography, capillary electrophoresis or supercritical fluid chromatography have been used. GC methods, however, require to include a derivatization step, which transforms the ionic species into tetrasubstituted organotin compounds in order to provide sufficient volatility for GC separation. Beside hydride generation with sodium borohydride, alkylation with sodium tetraalkylborates ($NaBR_4$ with R = ethyl or propyl) and with Grignard reagents (RMgX with R = methyl, ethyl,

propyl, pentyl or hexyl, and X = usually bromine or chlorine) are commonly used in organotin speciation. Grignard derivatization is considered less matrix dependent than alkylation with $NaBR_4$[10,11] but strict anhydrous conditions and aprotic solvents are required, and more handling steps are involved. Therefore, $NaBR_4$ is often preferred since derivatization can be carried out in aqueous media and liquid-liquid extraction of the tetrasubstituted species into an organic layer is performed simultaneously with the derivatization.

Most analytical procedures for organotin speciation in sediments require an extra cleanup step due to co-extracted substances, which may damage the analytical GC column. The majority of the cleanup procedures are based on adsorption chromatography with different adsorbents like alumina (basic or neutral), florisil or silica gel.

The objective of this work is to critically evaluate a sample preparation procedure for the speciation analysis of butyl- and phenyltin compounds in sediments. The present study is an extension of former investigations on the problems related to multi-step sample preparation procedures for organotin compounds in sediments[10,11,12,13,16]. The proposed procedure is based on a combination of two different sample preparation methods developed by Ceulemans and Adams[16]. A comprehensive study of all analytical steps involved in the sample preparation procedure was carried out in order to reveal problems and sources of errors. The optimized method was validated within an interlaboratory study for the certification of organotin compounds in a freshwater sediment (BCR RM 646) in order to produce a certified reference material (CRM) for tributyltin (TBT), triphenyltin (TPhT) and their degradation products. The certification campaign was organized in summer 1998 by the Standards, Measurements and Testing Program (formerly BCR, Community Bureau of Reference) of the European Commission[11,14].

EXPERIMENTAL

Reagents and materials

Analytical reagent grade chemicals and solvents obtained from Merck (Darmstadt, Germany) and Riedel de Haën (Seelze, Germany), respectively, were used unless stated otherwise. Milli-Q water (Millipore, Molsheim, France) was generally used. Basic alumina (Al_2O_3, for chromatography, type 5016 A) was obtained from Fluka (Buchs, Switzerland) and silanized glass wool from Supelco (Bellefonte, PA, USA). Butyl- and phenyltin compounds as chlorides and as ethylated

standards with purities of >98% were obtained from the Institute of Environmental Studies, IVM, Free University of Amsterdam (The Netherlands). Tetrabutyltin (TeBT) used as internal standard was obtained from Fluka. Stock solutions and dilutions were prepared in methanol by weight and stored at –20°C in the dark. A solution of ethyl magnesium bromide in diethyl ether (2 *M* EtMgBr in DEE) was obtained from Aldrich and sodium tetraethylborate ($NaBEt_4$) from Witco (Bergkamen, Germany). A 0.3% (w/w) aqueous solution of $NaBEt_4$ was freshly prepared before use. Handling of the hygroscopic and air sensitive reagent was performed only under argon. A solution of diethyl dithiocarbamic acid (DDTC) in n-hexane was prepared daily by dissolving 2.25 g of sodium diethyl dithiocarbamate (NaDDTC) in 10 mL of water. After addition of 20 mL of 0.5 *M* H_2SO_4 the free acid was extracted into 10 mL of n-hexane. The acetate buffer (pH 5, 0.1 *M*) was prepared by dissolving 27.2 g of sodium acetate trihydrate (AcONa × 3 H_2O) in 2 L of water and adjusting the pH to 5.00 ± 0.05 with concentrated acetic acid (AcOH, 100%). The buffer solution was stored in the laboratory at room temperature. Preliminary studies were carried out on a SETOC* sediment (SETOC 708, distributed in 1993). The candidate reference material (BCR RM 646, freshwater sediment) was obtained from the Community Bureau of Reference (BCR, Commission of the European Communities, Brussels, Belgium).

Instrumentation

Organotin species were separated on a HP Ultra 2 capillary column (25 m × 0.32 mm i.d. × 0.52 µm film thickness) using a HP Model 5890 Series II gas chromatograph and were detected by means of a HP Model 5921A atomic emission detector (Hewlett-Packard, Avondale, PA, USA). The gas chromatograph was equipped with a split-splitless injection port and an HP Model 7673A autosampler. The GC-AED parameters are listed in detail in Table I.

Procedure

Spiking

Method A (simple method)

1 g of freeze dried sediment sample was weighed in an Erlenmeyer flask and spiked with 100 µL of a mixed standard solution containing the analytes as chlorides (1 µg/mL as tin). The spiked sediment was allowed to stand for 15 min at room temperature before starting the extraction procedure.

* SETOC...International Sediment Exchange for Tests on Organic Contaminants, organized by the Department of Soil and Nutrition of the Wageningen Agricultural University (The Netherlands)

TABLE I GC-AED operating parameters

	HP 5890 Series II gas chromatograph
Injection technique	Splitless (purge delay: 1 min)
Injection volume	1 μL
Inlet temperature	300°C
Column	HP Ultra 2 (25 m × 0.32 mm i.d. × 0.52 μm film thickness) with retention gap (1.5 m × 0.32 mm i.d.)
Column head pressure	100 kPa
Carrier gas	Helium (purity >99.9996%)
Oven program	Init. temp.: 65°C, initial time: 3 min, with 20°C/min to 200°C,
	with 50°/min to 280°C, final time: 3 min (Σ 12.35 min)
	HP 5921A atomic emission detector
Transfer line temperature	280°C
Wavelength	Sn 270.651 nm, Pb 261.418 nm, C 247.857 nm
Hydrogen pressure	500 kPa
Oxygen pressure	70 kPa
Cavity pressure	10 kPa
Helium total flow	250 mL/min
Spectrometer purge flow rate	0.1 L N_2/min
Cavity temperature	280°C
Solvent vent	on: 0 min, off: 4 min

Method B (based on ENEA-method[15]).

Method B is based on the spiking method developed by R. Morabito (ENEA, Rome, Italy) and was adapted to the facilities of our laboratory. Briefly, 1 g of freeze dried sediment sample was weighed in an Erlenmeyer flask, moistened with 2 mL of MeOH and stirred magnetically for 30 min at room temperature without closing the flask. The slurry was then spiked with 2 mL of a methanolic solution containing the analytes as chlorides in the appropriate concentration and the stirring was continued overnight. Excessive MeOH was evaporated with a gentle stream of argon.

Extraction/leaching

Approximately 1 g of sediment was accurately weighed, placed in a 25 mL Erlenmeyer flask and suspended in a mixture of 4 mL of Milli-Q water and 1 mL

acetic acid (AcOH, 100%). After 5 min, 1 mL of DDTC in hexane was added and the solution was blended up to a volume of 25 mL with n-hexane/EtOAc (1:1). The mixture was sonicated for 30 min (Ultrasonic bath: USW 4000, 120–180 W, 29–32 kHz, Gerätebau Brieslang GmbH, Germany) and the organic layer was transferred into a 100 mL narrow-necked extraction vessel by means of a Pasteur pipette. For Grignard derivatization, the organic layers were collected in a 100 mL pointed flask. A second extraction was carried out with another portion of approximately 20 mL n-hexane/EtOAc (1:1) by magnetic stirring (500 rpm) for 30 min and the organic phases were combined. In order to remove the organic layer completely, the residue was placed in a 15 mL centrifugation tube and centrifuged for approximately 1–2 min. The combined organic extracts were evaporated to dryness under vacuum on a rotary evaporator with a water bath temperature between 30 and 35°C.

Derivatization

$NaBEt_4$ derivatization. 50 mL of buffer solution (pH 5, 0.1 *M*) was added to the evaporation residue in the extraction flask immediately after solvent evaporation, together with 100 µL of methanolic internal standard solution (TeBT, 1 mg/L as tin), 1 mL n-hexane and 1 mL of 0.3% $NaBEt_4$ in water. After shaking the mixture manually for 5 min, a second portion of reagent was added. The mixture was again shaken for 5 min and after phase separation the hexane layer was sampled for cleanup/analysis.

Grignard derivatization. The evaporation residue from the extraction was taken up with 1 mL of toluene. 100 µL of internal standard solution (TeBT, 1 mg/L as tin) was added and ethylation was carried out with 2 mL of 2 *M* EtMgBr in diethyl ether (exothermic reaction). The reaction mixture was swirled carefully and was allowed to stand for 20 min. Excess Grignard reagent was destroyed under ice bath cooling by addition of 10 mL of water. The formed hydroxides were dissolved by dropwise adding 37% HCl to the mixture. After phase separation, the aqueous phase was extracted 3 times with 2 mL of n-hexane each and the combined organic layers were passed through a funnel filled with 5 g of Na_2SO_4. The solvent was evaporated to a final volume of 1 mL and was subjected to cleanup before analysis.

Cleanup

A Pasteur pipette (150 mm length) was filled with approximately 1 g of basic alumina (activated at 130°C overnight) to form a plug of 5 cm length. The adsorbent was held in position by means of a small portion of silanized glass wool placed in the narrow part of the pipette. The cleanup column was condi-

tioned with 2 mL of the eluent used before introducing the derivatized sediment extract onto the column. After elution of the analytes with 3 mL of n-hexane/EtOAc (1:1), the extract was preconcentrated to a final volume of 1 mL using a gentle stream of nitrogen and was analyzed by GC-AED. The samples were stored at –20°C in the dark until analysis.

Figure 1 shows a schematic representation of the optimized procedure with $NaBEt_4$ derivatization.

Quantitation

TeBT was used as internal standard to correct for volume changes during the sample preparation procedure and variation of detector sensitivity during GC analysis.

Recovery

All experiments were carried out with 4 replicates each. Calibration samples for the recovery evaluation were prepared daily by derivatizing spiked buffer solutions with a spiking amount equal to that used for the sediment samples. The recoveries were then calculated on the basis of these daily calibrations. The standard deviation of the recovery measurements were calculated by considering both standard deviations of the calibration and the recovery measurements using the error propagation law. Overall recoveries were calculated by comparing the slopes of the graphs from external calibration with the slopes obtained by standard addition.

Linearity

Calibration graphs for the determination of the linearity range of the detector and the overall recovery of the method were obtained by dilution of ethylated standards in hexane and analysis with GC-AED. Since butyltin concentrations were expected to be one order of magnitude higher than those of phenyltin in the freshwater sediment, calibration graphs with nine concentration levels ranging from 0.2 to 5 ng/μL as cation for butyltin compounds and 0.02 to 0.5 ng/μL as cation for phenyltin compounds were prepared. The concentration of the internal standard TeBT (~ 70 pg/μL as tin) was the same in all calibrant solutions. Organotin response on the AED was found to be linear within 10% deviation in the range of 0.2 to 2 ng/μL as cation (= 200–2000 ng/g sediment as cation) for butyltin compounds and 0.02 to 0.2 ng/μL as cation (= 20–200 ng/g sediment as cation) for phenyltin compounds.

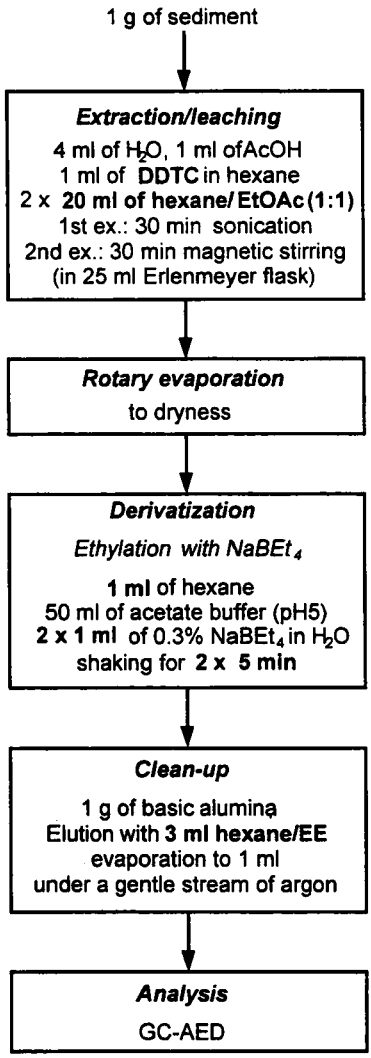

FIGURE 1 Schematic representation of the optimized procedure

Standard addition

Quantitation of organotin compound concentrations in sediments was carried out by standard addition in order to correct for matrix effects and for losses during the sample preparation procedure. The sediment (BCR RM 646) was spiked at 3

equidistant concentration levels (see Table 2) with methanolic standard solutions of the organotin chlorides according to Method B.

TABLE II Comparison of the results from the certification campaign (BCR 1998) of the candidate reference material BCR RM 646 (in ng/g as cation), spike levels used for standard additon (in ng/g as cation), overall recovery (in %) of the procedure applied to the certification campaign (n=5), and relative standard deviations (RSD, in %) of 5 replicates

	External calibration[a] (RSD)	*Standard addition[a] (RSD)*	*Indicative values[b]*	*Spike levels*	*Overall recovery[c]*
MBT	n.e.	n.e.	674 ± 102	40;80;120	< 10
DBT	765 ± 72 (9)	1215 ± 187 (15)[d]	770 ± 117	300;600;900	65
TBT	501 ± 41 (8)	578 ± 43 (7)	491 ± 65	200;400;600	100
MPhT	n.e.	n.e.	74.4 ± 22.5	20;40;60	< 10
DPhT	11.1 ± 1.9 (17)	27.1 ± 3.0 (11)	37.9 ± 9.4	20;40;60	43
TPhT	43.0 ± 5.4 (12)	39.0 ± 4.6 (12)	35.0 ± 5.1	40;80;120	126

n.e.. not evaluated

a. after dilution of the final extract by a factor of 3, evaluated by standard addition at 3 spiking levels (values submitted to the certification campaign) or with external calibration (without recovery correction)
b. preliminary results of the certification campaign (mean of mean values after first outlier correction)
c. evaluated by comparing the slopes of the standard addition graph and the external calibration graph
d. value for DBT was not accepted (declared as outlier)

RESULTS AND DISCUSSION

Since sample preparation procedures for the speciation of organotin compounds in sediments contain many different steps with their individual sources of errors, each step needs to be checked and optimized separately. In order to distinguish between procedure related problems and problems arising from matrix effects, preliminary optimizations were carried out excluding the matrix.

Extraction/Leaching

In most procedures applied to organotin speciation in sediments, leaching of the sediment with acidic solutions (aqueous acid solutions or mixtures of acids and organic solvents) is followed by an extraction step in order to transfer the organotin species into an organic solvent which then can be easily preconcentrated. The

addition of complexing agents like sodium diethyl dithiocarbamate (NaDDTC), ammonium pyrrolidine dithiocarbamate (APDC) or tropolone is mandatory in order to increase extraction yields, especially for mono- and disubstituted organotin compounds. The complexing agent not only increases the solubility of the analytes in the organic phase but may also decrease the matrix-analyte interactions.

First extraction experiments were carried out based on a combination of two procedures proposed by Ceulemans and Adams[16]. DDTC was chosen as complexing agent since it is cheaper compared to tropolone although it needs to be prepared daily due to its lower stability in solution. The two-step extraction with a combination of sonication and magnetic stirring was preferred to a single long extraction step as it seemed more efficient. On one hand sonication increases the interaction between the extraction medium and the sediment, whereas on the other hand the mixing and, thus, partitioning between the aqueous and the organic phase is improved by magnetic stirring.

Solvent polarity

The solvent polarity has a great influence on the solubility of the analyte-DDTC complex as can be seen in Figure 2. When using pure n-hexane as proposed by Ceulemans and Adams[16] for the extraction of the analyte-DDTC complexes from the aqueous AcOH solution, low recoveries of monobutyltin (MBT) and monophenyltin (MPhT) with strongly varying extraction yields were observed. However, Ceulemans and Adams[16] reported a pronounced increase in solubility of the MBT-tropolone complex by increasing the polarity of the organic solvent. Similar results were observed by Abalos et al.[13] who found increased extraction efficiences for tropolone complexes when using toluene instead of n-hexane. Due to the structure of the DDTC, also higher polarity of the organotin complexes can be assumed, which implies that increased solvent polarity should also enhance the solubility of the complex in the organic phase. Therefore, a mixture of hexane/EtOAc (1:1 v/v) was applied to the extraction procedure, which improved the extraction yields tremendously, leading to satisfying recoveries (85–100%) and low relative standard deviations (2–5%) for all investigated organotin compounds.

Solvent volume

In order to reduce the amount of organic solvent used in the extraction procedure, experiments were carried out with 5 mL and 10 mL instead of 20 mL of hexane/EtOAc. The effect of reduced solvent volume is shown in Figure 3. Reduction of the solvent volume to 5 or 10 mL per extraction step turned out to

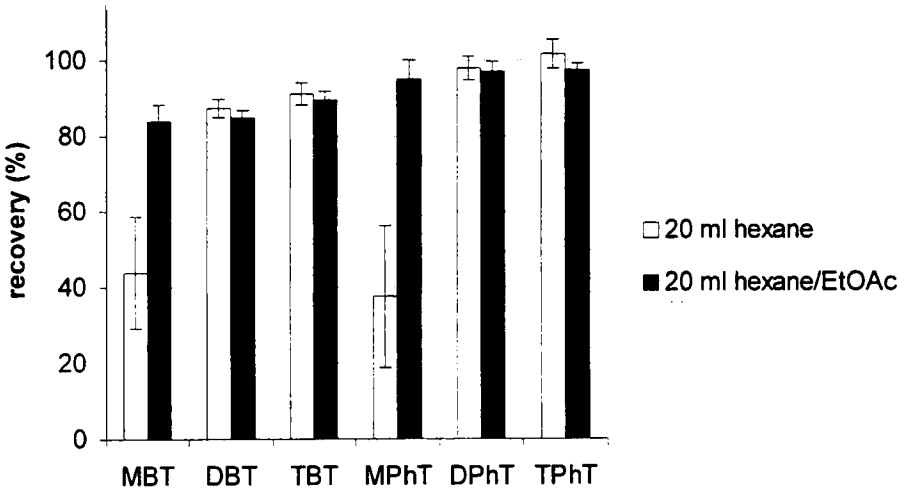

FIGURE 2 Influence of the solvent polarity on the extraction efficiency of the DDTC-organotin complex into the organic solvent

adversely affect the repeatability of the extraction yield, especially for MBT and MPhT. Repeating the extraction with 5 mL a third time using again magnetic stirring slightly improved the repeatability of the monosubstituted organotin compounds, but still extraction with two portions of 20 mL of hexane/EtOAc gave the best results.

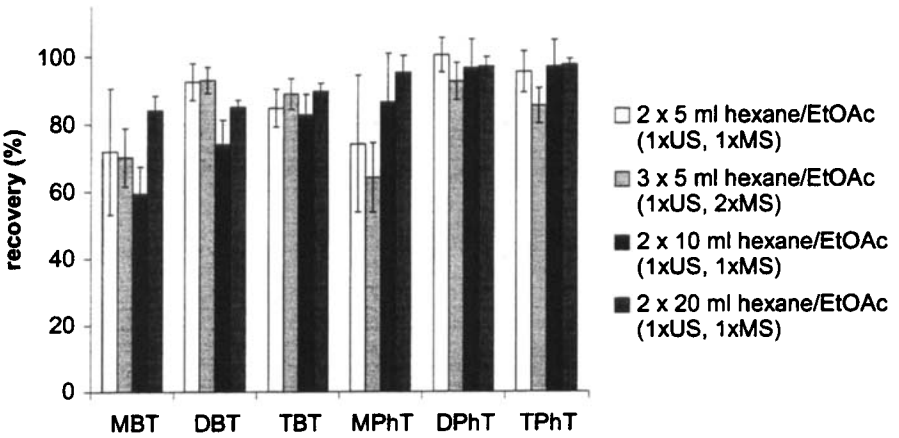

FIGURE 3 Influence of solvent volume on the extraction efficiency (MS...magnetic stirring, US...ultrasonic)

Influence of acid

As organotin compounds are supposed to bind only onto the surface of the sediment, a complete digestion of the material is not considered to be necessary. Most procedures employ acids in order to destroy the carbonates usually present in sediments and to release the analytes from the complex matrix. However, degradation may occur when organotin compounds are exposed to acids. In order to investigate the influence of HCl and AcOH on the extraction yield, 1 mL of concentrated acid (100% AcOH and 37% HCl) was added to the sediment slurry (1 g of sediment wetted with 4 mL of water) resulting in final acid concentrations of ~3 *M* AcOH and ~2 *M* HCl. The results are presented in Figure 4. In the case of dibutyltin (DBT) and TBT significantly lower recoveries, and for MPhT slightly higher results were observed when using HCl instead of AcOH although optimum parameters for HCl according to Ceulemans and Adams[16] were used. These results indicate faster degradation of the analytes when using HCl. While the recoveries of the phenyltin compounds were around 100%, the recoveries of the butyltin compounds were significantly lower, ranging from 75 to 90%.

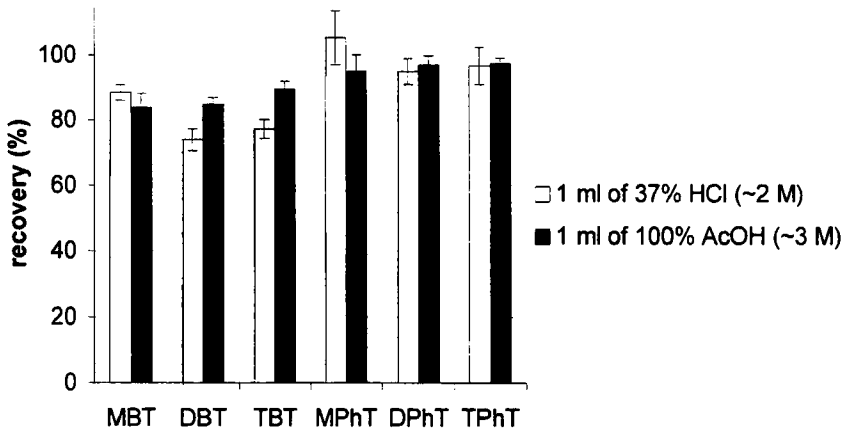

FIGURE 4 Influence of acid type on the analytes during extraction/leaching (1 mL of concentrated acid is added to 4 mL of water)

Preconcentration with rotary vacuum evaporation

Careful handling is necessary during preconcentration of extracts since losses of organotin compounds due to degradation or volatilization may occur either when exposing to high temperatures or evaporating nearly to dryness. The water bath temperature should thus generally be held below 40°C. Furthermore, acid traces

which remain in the organic phase after the extraction/leaching step, may induce degradation processes when evaporating to dryness.

In order to reveal potential losses during the preconcentration step, a pure hexane/EtOAc (1:1) mixture as well as a representative organic phase containing traces of AcOH were spiked and evaporated to dryness. To obtain such a representative organic phase with traces of AcOH, a pure aqueous solution of AcOH (16 mL H_2O + 4 mL AcOH) was extracted according to the proposed extraction/leaching procedure. As Figure 5 shows, preconcentration to dryness without traces of acid in the organic phase has no effect on the recovery of the organotin species, excluding possible losses due to volatilization under the given conditions. But in the presence of acid traces, some degradation of TBT and TPhT in the order of 10 % occurs when the extract is evaporated to dryness. However, considering the problems caused by the matrix effects, these losses are negligible. In order to minimize degradation losses, the buffer solution must be added immediately after preconcentration in order to minimize the time span the analytes are in contact with the acid traces left in the extract residue.

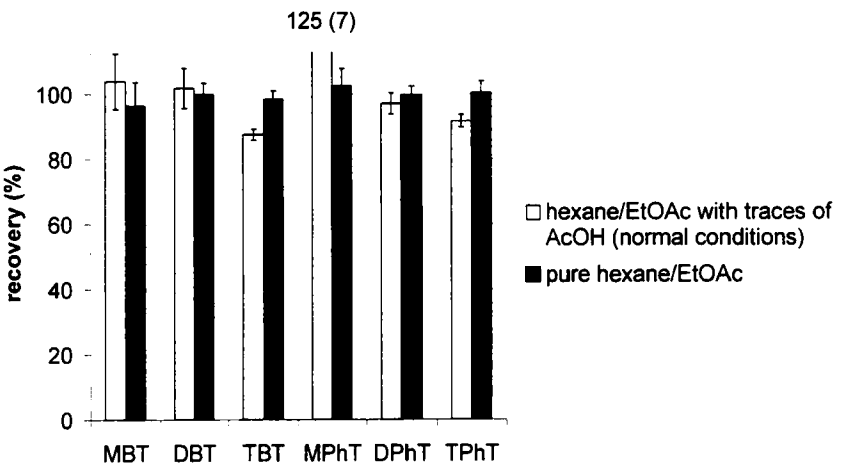

FIGURE 5 Comparison of losses of organotin species during the evaporation of the organic phase to dryness using rotary evaporation without and in the presence of acid traces, respectively

Derivatization

Ethylation with $NaBEt_4$ was preferred to Grignard alkylation since derivatization is carried out in aqueous medium and extraction of the derivatives into a small volume of organic solvent takes place simultaneously. Therefore, fewer analytical steps are involved as drying of the solvent and preconcentration of the final

extract are not necessary. This makes the derivatization step much faster and easier to handle. Both derivatization procedures give comparable results when applied to standard solutions[17], which was also confirmed in our laboratory. According to the experience of other laboratories, Grignard derivatization produces slightly better results when applied to real matrices like biotic material[10] and sediments[11]. However, it has to be stated in this context, that the amount of reagents normally used for derivatization with Grignard and $NaBEt_4$ may differ by a factor of 30 to 200, considering the fact that normally 1 to 2 mL of 2 *M* RMgX solution and only 1 mL of a 0.02 to 0.06 *M* $NaBEt_4$ solution in water (corresponding to 0.3 to 1 % $NaBEt_4$ in water), respectively, are used. The matrix influence on the derivatization observed in this work is discussed in the following. Improvement of repeatability and derivatization efficiency of organotin compounds under matrix influence can be achieved by using successively two portions of reagent for the derivatization with $NaBEt_4$ (see Figures 6 and 7).

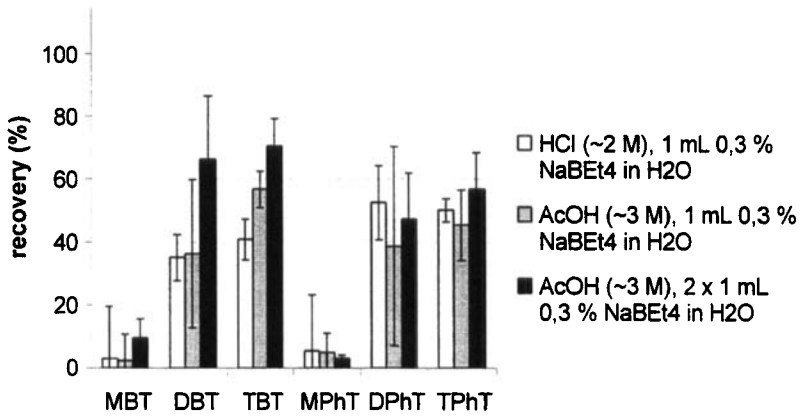

FIGURE 6 Influence of the amount of derivatization reagent on the derivatization in the presence of a sediment matrix (SETOC 708) after extraction with either HCl or AcOH

Linearity range of derivatization with $NaBEt_4$

According to the results of the preliminary intercomparison study for organotin speciation in a freshwater sediment[11], relatively high butyltin concentrations in the range of 100–1200 ng/g as cation (= app. 50–800 ng/g as tin) could be expected since the BCR RM 646 was stated to have a similar content of organotin compounds. Therefore, the linearity of derivatization with $NaBEt_4$ was checked in the range of 100 to 1000 ng as tin for all butyl- and phenyltin compounds by spiking 25 mL of buffer solution with 10 to 100 µL of a 10 µg Sn/mL standard solution in equidistant steps (10 levels) and derivatization as described

(with two portions of reagent, TeBT: 100 ng as tin, 100 µL of a 1 µg Sn/mL solution). The derivatized extracts were diluted by a factor of two (50 µL hexane and 50 µL sample) in order to assure that the linear range of the detector was not exceeded. Derivatization with $NaBEt_4$ was found to be linear in a range of 100 to 1000 ng as tin within 10% deviation for all butyl and phenyltin compounds (except for diphenyltin (DPhT) which was linear in the range of 300 to 1000 ng as tin).

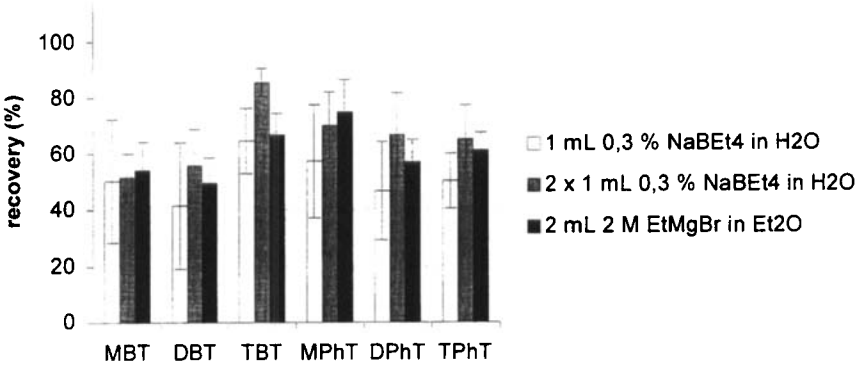

FIGURE 7 Influence of the sediment extract on the derivatization efficiency with $NaBEt_4$ and EtMgBr, respectively, as derivatization reagents

Influence of sediment matrix on organotin recovery

The optimized extraction procedure was applied to a spiked sediment sample (Setoc 708) using the simple spiking method (Method A) as described earlier. Recoveries were evaluated by analyzing the spiked sediment, subtracting the organotin concentrations that are naturally present in the sediment, and comparing the results with those obtained from the analysis of derivatized standard solutions. A very high matrix influence on the recovery of all organotin compounds was observed. While only 40 to 70 % of the di- and trisubstituted tin species were recovered, MBT and MPhT were lost completely (see Figure 6), irrespective of the acid type used. Particularly high standard deviations were observed when leaching was performed with AcOH.

There are two possible explanations for this large scatter of the recoveries: (i) derivatization is strongly inhibited by co-extracted compounds or (ii) desorption of the organotin compounds, especially of MBT and MPhT, from the particular matter is incomplete when using the proposed procedure.

In order to investigate the influence of co-extracted compounds on the efficiency of the derivatization with $NaBEt_4$ and EtMgBr, sediments were extracted

as described, but only the organic extract was spiked after phase separation and centrifugation. As can be seen in Figure 7, the derivatization of all organotin compounds is inhibited to a great extent in the presence of co-extracted compounds resulting in recoveries of 40 to 85%. Especially when using only a single portion of 0.3% $NaBEt_4$ solution, relative standard deviations were extremely high (20–50%). Repeatability of the derivatization with $NaBEt_4$ was improved by addition of a second portion of reagent solution and further shaking for 5 min, resulting in standard deviations of 6 to 23%. Usage of Grignard reagent on the spiked extract did not lead to significant improvement of derivatization efficiencies as observed by other laboratories[10,11].

These findings lead to the conclusion that the derivatization efficiencies of all organotin compounds are strongly affected by co-extracted compounds, but the extreme losses of MBT and MPhT observed when extracting spiked sediments, are mainly caused by incomplete desorption from the sediment matter. Studies on the adsorptive behavior of organotin compounds have indicated that the strength of adsorption obeys the following order: MBT > TBT > DBT[18,19,20,21,22]. TBT adsorption is favored by hydrophobicity and complex formation of the tin atom to charged ligands of humic acids[20] whereas MBT adsorption is mainly driven by polarity and cationic exchange mechanisms[19]. This may explain the high affinity of monosubstituted tin species for particular matter that cannot be easily overcome by the organic solvent mixture used in this study, although a combination of acid and complexing agent was applied in order to overcome the interaction of organotin compounds with the matrix.

Cleanup

Sediment extracts contain a fraction of non-volatile substances which may contaminate the chromatographic column. Such column contaminations lead to a decrease in separation efficiency and/or higher baseline noise which adversely affects the limit of detection, thus making a cleanup step mandatory[12,16]. Most cleanup procedures are based on adsorption chromatography using a variety of different adsorbents like alumina, florisil or silica gel and are carried out after the derivatization step.

Basic alumina was chosen as adsorbent due to its widespread use. Preliminary experiments with 1 g of basic alumina filled in a Pasteur pipette, conditioning and elution with hexane, showed that all ethylated analytes except TPhT were eluted nearly quantitatively in the first 5 mL of hexane, whereas TPhT was not eluted at all under these conditions. TPhT is the compound with the highest polarity among the investigated analytes, indicating that an eluent with higher polarity is needed.

With additional 5 mL of hexane/EtOAc (1:1 v/v) TPhT was also eluted completely from the cleanup column. Therefore, hexane/EtOAc (1:1) was chosen as eluent for further experiments. To evaluate the solvent volume needed for quantitative elution of the analytes, four fractions of 2 mL each were taken with the first fraction consisting of 1 mL of hexane containing the derivatized standards and an additional milliliter of the eluent (hexane/EtOAc = 1:1). As can be seen in Figure 8, all analytes were removed quantitatively from the cleanup column within 3 mL of hexane/EtOAc (1:1). While the butyltin compounds are eluted mainly in the first fraction, the phenyltin compounds, especially TPhT, show higher retention on the column and need a larger solvent volume for elution.

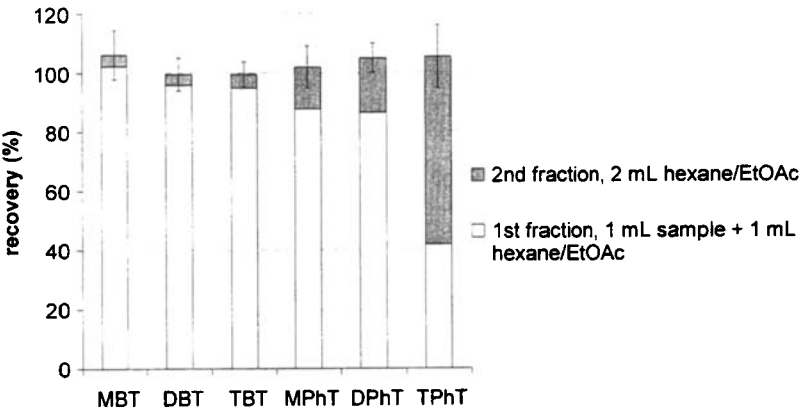

FIGURE 8 Optimization of the solvent volume needed for the quantitative recovery of the analytes from the alumina cleanup column

De la Calle-Guntinas et al.[10] and Ariese et al.[11] mentioned the possibility of losses of ethylated and propylated organotin compounds, when preconcentrated to a volume smaller than 0.3 mL or to dryness. However, we could show that no losses of ethylated species occurred during the evaporation of the extract to 1 mL with a gentle stream of Argon, indicating that even ethylated organotin compounds may be preconcentrated if carefully done e.g. avoiding elevated temperatures and reduction to volumes smaller than 1 mL.

Method validation

The optimized sample preparation procedure (extraction: H_2O/AcOH, DDTC, n-hexane/EtOAc, sonication and magnetic stirring, derivatization: $NaBEt_4$,

cleanup: basic alumina, n-hexane/EtOAc, analysis: GC-AED) was applied to a freshwater sediment (BCR candidate reference material 646). The mean results of 5 independent determinations obtained by standard addition (values submitted for certification) and by external calibration are presented in Table II.

The results of TBT, TPhT and DPhT evaluated with standard addition agree well with the values submitted by the other laboratories and were accepted for certification. The DBT concentration determined in this study, however, was much higher compared to the submitted values of other laboratories. Evaluation of TBT and DBT with external calibration (without recovery correction) showed surprisingly good agreement with the indicative values after outlier correction. This contrasts previous experiments that revealed strong matrix influences on the recovery of all organotin compounds (see Figure 6).

Further investigations were carried out in order to explain the high DBT concentrations found in the proposed procedure. The following three reasons were considered to give possible explanations for the overestimation of DBT: *(i)* decomposition of TBT to DBT and MBT during the sample preparation procedure, *(ii)* exceeding the linear range of detection and *(iii)* exceeding the linear range of derivatization. All laboratories used the same standards for calibrations provided by the organizers which excludes a possible systematic error due to inconsistent calibrants. The potential error sources are discussed in the following:

(i) Decomposition

All sample preparation steps were checked carefully for possible errors due to degradation or losses of organotin species, as described earlier. But during the whole optimization study no DBT overestimation due to decomposition of the parent compound TPhT was observed in any of the investigated steps that could explain the high DBT concentrations found in the candidate reference material. Only during the preconcentration step decomposition of especially the phenyltin compounds was observed with a MPhT overestimation of 25%. Nevertheless, the use of standard addition for quantitation would compensate for such systematic errors.

(ii) Linear range of detection

Working beyond the linearity range of the detector results in standard addition graphs with too small slopes which leads to overestimation. The highest concentration of DBT used for the standard addition experiments (max. 1600 ng/g as cation, if no losses occur during the sample preparation) was near the upper limit of the AED linearity range (<1000–2000 ng/g as cation). In order to exclude pos-

sible effects due to exceeding the linear working range, the standard addition samples were diluted by a factor of 3 (1:2 v/v) and 5 (1:4 v/v) and analysis was repeated. Dilution by a factor of 3 decreased the DBT result drastically from 1965 ± 416 ng/g as cation to 1215 ± 187 ng/g as cation, which strongly indicates that the linear working range was exceeded with the undiluted extracts. TBT showed the same effect as DBT, but less pronounced (decrease from 807 ± 95 to 578 ± 43 ng/g as cation). DPhT and TPhT showed no significant difference between the results determined from the original extracts and the diluted one. Further dilution of the extracts had no significant effect on the DBT results. This indicates that a dilution factor of 3 was enough to shift the standard addition graph into the linear working range of the AED. Therefore, the results obtained with a dilution factor of 3 were submitted. Still the DBT concentration was higher compared to the results of other laboratories and therefore were not accepted for certification.

(iii) Linear range of derivatization

The linearity range of the derivatization has been previously discussed and was found to be given at least in the considered range from 200 to 2000 ng as DBT within 10% deviation. This excludes a concentration-dependent reaction yield in the standard addition experiments as a possible explanation for the higher results of DBT compared to the other laboratories.

CONCLUSION

This study reveals problems and sources of errors that can occur when applying multi-step procedures as generally necessary for the speciation analysis of organotin compounds in sediments. Although each individual step was optimized in this multi-step extraction and derivatization procedure, the extraction efficiency of the monosubstituted organotin compounds was very poor when applied to sediment samples and did not allow their quantitative determination. The proposed method was applied to the determination of di- and trisubstituted organotin compounds in the BCR candidate reference material 646 (freshwater sediment). The values obtained for TBT, TPhT and DPhT by standard addition were in good agreement with the consensus values while the DBT value was significantly overestimated compared to the other laboratories participating in the intercomparison study. No satisfactory explanation has been found for this behavior yet, although it seems that over-correction of the results has led to this overestimation, since the result

of DBT obtained by external calibration would have been in good agreement with the consensus value.

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