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A quantitative extraction method for the determination of trace amounts of both butyl- and phenyltin compounds in sediments by gas chromatography-inductively coupled plasma mass spectrometry

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A simple and reliable extraction method was developed for quantitative determination of both butyl- and phenyltin compounds in sediments by capillary gas chromatography combined with inductively coupled plasma mass spectrometry (GC-ICP-MS). Both types of organotin compounds were extracted quantitatively from sediment by mechanical shaking into tropolone–toluene and HCl–methanol. After phase separation and pH adjustment, these organotins were ethylated with sodium tetraethylborate. The method was evaluated by analyzing PACS-2 and NIES No.12 sediment certified reference materials. The dibutyltin (DBT; 1.14 ± 0.02 μg g^{-1}) and tributyltin (TBT; 1.01 ± 0.04 μg g^{-1}) values observed in PACS-2 sediment closely matched the certified values (DBT, 1.09 ± 0.15; TBT, 0.98 ± 0.13 μg g^{-1} as tin). The monobutyltin (MBT) value was higher (0.62 ± 0.02 μg g^{-1}) by more than two fold over the reference value (0.3 μg g^{-1} as tin). The concentrations of TBT (0.18 ± 0.04 μg g^{-1}) and triphenyltin (TPhT; 0.0099 ± 0.002 μg g^{-1}) in the NIES No.12 sediment were also in good agreement with the certified and reference values of TBT (0.19 ± 0.03 μg g^{-1} as compound) and TPhT (0.008 μg g^{-1} as compound), respectively. Recoveries of TBT, tripentyltin (TPeT) and TPhT from spiked sediments were satisfactory (TBT, 102 ± 3.4%; TPrT, 96 ± 3.4%; TPhT, 99 ± 8.5%). The detection limits as tin were in the range 0.23–0.48 ng g^{-1} for a 0.5 g sample size. It is also noteworthy that clean-up of the extract is not necessary because of the superior selectivity of ICP-MS detection. The present method was successfully applied to marine sediment samples.

Introduction

Organotin compounds have been used in antifouling paint formulations, as well as polymer additives, biocides and fungicides, for the past few decades. Pollution, as a result of using such compounds, particularly tributyltin (TBT)- and triphenyltin (TPhT)-based antifoulants, has been of great concern due to the high bioaccumulation potential, persistence in sediment for periods of up to several years and their high toxicity to marine organisms particularly molluscs[1–4] but also to marine mammals.[5] Their widespread use in conjunction with ships and fishing nets resulted in severe contamination of aquatic environments during the 1980s and early 1990s. Since 1982, the application of TBT-based paints has been prohibited on vessels smaller than 25 m in France. Similar regulations followed in Europe, North America and Australia. In Japan, the use of TBT- and TPhT-based paints on any new hulls constructed since 1990 and on all maintenance since 1992 has been prohibited.[6] However, based on recent reports, it can be concluded that organotin levels in water and in sediment samples from marine environments remain relatively high, and continue to pose a toxicological risk to marine organisms.[7–10] Furthermore, TBT is not only a concern in near-shore urban waterways but is also an important issue in the open ocean area, because of its continued use on large ships, which sail internationally.[11]

Sediments are recognized as the ultimate sink for contaminants such as organotins.[12] The accumulated species can be released into the water column through diffusive flux and resuspension/desorption phenomena,[13] thus creating an ecotoxicological risk long after such anthropogenic sources have banned. In spite of the importance of organotin compounds, their quantification in sediments has not proved to be an easy task, because of the difficulty in their complete extraction from sediment samples. Zhang *et al.*[14] evaluated ten different extraction techniques developed during the 1980s for butyltin compounds in sediment and found that only three resulted in a satisfactory recovery of TBT, and that none was applicable to the quantitative extraction of monobutyltin (MBT). Since then, considerable advances in extraction methods and analytical techniques have been achieved. Microwave-assisted extraction,[15] supercritical fluid extraction (SFE)[16] and solid-phase microextraction (SPME)[17,18] represent emerging extraction techniques for organotin compounds from solid matrices in terms of solvent consumption and the reduction in analysis time. Based on presently available data, phenyltin determination in sediments along with butyltins was rarely reported. Among the above-mentioned methods the authors initially used microwave-assisted extraction for butyltins with good results, but sometimes a low recovery of TPhT was observed. The low recoveries of TPhT were frequently observed in the absence of the sediment matrix, but not so often for cases where TPhT was added to the sediment. This suggests that TPhT is partially decomposed under the acidic conditions used in microwave-assisted extraction and that the decomposition is inhibited, to some extent, by the sediment matrix. It was reported that acidification with HCl to pH 2 caused the degradation of TPhT through nucleophilic attack by HCl,[19] although less active acetic acid was used instead of HCl in the microwave-assisted extraction.

Dibutyltin (DBT) and MBT (products of TBT degradation) do not cause imposex in gastropods, but strong toxicity has been reported.[20] Because of their toxicity and environmental occurrence, their simultaneous monitoring is necessary. For the same

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reason, monitoring of monophenyltin (MPhT) and diphenyltin (DPhT) is required along with TPhT. The purpose of the present study was the development of a simple and reliable analytical method by means of developing a sample preparation method which could be used for the simultaneous extraction of butyl- and phenyltins from sediment samples and by application of highly sensitive and selective analysis by GC-ICP-MS. Analytical figures of merit in terms of repeatability and detection limit are also presented.

Experimental

Instrumentation

A gas chromatograph coupled to an inductively coupled plasma mass spectrometer (GC-ICP-MS) was used for the quantification of organotins. The GC (HP 6890, Agilent Technologies, Wilmington, DE, USA) was coupled to the ICP-MS (HP 4500, Yokogawa, Tokyo, Japan) by a laboratory-made transfer line, as described in a previous paper.[21] The ICP was operated with a shield torch under normal plasma conditions, which enhanced the signal intensity by two orders of magnitude, compared with not using the shield torch.[10] A DB-1 (30 m × 0.32 mm id, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) capillary column was used. One microliter of the final extracts was injected into the GC using an HP 6890 series automatic injector in the pulsed splitless injection mode. The concentration of the analyte was calculated by comparing the peak area of the analyte with that of the internal standard (tripropyltin). Details of the optimum GC-ICP-MS operating conditions are given in Table 1.

Reagents

Monobutyltin (MBT) chloride, monophenyltin (MPhT) chloride, diphenyltin (DPhT) chloride and tripentyltin (TPeT) chloride were purchased from Aldrich (Milwaukee, WI, USA). Dibutyltin (DBT) chloride and tributyltin (TBT) chloride were obtained from Wako Pure Chemicals (Osaka, Japan). Triphenyltin (TPhT) chloride was purchased from Kanto Chemicals (Tokyo, Japan). Tripropyltin (TPrT) chloride and tetrabutyltin (TeBT) were purchased from Merck (Darmstadt, Germany). Monooctyltin (MOT) chloride, dioctyltin (DOT) chloride and trioctyltin (TOT) chloride were purchased from AZmax (Chiba, Japan). Stock standard solutions of individual organotin compounds (1 g dm^{-3} as Sn) were prepared by dissolving appropriate amounts of the respective compounds in methanol (pesticide analysis grade, Wako), and were stored at 4 °C until required for use. Working standard solutions were prepared each time by dilution of an aliquot of the stock solutions. TPrT chloride was used as an internal standard for both sample preparation and GC-ICP-MS determination. Sodium tetraethylborate ($NaBEt_4$) was purchased from Strem Chemicals (Newburyport, MA, USA). The purification procedure for $NaBEt_4$ has been discussed in detail elsewhere.[10] Briefly, a 5% m/v $NaBEt_4$ solution was filtered with a 0.2 μm poly(tetrafluoroethylene) (PTFE) membrane filter (Millipore) and then centrifuged at 2000 rpm for 3 min to remove particulate organotin impurities. The supernatant was transferred into another centrifuge tube and extracted three times with one-tenth volume of hexane, in order to remove dissolved organotin impurities. Hydrochloric acid, acetic acid and ammonia solution were of ultrapure grade (Merck). Acetate buffer (1 mol dm^{-3}, pH 5) was prepared by mixing acetic acid and ammonia solution. A 1 mol dm^{-3} HCl–methanol solution was prepared by mixing HCl with methanol (pesticide analysis grade, Wako). Sodium chloride (analytical reagent grade, Wako) and anhydrous sodium sulfate (pesticide analysis grade, Wako) were heated at 500 °C for 20 h to decompose organotin impurities. Tropolone (95%, Wako) was used without purification and 0.1% m/v tropolone was freshly prepared in toluene (pesticide analysis grade, Wako) for each batch of extraction. The water used throughout the experiments was purified by a Milli-Q water purification system (MilliQ-ICP-MS, Nihon Millipore Kogyo, Tokyo, Japan). The Xe gas (981 ppm diluted in Ar gas), used for optimization of the ICP-MS parameters, was purchased from Takachiho Kagaku (Tokyo, Japan). Organotin concentrations given in this paper are expressed as tin, unless stated otherwise.

Table 1 GC-ICP-MS optimum operating conditions

GC Parameter—	
Injection mode	Pulsed splitless
Inlet temp./°C	250
Column	DB1, 30m × 0.32 mm id × 0.25 μm
Oven temp./°C (hold time/min))	55 (0)–100 (0)–300 (1.33)
Oven temp. ramp rate/°C min^{-1})	15 (from 55 to 100 °C), 30 (from 100 to 300 °C)
Sample volume/μl	1
He carrier gas flow rate/ml min^{-1})	2 (constant flow mode)
ICP-MS parameters—	
Forward power/kW	1.2
Ar plasma gas flow rate/l min^{-1}	16.0
Ar auxiliary gas flow rate/l min^{-1}	0.91
Ar makeup gas flow rate/l min^{-1}	1.18
Sampling depth/mm	4.0
Measured *m/z*	120
Dwell time/ms	100
Transfer line parameters/	
Transfer line column	Inactivated capillary column (1.5 m × 0.32 mm id)
Heater-1 (torch side) temp./°C	240
Heater-2 (GC side) temp./°C	280

Sample preparation

Approximately 0.5 g of sediment was accurately weighed and placed in a glass-stoppered 30 ml centrifuge tube, and 0.1 ml of TPrT (*ca.* 3 μg ml^{-1}) internal standard was added. After 10 min, 2 g of NaCl, 12 ml of toluene containing 0.1 % tropolone and 10 ml of 1 mol dm^{-3} HCl methanol solution were added, and the resulting mixture was extracted using a mechanical shaker for 60 min. A 10-ml aliquot of pure water was added into the tube, which was again shaken for 10 min. The tube was centrifuged at 2000 rpm for 2 min in order to achieve a clear phase separation. The upper toluene layer was transferred into another centrifuge tube and evaporated to 5 ml under a nitrogen gas stream. After evaporation, 5 ml of 1 mol dm^{-3} acetate buffer of pH 5, 15 ml of pure water and 0.2 ml of 5% $NaBEt_4$ were added to the extract, and the tube was shaken for 10 min using a mechanical shaker for ethylation and extraction. Centrifugation was then performed for 2 min at 2000 rpm in order to achieve phase separation. The toluene layer was collected by a Pasteur pipette in a centrifuge tube, mixed with 2 g of anhydrous sodium sulfate, and shaken manually to remove the water. Finally the tube was centrifuged at 2000 rpm for 2 min. A portion of the extract was then transferred into a glass vial for the automatic injector and stored at −20 °C until analysis by GC-ICP-MS. One microliter of the final extract was used for analysis. Any contact of the sample with plastics was avoided throughout the sample preparation in order to eliminate possible contamination by plastic additives and stabilizers.

Certified reference material

Certified reference materials are useful for validating newly developed sample preparation methods or analytical instru-

ments. In this study the sediment certified reference materials PACS-2 (National Research Council of Canada, Ottawa, Canada) and NIES No.12 (National Institute for Environmental Studies, Tsukuba, Japan) were used for validation. Two different containers of PACS-2 were used. One is designated as PACS-2 (new) and the other as PACS-2 (old). The former was used for validation but the latter was used only for the optimization of sample preparation. Because 2 years had passed since the PACS-2 (old) was stored at room temperature, some degradation of the organotins was suspected as mentioned later. For the same reason, the PACS-1, which had been stored for 5 years at room temperature, was not used for validation but only for a comparison study on different extraction methods. The PACS-2 (new) was recently purchased and the NIES No.12 had been stored for about 2 years at −20 °C without opening until used for this validation.

Results and discussion

Optimization of sample preparation steps

In selecting an extraction system, the following experimental results reported thus far were taken into account: (1) The use of toluene, in combination with tropolone as a chelating agent, improved the extraction efficiency of MBT substantially.[22] (2) Direct extraction of sediment with an organic solvent with/without a chelating agent was not sufficient for the extraction of DBT and TBT, and acid leaching of sediment appeared to be indispensable.[14] (3) TPhT should not be kept in a strongly acidic medium to prevent decomposition.[19,23] To satisfy all these conditions, HCl–methanol was selected as a leaching solvent and tropolone–toluene as an extraction solution. Both solutions were mixed with the sediment samples and shaken vigorously in the present system. Organotin species leached by HCl–methanol from the sediment are thought to be transferred rapidly into the toluene layer by forming an ion-pair with chloride (TBT, TPhT, *etc.*) or a chelate with tropolone (inorganic tin, MBT, DBT, *etc.*), and the chance of degradation of organotins such as TPhT would be expected to be low.

In all the experiments for the optimization of sample preparation, the PACS-2 (old) reference material was used.

Effect of leaching/extraction time. The effect of the leaching/extraction time on the recovery of organotin compounds from the sediment was investigated and the results are shown in Fig. 1. The experimental conditions were as follows: sediment, 0.5 g; 1 mol dm^{-3} HCl–methanol, 10 ml; toluene containing 0.1% tropolone, 12 ml; NaCl, 2 g; 5% $NaBEt_4$, 0.2 ml; derivatization time, 10 min. The results show that, for the most part, the organotins are easily leached from the sediment even at 15 min. However, the concentrations of inorganic tin and MBT gradually increased on increasing the shaking time from 15 to 60 min, reaching a nearly constant level after 60 min. Therefore, the shaking time was set at 60 min in all the subsequent experiments. The TBT released from sediment on sonication with methanolic HCl was reported to resorb immediately onto the sediment,[24] but this was not observed with the present extraction system, because the TBT released from the sediment was immediately transferred into the toluene layer. Since the concentration of TPhT originally present in the PACS-2 was very low and the precision of the TPhT determination was poor, the extractability of the added standard of TPhT was examined in the subsequent optimization experiments.

Effect of tropolone–toluene volume. Tropolone has been used to improve the extractability of mono- and disubstituted organotins into low polarity solvents.[25] No significant differences in extraction efficiency as a function of tropolone concentration from 0.01 to 0.5% have been found.[26] In the present experiment, the effect of the volume of toluene which contained 0.1% tropolone was examined instead of changing the tropolone concentration. Thus, the observed effect appears to be a combined effect of the absolute amounts of tropolone and the volume of toluene. The results are shown in Fig. 2. The extractability of inorganic tin and MBT increased markedly

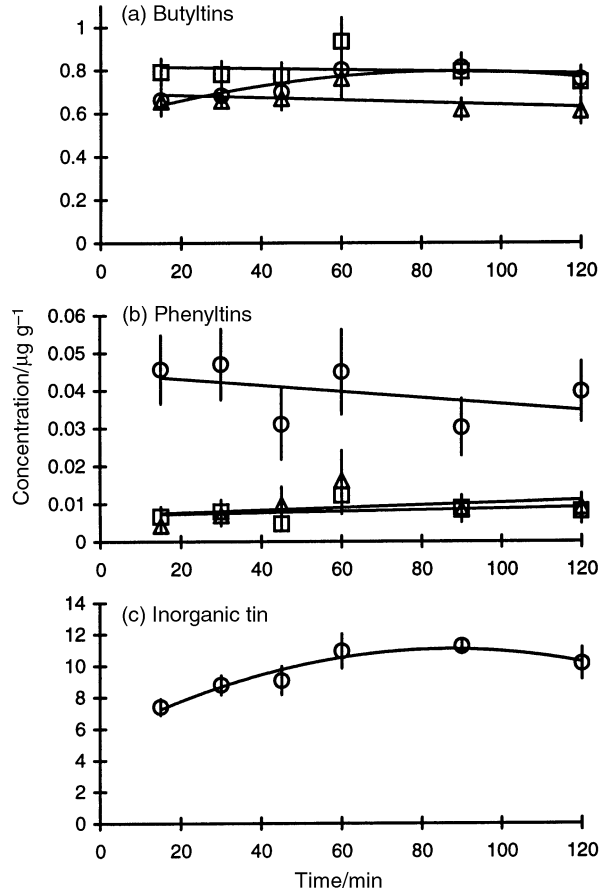

Fig. 1 Effect of leaching/extraction time on recovery of tin species. (a) ○: MBT; □: DBT; △: TBT, (b) ○: MPhT; □: DPhT; △: TPhT, and (c) ○: inorganic tin. Error bars represent the standard deviation of four replicate analyses.

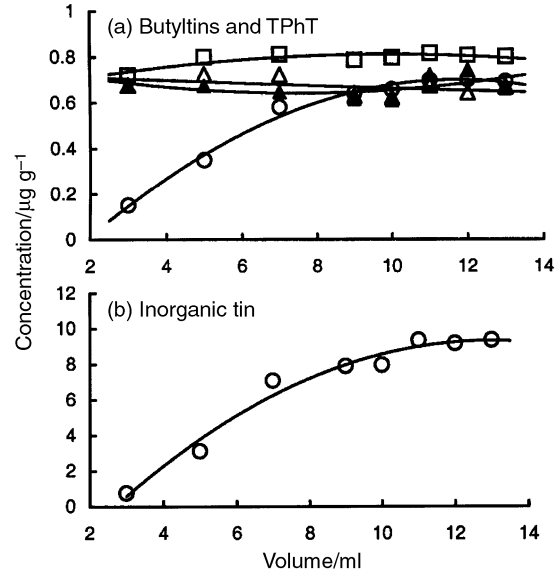

Fig. 2 Effect of tropolone–toluene volume on recovery of tin species. (a) ○: MBT; □: DBT;△: TBT; ▲: TPhT and (b) ○: inorganic tin.

with increasing toluene volume and DBT showed a slight increase in extractability. In contrast, the extractability of TBT and TPhT was nearly independent of the volume of toluene. In order to examine the effect of toluene volume alone, the extraction was also carried out with the same absolute amounts of tropolone, that is, the extraction capability of 6 ml of toluene containing 0.2% tropolone was compared with that of 12 ml of toluene containing 0.1% tropolone. The extraction capability of the former extractant was 79% for inorganic tin, 85% for MBT, 91% for DBT and 96% for TBT compared with that of the latter condition, which means that the volume of toluene itself is an important factor in the extraction. Consequently, the optimum volume of tropolone/toluene solution was selected to be 12 ml in all the subsequent experiments. Since the use of tropolone also enhances the solubility of co-extracted compounds from the sediment matrix, it has been reported that a clean-up step prior to the GC determination is mandatory.[27] However, there is no need for a clean-up with the present method because of the superior selectivity of ICP-MS detection and the large dilution factor. Organotins and co-extracted compounds contained in 0.5 g of sediment sample were finally dissolved in 5 ml of toluene with the present method, compared with which those in 1 g of sediment sample were finally dissolved in 0.5 ml of hexane in the literature.[27] Even with this large dilution factor, a satisfactorily lower detection limit was obtained due to the superior detection capability of ICP-MS. In addition, no degradation of the GC separation was observed during the experimental period of approximately 6 months.

Effect of $NaBEt_4$ volume. The effect of the volume of a 5% $NaBEt_4$ solution on ethylation efficiency was examined for a constant reaction time at 10 min. No significant differences in the efficiency from 0.1 to 0.5 ml were detected. In order to decrease the reagent blank, a smaller volume is preferable, but in order to achieve quantitative ethylation efficiency, which might sometimes be reduced by accompanying substances in real samples, the presence of excess amounts of $NaBEt_4$ appears to be necessary. Therefore, the $NaBEt_4$ volume was set at 0.2 ml.

Effect of derivatization time. To derivatize the tin compounds which were extracted into the toluene layer, these compounds must be first transferred to the aqueous layer adjusted to pH 5, then reacted with $NaBEt_4$, and finally extracted back into the toluene layer. Therefore, it was predicted that a longer time would be necessary for the derivatization, compared with the time for water samples in which the tin compounds were already in the aqueous layer. The effect of shaking time from 2 to 10 min on the derivatization efficiency was examined with a constant value of 0.2 ml of 5% $NaBEt_4$. After 2 min, more than 90% of all the organotin compounds examined and about 85% of the inorganic tin were found to be already derivatized. A slightly longer time was required to derivatize inorganic tin and MBT because these species must react with $NaBEt_4$ four and three times, respectively, for full derivatization. After 6 min the derivatization efficiency for all the tin species was nearly constant. These results suggest that the ethylation and extraction took place more rapidly than predicted. The derivatization time was set at 10 min to achieve quantitative derivatization in all the subsequent experiments.

Effects of other parameters, such as amounts of NaCl and phase separation time, on the recovery of organotins were also examined and their role in the extraction was found not to be critical. The optimum conditions for sediment samples were concluded to be as follows: leaching/extraction time, 60 min; toluene containing 0.1% tropolone, 12 ml; NaCl, 2.0 g; phase separation time, 10 min; ethylating reagent, 0.2 ml of 5% $NaBEt_4$; and derivatization time, 10 min.

Detection limits, repeatability and recovery

The method detection limit is defined as the concentration which would give three times the standard deviation of the peak areas for six replicates of the blank. The value for each organotin species ranged from 0.23 to 0.48 ng g^{-1}, mainly depending on the impurity levels in $NaBEt_4$, when 0.5 g of sample was used and the volume of the final extract was 5 ml. Table 2 compares the detection limits thus for reported. It can be seen that the detection limit obtained in this work belongs to the lowest group obtained thus far. Repeatability was evaluated from six replicates of 0.5 g of PACS-1 spiked with 0.1 ml of a mixed standard containing *ca.* 3 μg ml^{-1} of TBT, TPrT and TPhT. The relative standard deviations of peak area with internal standardization by TPrT were 4.0% for TBT and 6.9% for TPhT. The recovery of standards of TBT, TPrT and TPhT spiked into the sediment was also examined, and a recovery of almost 100% (TBT, 102 ± 3.4%; TPrT, 96 ± 3.4%; TPhT, 99 ± 8.5%) was obtained for these organotins.

Validation with certified reference materials

The determination of butyltin species (MBT, DBT and TBT) in PACS-2, and butyl- and phenyltin species (TBT and TPhT) in NIES No.12 sediment reference materials was carried out for

Table 2 Comparison of method detection limits

Detection limit/ng g^{-1} dry weight	Method	Detection technique	Reference
0.05–2	HCL–diethylether extraction	GC-FPD	Fent and Hunn[32]
0.5	HCl-THF extraction	GC-FPD	Harino *et al.*[33]
1.5–5.8	Supercritical fluid extraction	GC-FPD	Cai *et al.*[34]
10	Microwave-assisted extraction	GC-FPD	Lalere *et al.*[35]
5[a]	HCl/acetone extraction	GC-FPD	Harino *et al.*[9]
1	HCl–CH_3Cl extraction	GC-FPD	Hwang *et al.*[36]
2–3	HCl–CH_3Cl extraction	GC-FPD	Shim *et al.*[37]
0.1–0.44	Methanolic HCl–sonication	GC-AAS	Cai *et al.*[38]
9–38	Methanolic HCl–sonication	GC-AAS	Cai *et al.*[39]
25 ± 5[b]	Hexane extraction	GC-AAS	Kuballa *et al.*[40]
2	HCl–hexane extraction	ETAAS	de Mora *et al.*[41]
0.2–10	Soxhlet extraction	GC-MSD/GC-MIP-AED	Stab *et al.*[42]
2	Microwave-assisted extraction	GC-MIP-AED	Szpunar *et al.*[15]
0.1	Hexane extraction	GC-ICP-MS	Jantzen and Prange[43]
0.34–2.1	SPME	GC-ICP-MS	Moens *et al.*[17]
0.019–0.06	Headspace SPME	GC-ICP-MS	De Smaele *et al.*[18]
0.23–0.48	Methanolic HCl–toluene extraction	GC-ICP-MS	Present study

[a] As cation. [b] pg μl^{-1} injected volume.

validation of the present method. The results are shown in Table 3. Concentrations obtained for DBT and TBT in PACS-2 (new) and TBT in NIES No.12 were in good agreement with the respective certified values. The value for TPhT was also similar to the reference value of NIES No.12. The value for MBT in PACS-2 (new) was much higher than the information value. However, it is recognized that different concentration values ranging from 0.03 to 1.03 μg g^{-1}, most of which were higher than the certified value, have been reported for MBT in PACS-1.[15] This is probably the result of the inefficient extraction of MBT in the certification process of PACS-1. Considering the fact that PACS-2 is a second generation certified reference material of PACS-1, the higher value observed for MBT in PACS-2 does not necessarily mean that the present method is inappropriate, but rather indicates that the method has a stronger extraction capability for MBT than that used for obtaining the information value for PACS-2. A higher value of 0.51 ± 0.02 μg g^{-1} was also reported for MBT in PACS-2 by using microwave-assisted extraction.[28] Concerning the value for acid-leachable inorganic tin obtained here, we presume that the value does not include inorganic tin involved in mineralogical processes but includes inorganic tin bound to organic matter and carbonates, and might be a good indicator of anthropogenic input into the environment. It has been reported that the extraction of inorganic tin was neither reproducible nor quantitative with a similar extraction system in which HCl–water and hexane–ethyl acetate were used instead of HCl–methanol and toluene, respectively.[27] In contrast, the value for inorganic tin obtained with the present extraction system was found to be fairly reproducible. Certified values for total tin in PACS-2 and NIES No.12 were 19.8 ± 2.5 and 10.7 ± 1.4 μg g^{-1}, respectively. The amount of acid-leachable inorganic tin in total inorganic tin, which was calculated by subtracting the organotins from the total tin, was 61 and 47% for PACS-2 and NIES No.12, respectively.

It should be noted that we also analysed PACS-1 and PACS-2 (old), the values obtained for TBT and DBT were significantly lower than the certified values. It is inferred from the lower levels that the degradation of TBT and DBT took place during storage. It is unlikely that the degradation was involved in the present sample preparation step, because PACS-2 (new) and the NIES No.12 values matched with the respective certified values. It is not clear at present, but degradation seems to be one of the potential sources of the unmatched values for the PACS-2 (old) and PACS-1. The occurrence of abiotic degradation of TBT in sediment samples, as well as biotic degradation, has been reported.[29] Caricchia *et al.*[30] evaluated the stability of both butyl- and phenyltins in freeze-dried mussel tissue and found some degradation at room temperature in the presence of light; they also found good stability of tin compounds at −20 °C in the dark for periods of up to 1 year. Studies on the stability of organotins in certified reference materials under different storage conditions would be useful.

Table 3 Concentration of tin species (as Sn μg g^{-1} dry weight) in sediment certified reference materials

Sediment	Species	Certified value[a]	Obtained value[b]
NIES No.12[c]	Inorganic	n.a[d]	4.8 ± 0.34 (6)
	MBT	n.a	0.125 ± 0.008 (6)
	DBT	n.a	0.142 ± 0.007 (6)
	TBT	0.19 ± 0.03	0.18 ± 0.04 (5)
	MPhT	n.a	0.058 ± 0.08 (3)
	DPhT	n.a	0.0033 ± 0.0008 (3)
	TPhT	0.008[e]	0.0099 ± 0.0020 (3)
PACS-2 (new)	Inorganic	n.a	10.5 ± 0.6 (6)
	MBT	0.3[f]	0.62 ± 0.02 (6)
	DBT	1.09 ± 0.15	1.14 ± 0.02 (6)
	TBT	0.98 ± 0.13	1.01 ± 0.04 (6)
	MPhT	n.a	0.028 ± 0.008 (5)
	DPhT	n.a	0.017 ± 0.015 (5)
	TPhT	n.a	0.026 ± 0.017(5)

[a] The uncertainties represent 95% confidence limits. [b] All values are expressed as mean ± *s* (standard deviation); figures in parentheses are the number of independent analyses. [c] Concentration as compound. [d] Not available. [e] Reference value. [f] Information value.

Comparison with other extraction methods

PACS-1 was subjected to microwave-assisted extraction[15] and ultrasonic extraction in order to compare the extractability of the present extraction method for organotins, particularly for phenyltins. It is obvious that the present method yielded a higher extraction efficiency than the other two methods [Fig. 3(a)]. The extraction efficiency for phenyltins in PACS-2 (new) and NIES No.12 was also poorer (no DPhT and TPhT in NIES No.12) with the microwave-assisted extraction than with the present method [Fig. 3(b) and (c)]. As stated before, TPhT undergoes decomposition in strongly acidic media, and therefore, it would be difficult to determine both butyl- and phenyltins simultaneously with microwave-assisted extraction or ultrasonic extraction. It is noteworthy that no decomposition was observed with the present extraction method.

Application to real samples

Marine sediment samples, collected from the Seto Inland Sea near Hiroshima, Japan in May 1999 with an Eckman–Barge grab sampler, were analyzed. Fig. 4 shows the sampling stations. Fig. 5 shows chromatograms of the standard, procedural blank and a sample collected at St. 1, respectively. Although both TPrT and TPeT were added to the blank and the sample as internal standards, only TPrT was used for the

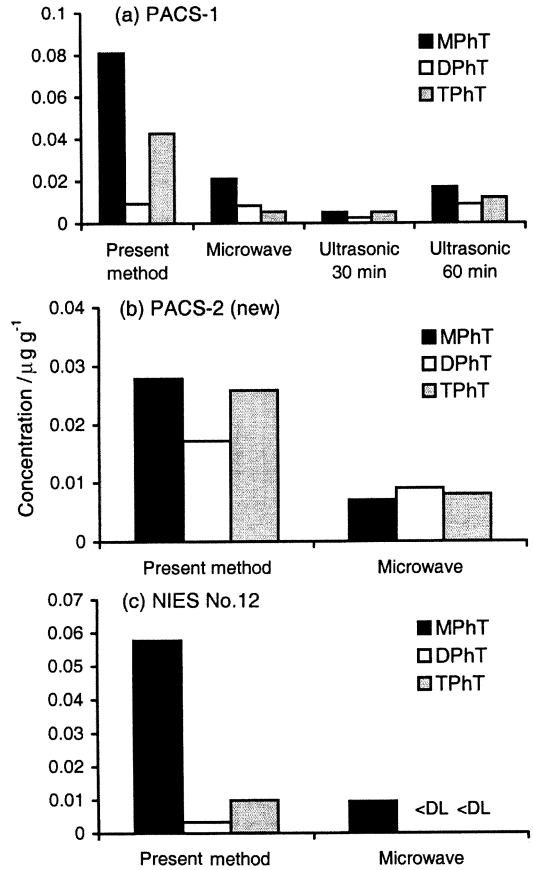

Fig. 3 Comparison of extractability of phenyltin compounds from certified reference materials. (a) PACS-1, (b) PACS-2 (new) and (c) NIES No. 12.

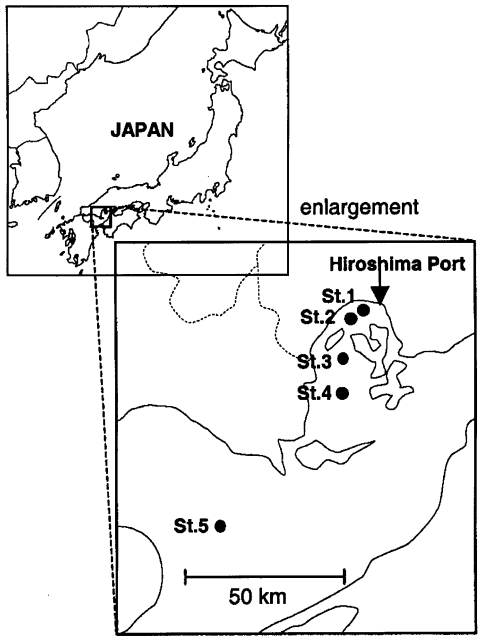

Fig. 4 Sampling stations of marine sediment samples.

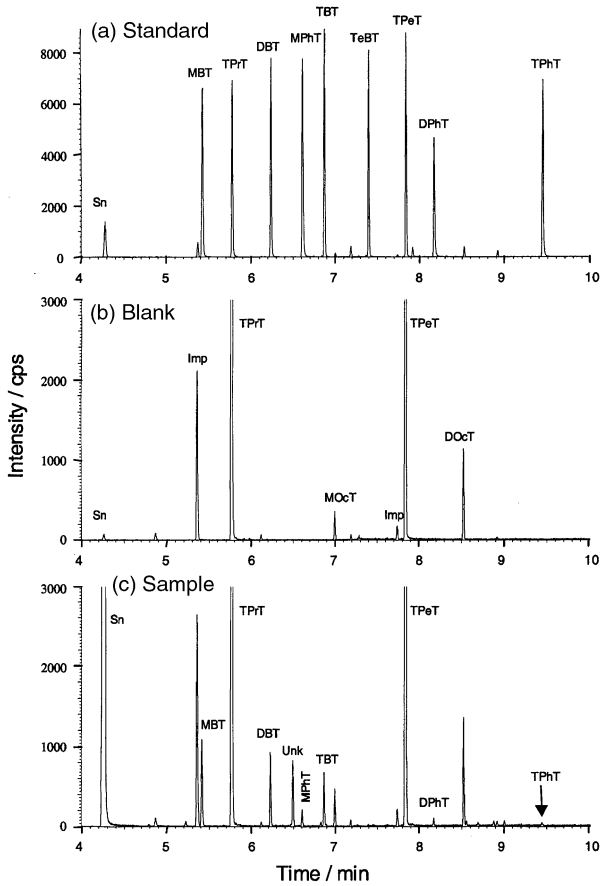

Fig. 5 Chromatograms of (a) the standard containing MBT (10 pg), TPrT (10 pg), DBT(10 pg), MPhT (11 pg), TBT (11 pg), TeBT (10 pg), TPeT (11 pg), DPhT (7 pg) and TPhT (10 pg), (b) the procedural blank and (c) the sample collected at St. 1. Sn, inorganic tin; Imp, impurity; Unk, unknown compound.

quantification because unidentified peaks were sometimes observed at the same retention time as that of TPeT. Peaks at 5.35 and 7.74 min were due to impurities in the TPrT and TPeT reagents, respectively. Although octyltins, such as MOcT and DOcT, were detected in the samples, they were also detected in the procedural blank and the origin was considered to be

Table 4 Concentrations of tin species in marine sediments from the Seto Inland Sea

Sample	Concentration/μg g^{-1} dry weight						
	Inorganic	MBT	DBT	TBT	MPT	DPhT	TPhT
St. 1	1.28	0.0181	0.0128	0.0093	0.0018	0.0008	0.0002
St. 2	0.80	0.0122	0.0065	0.0065	0.0009	0.0005	0.0006
St. 3	0.49	0.0069	0.0037	0.0039	0.0005	0.0003	n.d.[a]
St. 4	0.51	0.0063	0.0032	0.0022	0.0005	0.0003	n.d
St. 5	0.12	n.d	n.d	n.d	n.d	n.d	n.d

[a] Not detected.

impurities in the $NaBEt_4$ reagent. Several unidentified peaks can be seen, and a relatively large peak at 6.48 min was present. Considering its retention time, a possible candidate is tributylmethyltin which is produced through the biomethylation of TBT as pointed out by Amouroux *et al.*[31] The identification is now underway. Butyltins and phenyltins, except for TPhT, were quantified in all the stations except for St. 5 and TPhT was quantified in two of the five stations (Table 4). A significant correlation between the distance from the port of Hiroshima and organotin concentrations in marine sediments was observed, thus demonstrating the usefulness of the present method in clarifying the behavior of trace organotins. The occurrence of the acid-leachable inorganic tin was similar to those of organotins. This result is consistent with the view that the acid-leachable inorganic tin reflected the anthropogenic input into the environment.

Conclusion

A simple and reliable extraction method for both butyl- and phenyltins was developed and combined with a highly sensitive and selective determination by GC-ICP-MS. Although no clean-up of the extract was involved prior to analysis by GC-ICP-MS, column contamination and background interference were not encountered. This method permits the simultaneous determination of butyl- and phenyltins down to the level of 0.23–0.48 ng g^{-1} (as tin). The method was validated by analyzing certified reference materials and was applied to actual marine sediments.

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