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Gas chromatography–high-resolution mass spectrometry based method for the simultaneous determination of nine organotin compounds in water, sediment and tissue

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Abstract

A GC–HRMS based method for the accurate and sensitive determination of nine organotin compounds, tetrabutyltin (TeBT), tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), triphenyltin (TPhT), diphenyltin (DPhT), monophenyltin (MPhT), tricyclohexyltin (TCyT), and dicyclohexyltin (DCyT) in sediment, tissue and water samples is presented and discussed. Mass spectral features of these analytes via both low resolution quadrupole and high resolution magnetic sector, GC–HRMS conditions under selective ion monitoring mode and QA/QC criteria for the positive identification of analyte are all provided. Linearity of response and minimal detectable limits are illustrated for each of the nine compounds monitored and the estimates of method limits-of-detection were 7–29 ppt for water and 0.35–1.45 ppb for tissue or sediments. Sample preparation considerations and precision are discussed for spiked water and sediment samples, whereas method accuracy was established by analysing a certified reference material (CRM) mussel sample and comparing our results to the assigned values. Good agreement was found between our results and assigned or indicative values for MBT, DBT, TBT, DPhT and TPhT (cyclohexyl-tins were not present in the CRM).
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Keywords: Organotin compounds

1. Introduction

Organotin (OT) compounds, characterised by containing at least one carbon–tin bond and generally represented as R_nSnX_{4-n} (n=1–4; R=alkyl or aryl; X=H, OR′, halogen, etc.), are produced globally at ~51 000 tonnes per annum [1]. The biocidal properties of trisubstituted organotins (i.e. n=3) have been recognised since 1954, and as a result, tributyl-, triphenyl- and tricyclohexyl-organotin compounds have been incorporated into many industrial and agricultural biocides, and wood-preserving and antifouling agents [2]. However, a variety of non-target effects of these compounds have been identified as a result of their emission into aquatic environments and high bioaccumulation properties. The most well documented of these effects is caused by tributyltin (TBT) leached in to the environment from antifouling marine paints used on the outer hulls of oceanic vessels and on fishnets or other fishing equipment.

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The effects of TBT on aquatic organisms include high larval mortality and severe malformation of shells in oysters, imposex in dogwhelk populations, growth retardation in mussels and micro-algae, and deformities in fiddler crabs (reviewed in Ref. [3]). Environmental monobutyltin (MBT) and dibutyltin (DBT) concentrations are also of interest since they are the major metabolites of TBT, and are also used industrially as stabilisers in chlorinated polymers (e.g. PVC) [4]. In addition, triphenyltin (TPhT) has been used as a co-toxicant with TBT in some antifouling paints as well as on its own in certain fungicides for various crops. Thus, not surprisingly, TPhT and its metabolites, diphenyltin (DPhT) and monophenyltin (MPhT), have been reported in biomonitoring organisms captured at coastal locations with potential TPhT inputs [5–7]. Finally, tricyclohexyltin (TCyT) which is predominantly used as an acaricide (i.e. kills mites and ticks), has been reported in marine organisms by Stäb et al. [6].

The importance of accurate OT chemical speciation with a low ppb limit-of-detection (LoD) in environmental samples has been acknowledged by various scientists performing monitoring and ecotoxicological studies on these compounds. Many modern methods for the analysis of organotins focus on the simultaneous determination of various co-occurring compounds [8–11]. Most organotin methods published determine environmental concentrations of TBT, DBT, MBT, TPhT, DPhT, MPhT, TCyT, dicyclohexyltin (DCyT), tetrabutyltin (TeBT), fenbutatin oxide (FBTO) and/or various methyl-, ethyl- and/or propyl-tin compounds (reviewed in Refs. [11,12]). Moreover, in order to perform comprehensive environmental monitoring and ecotoxicological risk assessments for organotins, the levels of toxic OT compounds in potential biomonitoring species (i.e. molluscs), sediments and water should be examined.

Although they are generally quite lipophilic, suggesting high solubility in non-polar solvents (e.g. hexane, isooctane, etc.), tri-, di- and mono-substituted organotin compounds occur as salts with various counter-ions, which hampers their uptake into non-polar organic solvents. This effect is particularly noticed for mono-substituted organotins such as MBT as evidenced by the persistently low recoveries published for this compound [13]. Ashby et al. [14] developed a derivatization method employing sodium tetraethylborate ($NaB(Et)_4$) which converts ionic organotin compounds (i.e. R_nSnX_{4-n}) into non-polar volatile derivatives (i.e. $R_nSn(Et)_{4-n}$) in situ, eliminating the problems associated with extracting ionic compounds into an organic solvent from an aqueous matrix. This technique of OT derivatization has replaced alkylation with Grignard reagents [8,10,15], the latter of which involves several steps, and is sensitive to even trace amounts of water present in the sample or sample extract [16]. In more complex matrices (i.e. sediments, tissue, etc.), in situ $NaB(Et)_4$ derivatization may lead to a large amount of co-extracted components which could interfere with the analysis and/or lead to incomplete derivatization of target analyte. However, extraction prior to derivatization has been enhanced (particularly for highly charged OTs) by extracting samples using a low-to-medium polarity solvent containing tropolone (0.01–1.5%, w/v), which acts as a complexing agent enhancing the solubility of organotin cations in organic solvents (reviewed in Ref. [12]). Furthermore, a sample clean-up stage (i.e. silica, alumina or florisil) is usually employed to remove all co-extracted sample matrix components that may interfere with GC determination.

GC analysis is performed in most cases, utilising one of many suitable detection methods including atomic absorption spectrometry (AAS), atomic emission detection (AED), microwave induced plasma-atomic emission spectroscopy (MIP-AES), flame photometric detection (FPD), pulsed flame photometric detection (PFPD), mass spectrometry (MS), and inductively coupled plasma-mass spectrometry (ICP-MS) [11,17,18] (for reviews see Refs. [19–21]). However, the main limitations and drawbacks of most detectors used in organotin analysis are a lack of selectivity and/or sensitivity towards the analytes of interest. FPD suffers from interference due to co-extracted sulphur and/or phosphorous compounds. The PFPD technique is based on a discontinuous air–hydrogen flame in which each species formed has its own time photometric emission profile. This approach substantially reduces the interferences experienced with the FPD technique and provides a much higher analyte specificity. The PFPD technique however has a narrow dynamic range, a limitation inherent to flame photometric

systems [17]. The ICP-MS based technique has sub-picogram detection limit capabilities for several OTs in various environmental samples, however a recent study has shown that it is difficult to maintain long-term signal stability with this technique (i.e. signal can decrease by 60% during a working day) [17]. Also, the GC–ICP-MS interface used in the study was custom built which could limit the utilization of such a technique in other laboratories until well-characterised and reliable GC–ICP-MS systems become more readily available. Low resolution MS suffers from interference in the low-molecular-mass region where most diagnostic ions for organotins appear [12]. To date, high resolution mass spectrometry (HRMS) has not been evaluated for organotin analysis in environmental samples. This technique is very sensitive and can be extremely selective due to the narrow mass channels (10 000 resolution) used to monitor eluting analyte. In this paper, we describe a GC–HRMS based methodology for the determination of nine organotin compounds (TeBT, TBT, DBT, MBT, TPhT, DPhT, MPhT, TCyT, DCyT) in water, sediment and mussel tissue including: (a) mass spectral features of the organotin analytes including a comparison between quadrupole and magnetic sector (MS); (b) illustration of the selected ion monitoring (SIM) GC–HRMS (magnetic sector) method including linearity of response, instrument minimal detectable limits (MDLs), and method precision and accuracy for all nine OT analytes; and (c) sample preparation and extraction considerations for the efficient recovery of all analytes.

2. Experimental

2.1. Sample origin

Milli-Q® deionized water was used for all water sample trials. Sediment samples were collected at the inter-tidal zone on Botanical Beach, Port Renfrew, Vancouver Island in December of 1999. Samples were kept frozen (−20 °C) in Teflon® lined screw-top glass jars until analysis at which point the samples were thawed overnight, and an appropriate aliquot from each was measured. Dry mussel homogenate (CRM# QSP001BT) was supplied by the Quality Assurance Laboratory Performance Studies for Environmental Measurements in Marine Samples Project (Quasimeme, Aberdeen, UK) for round 12 of their international laboratory performance studies.

2.2. Reagents and chemicals

Di(*n*-propyl)tin dichloride (>99%; internal standard) was from Aldrich (Milwaukee, WI, USA). Tetra(*n*-pentyl)tin (>99%; performance standard), mono(*n*-butyl)tin trichloride (>99%), di(*n*-butyl)tin dichloride (>99%), tri(*n*-butyl)tin monochloride (>99%), mono(*n*-phenyl)tin trichloride (>99%), di(*n*-phenyl)tin dichloride (>98%), tri(*n*-phenyl)tin monochloride (>99%), di(*n*-cyclohexyl)tin dichloride (>99%), and tri(*n*-cyclohexyl)tin monochloride (>99%) were obtained from the Quasimeme programme at Vrije Universiteit, Amsterdam (Amsterdam, Netherlands). Tropolone (98%), 25% tetramethyl ammonium hydroxide (TMAH; reagent grade), sodium acetate (NaOAc; 99.995%), potassium hydroxide (KOH; 99.99%; semiconductor grade) and dichlorodimethylsilane (DCDMS; 99%) were purchased from Aldrich. Sodium chloride (NaCl; 99.9%) was purchased from BDH (Toronto, Ont., Canada). Sodium tetraethylborate ($NaB(Et)_4$; 98%) was obtained from Alpha Aestar (Ward Hill, MA, USA). Acetic acid (99.8%; Omnitrace) was obtained from EM Science (Gibbstown, NJ, USA). Dichloromethane (DCM; reagent grade), acetone (reagent grade), cyclohexane (hex; reagent grade), toluene (reagent grade) and methanol (MeOH; HPLC grade) were obtained from Caledon Laboratories (Georgetown, Ont., Canada). Diethyl ether (Et_2O; analytical grade) was purchased from BDH. Basic alumina (aluminum oxide) was Brockmann I, standard grade, ~150 mesh, 58 Å, obtained from Aldrich.

2.3. Glassware cleaning/treatment

In order to eliminate the problem of high background levels due to analyte adsorption on glassware surfaces, commonly encountered in trace metal analysis, the following cleaning procedure was adopted for all routine glassware used in this work. Initially, each piece of glassware was rinsed several times with hot tap water, soaked in 2% nitric acid (HNO_3),

and rinsed with hot tap water again. The glassware was then rinsed in a laboratory dishwasher using distilled water, each piece was rinsed twice with acetone and twice with DCM, treated with 5% DCDMS in DCM to deactivate the glassware surfaces, and finally rinsed twice with DCM to remove excess DCDMS. Next, the glassware was oven baked for 6 h at 325 °C and finally rinsed twice with acetone and twice with hexane before use. Due to potential loss of calibration of volumetric flasks upon heating at high temperatures, this step as well as prior silylation with DCDMS was avoided and replaced by simply air-drying for this type of glassware.

2.4. Sample extraction and derivatization

Deionized water was measured out into a 250-ml separatory funnel. Di(*n*-propyl)tin (DPrT) dichloride (1.00 ng cation/μl in MeOH) internal standard (I.S.), and NaOAc buffer (adjusted to pH 4.5 by adding AcOH) was added to each sample (Fig. 1). After shaking, $NaB(Et)_4$ in methanol was added to each sample. Samples were immediately shaken for ~1 min, hexane was added, and samples were shaken again for ~1 min. The samples were then allowed to react at room temperature for 30 min, and finally shaken for ~1 min again. The organic layer was collected, and another aliquot of $NaB(Et)_4$ was added to the aqueous layer of each sample to ensure complete derivatization in the presence of non-target consumption of $NaB(Et)_4$. The hexane extraction was repeated except without the 30-min reaction period. Both organic layers were combined, reduced by rotoevaporation followed by evaporation under a gentle stream of nitrogen (temperature <30 °C in

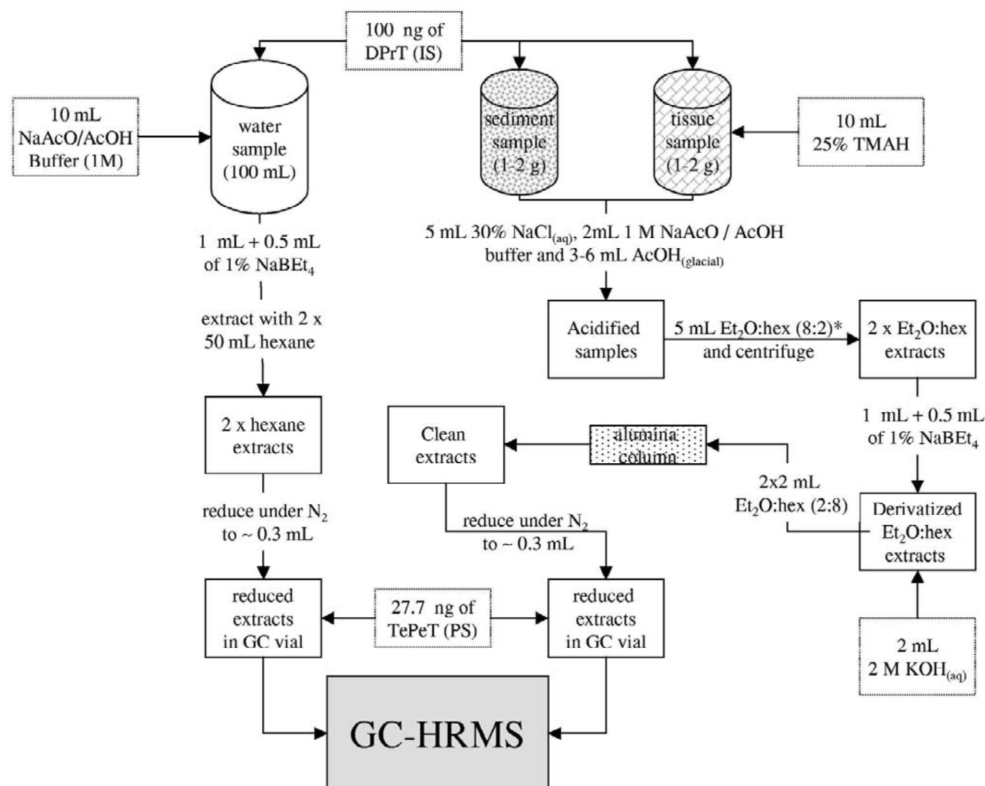

Fig. 1. Sample preparation scheme used in this work for water, sediment and tissue samples. *Indicates 0.02% tropolone.

both cases) and tetra(*n*-pentyl)tin (TePeT) performance standard (P.S.) was added prior to GC–HRMS analysis.

Wet tissue or sediment was weighed into a new 60-ml I-Chem Vial. For tissue samples only, each sample was sonicated for 1 h with TMAH acting as a tissue liquefier breaking down lipids to facilitate the extraction of OTs from the matrix. The I.S. was added as in the water trails, then glacial acetic acid, NaOAc buffer (pH 4.5) and $NaCl_{(aq)}$ was added to each tissue and sediment sample (Fig. 1). The acidic mixture was extracted with Et_2O:hexane (8:2) (with tropolone) by shaking for 1 h on an electric shaker. Subsequently, each sample was centrifuged at 700–1160 RCF (*g*-force) for 5–10 min and the supernatant (i.e. organic layer) was transferred to a 15-ml glass centrifuge tube. The extraction with the tropolone solution was repeated, and the final extract was combined with the former in the glass centrifuge tube. The extracts were reduced (~2 ml) under nitrogen and $NaB(Et)_4$ in MeOH was added. The solutions were vortexed, an additional aliquot of $NaB(Et)_4$ was added and the solutions were vortexed again. After the solutions sat overnight at room temperature, $KOH_{(aq)}$ was added and the samples were transferred/extracted with Et_2O:hexane (2:8).

2.5. Sample clean-up

Derivatized extracts from tissue or sediment (i.e. containing large amounts of co-extracted matrix components) were evaporated under nitrogen (~0.5 ml) and then loaded onto 6 cm of activated basic alumina packed in a 9-inch Pasteur pipette with filter paper functioning as a frit. The column was eluted with 10–15 ml of Et_2O:hexane (2:8), and the eluate was collected in a second 15-ml polyethylene tube. The samples were reduced and the P.S. was added prior to GC–HRMS analysis.

2.6. GC–MS analysis

A 1-μl aliquot of each sample was injected (splitless mode) onto a Hewlett-Packard 5890 Series II Gas Chromatograph coupled to a VG AutoSpec magnetic sector mass spectrometer (Micromass UK, Manchester). Samples were resolved on a DB-5 (30 m×0.25 mm I.D., 0.25 μm film thickness, J&W, Folsom, CA, USA) fused-silica capillary column with helium as a carrier gas at constant pressure (~60 kPa). The injector temperature was set at 250 °C, and the GC temperature program featured an initial column temperature of 80 °C with a ramp of 5 °C/min to 130 °C, then a 10 °C/min ramp to 280 °C which was held for 5 min. The GC–MS transfer line was set at 250 °C, and the ionisation source at 280 °C. The electron impact energy was 33 eV, and the electron multiplier voltage was 350 V. The magnetic sector was operated at 10 000 resolution. Selected ion monitoring (SIM) was used to detect ethylated analyte compounds as outlined in Table 1.

Low resolution mass spectra of organotin standard

Table 1
Summary of positive ions monitored for each OT analyte by GC–HRMS

Analyte	194.94 *m/z*	196.94 *m/z*	233.05 *m/z*	235.05 *m/z*	231.03 *m/z*	233.04 *m/z*	261.08 *m/z*	263.08 *m/z*
TePeT[a]							$Sn^{118}(Pe)_2H$	$Sn^{120}(Pe)_2H$
DPrT[b]			$Sn^{118}(Pr)_2Et$	$Sn^{120}(Pr)_2(Et)$				
MBT			$Sn^{118}(Bu)(Et)_2$	$Sn^{120}(Bu)(Et)_2$				
DBT			$Sn^{118}(Bu)_2H$	$Sn^{120}(Bu)_2H$				
TBT			$Sn^{118}(Bu)_2H$	$Sn^{120}(Bu)_2H$				
TeBT			$Sn^{118}(Bu)_2H$	$Sn^{120}(Bu)_2H$				
MPhT	$Sn^{118}(Ph)$	$Sn^{120}(Ph)$						
DPhT	$Sn^{118}(Ph)$	$Sn^{120}(Ph)$						
TPhT	$Sn^{118}(Ph)$	$Sn^{120}(Ph)$						
DCyT					$Sn^{118}(Cy)(Et)H$	$Sn^{120}(Cy)(Et)H$		
TCyT					$Sn^{118}(Cy)(Et)H$	$Sn^{120}(Cy)(Et)H$		

Bu = butyl; Cy = cyclohexyl; Et = ethyl; Pe = pentyl; Ph = phenyl.
[a] Performance standard.
[b] Internal standard.

solutions were produced on a Finnigan Voyager GC–MS system using +EI (electron impact) ionisation and a scan range of 100–400 m/z. The injector temperature was set at 250 °C, and the temperature program started at 100 °C for 10 min with a ramp of 5 °C/min to 250 °C, then a 10 °C/min ramp to 280 °C which was held for 2 min. The transfer line was set at 250 °C, and the ionisation source at 200 °C. The electron impact energy was 70 eV, and the electron multiplier voltage was 450 V.

2.7. Quality assurance/quality control (QA/QC) and quantitation

The criteria for quality assurance were adapted from our in-house protocol for ultra-trace analysis by GC–HRMS [22] and include: (a) two isotopes of the specific analyte are detected at their exact m/z and a minimum 10k resolving power during the entire chromatographic run; (b) both of the isotope signals must be present, and must maximise within ±2 s of one another; (c) the retention time (RT) of a specific analyte must be within 3 s of that obtained during analysis of the authentic compounds in the calibration standards; (d) signal-to-noise ratio of each of the isotope m/z channels must be ≥3; (e) the ratio of the two isotopic peaks (i.e. Sn^{118}/Sn^{120}) must be 0.73±0.15, otherwise it was reported as NDR (not detected due to incorrect isotopic ratio).

For calibration purposes, 50 μl of the OT standard mixture (~1 ng cation/μl of TBT, MBT, TPhT, DPhT, MPhT, TCyT and DCyT, ~2 ng cation/μl of DBT, and ~10 ng/μl of TeBT in MeOH) was spiked into a 15-ml glass centrifuge tube and 1 ml of acetic acid buffer was added. The standard was derivatized by adding 1 ml of 1% $NaB(Et)_4$ in MeOH as with the samples, except no second aliquot of $NaB(Et)_4$ was needed. Finally, the OT standard mixture was extracted with 3×2 ml hexane in the water analysis, and 2×2 ml Et_2O:hex (2:8) in the tissue and sediment analysis, the extracts combined, reduced and 10 μl of the P.S. were added. Samples were processed in batches of 13 which contained nine samples, one duplicate, one blank, one spiked sample and one certified reference material (CRM). The OT standard mixture was run on the GC–HRMS at the beginning and at the end of each batch of 13 samples for initial calibration and to ensure no large drifts in calibration occurred throughout the analysis.

Table 2
Average relative response factors (RRFs) for the target compounds based on internal standard

Analyte	RT (min)	RRF (RSD; $n=6$)
DPrT (I.S.)	10.00	0.15[a] (27)
MBT	10.24	0.36 (11)
DBT	14.11	0.48 (6)
MPhT	16.00	0.51 (37)
TBT	17.00	0.49 (14)
TeBT	19.12	0.18 (17)
DCyT/DPhT	22.18	1.23 (19)/0.61 (38)
TCyT/TPhT	26.55	0.90 (44)/0.63 (43)

[a] Relative to performance standard TePeT.

Quantitation of the nine OT analytes, TeBT, TBT, DBT, MBT, TPhT, DPhT, MPhT, TCyT and DCyT, monitored in this work was based on the internal standard (I.S.), DPrT dichloride. A performance standard, TePeT, was added following sample preparation, and was used to determine the amount of I.S. lost throughout the sample preparation process. Eq. (1) was used to determine the percentage recovery of I.S. and Eq. (2) allowed the determination of the recovery corrected concentrations for each OT compound in the sample.

$$Recovery\ (\%) = [(27.7\ \text{ng} \times A_{\text{I.S.}})/(A_{\text{P.S.}} \times 100\ \text{ng} \times RRF_{\text{I.S.(P.S.)}})] \times 100\% \quad (1)$$

$$*C = (100\ \text{ng} \times A_{\text{unk}}/A_{\text{I.S.}} \times RRF_{\text{unk(I.S.)}})/SW \quad (2)$$

where $*C$ is recovery corrected concentration (ng/g) of analyte in the sample, A is peak area, RRF is relative response factor and SW is sample weight (g). All OT concentrations or amounts in this work are based on weight of cationic species. Table 2 outlines the RRFs for all the nine analytes monitored in this work.

3. Results and discussion

3.1. Mass spectrometry of organotins: magnetic sector versus quadrupole

Our work is based on magnetic sector analysis due

to advantages in selectivity and sensitivity (e.g. typically 4–10-fold greater than quadrupole) by this HRMS technique. However, as many MS based environmental analytical methods and most mass spectral libraries (e.g. Wiley, NIST) employ quadrupole MS, a qualitative comparison between magnetic sector and quadrupole MS will be of value to practitioners of this technique.

The mass spectra produced by the magnetic sector MS used in this work, will vary from those reported in the literature generated using quadrupole or other MS techniques, largely due to the selective ion transmission characteristics inherent in other mass spectrometers compared to magnetic sector mass spectrometers. The differences in intensities of the major mass spectral ions between magnetic sector spectra and quadrupole MS are illustrated in Fig. 2 for ethylated TPhT/TCyT. The ion transmission in magnetic MS at constant acceleration voltage, is uniform throughout the mass range. In quadrupole MS (see insert in Fig. 2) the ion transmission is mass dependent and is governed by a number of operational variables [23]. Thus, the intensity of $SnPh_3^+$ at 351 *m/z* relative to $SnPh^+$ at 197 *m/z* in the magnetic sector spectrum is more than twice that of the quadrupole due to the lower transmission efficiency of the heavier fragment (i.e. $SnPh_3^+$) in the latter technique. A comprehensive table illustrating the differences in intensities of predominant ions by quadrupole MS versus magnetic sector MS is given in Table 3. In most cases, there is an increase in relative intensity of mass ions >200 *m/z* in magnetic sector versus quadrupole.

3.2. Optimisation of GC–HRMS (magnetic sector)

Most EI mass spectra that are published or included in MS libraries, are generated at an electron energy of 70 eV [24]. However, an electron energy of 33 eV was used in the fragmentation process of OT analytes in this work. This soft ionisation was ideal to produce a favorable fragmentation pattern of all nine OT analytes. Fig. 2 (main figure) illustrates the full-scan mass spectrum of ethyl derivatives of TPhT and TCyT monitored in this work. In the selection of SIM channels for each analyte, there was generally a trade-off between using the most intense ions and thus maximise sensitivity, and choosing distinct ions (i.e. not present in any coeluting interference components) and thus minimising interference. The mass ions listed in Table 1 were used in the SIM of all nine organotin analytes. Monitoring two distinct isotopes of tin (i.e. Sn^{118} and Sn^{120}) for each analyte, provides a peak purity check as described in the QA/QC criteria discussed in Section 2.7.

Most ethylated OT analytes are resolved well by the chromatographic conditions described in the experimental section. Fig. 3 shows the SIM (8-channel) profiles of the OT standard mixture run by the GC–HRMS method. Due to similarities in boiling point and polarity to some degree, the phenyltins (F and H) co-elute with cyclohexyltins (G and I) of the same order. However, since the phenyltins produce a predominant $[Sn(C_6H_5)]^+$ (monitored at 194.94 and 196.94 *m/z*) which occurs at a separate *m/z* than $[Sn(C_6H_{11})H_2]^+$ produced by the cyclohexyltins, these analytes can be quantified separately. Furthermore, the cyclohexyltins produce a predominant $[Sn(C_6H_{11})(C_2H_5)H]^+$ (monitored at 231.03 and 233.04 *m/z*) by EI-MS, which is free of interference from phenyltin fragment ions.

Linear calibration curves were established using seven concentrations of the OT standard mixture each in triplicate with an additional blank. The concentration ranges chosen encompassed those of the final extracts for all samples encountered. All peak areas were normalised to the response of 100 ng of the internal standard (DPrT). Table 4 summarises the linear regression of the calibration data for each of the nine target OT analytes in this work. The coefficient of determination (R^2) value is given for the linear regression of each analyte together with the $(R_{critical})^2$ for significance at a 95% confidence level, along with the standard error of the estimate (SEE) which is a measure of the goodness-of-fit despite differences in calibration curve slope between analytes. Additionally, MDLs were determined for a signal-to-noise level of 3 based on the least concentrated calibration solution. MDLs ranged from 0.13 pg for MPhT to 5.9 pg for TeBT, and are well within the range published for other sensitive and selective detection methods as evaluated by Aguerre et al. [17].

The R^2 of most analytes was ~0.99, indicating the response is highly linear for the concentrations

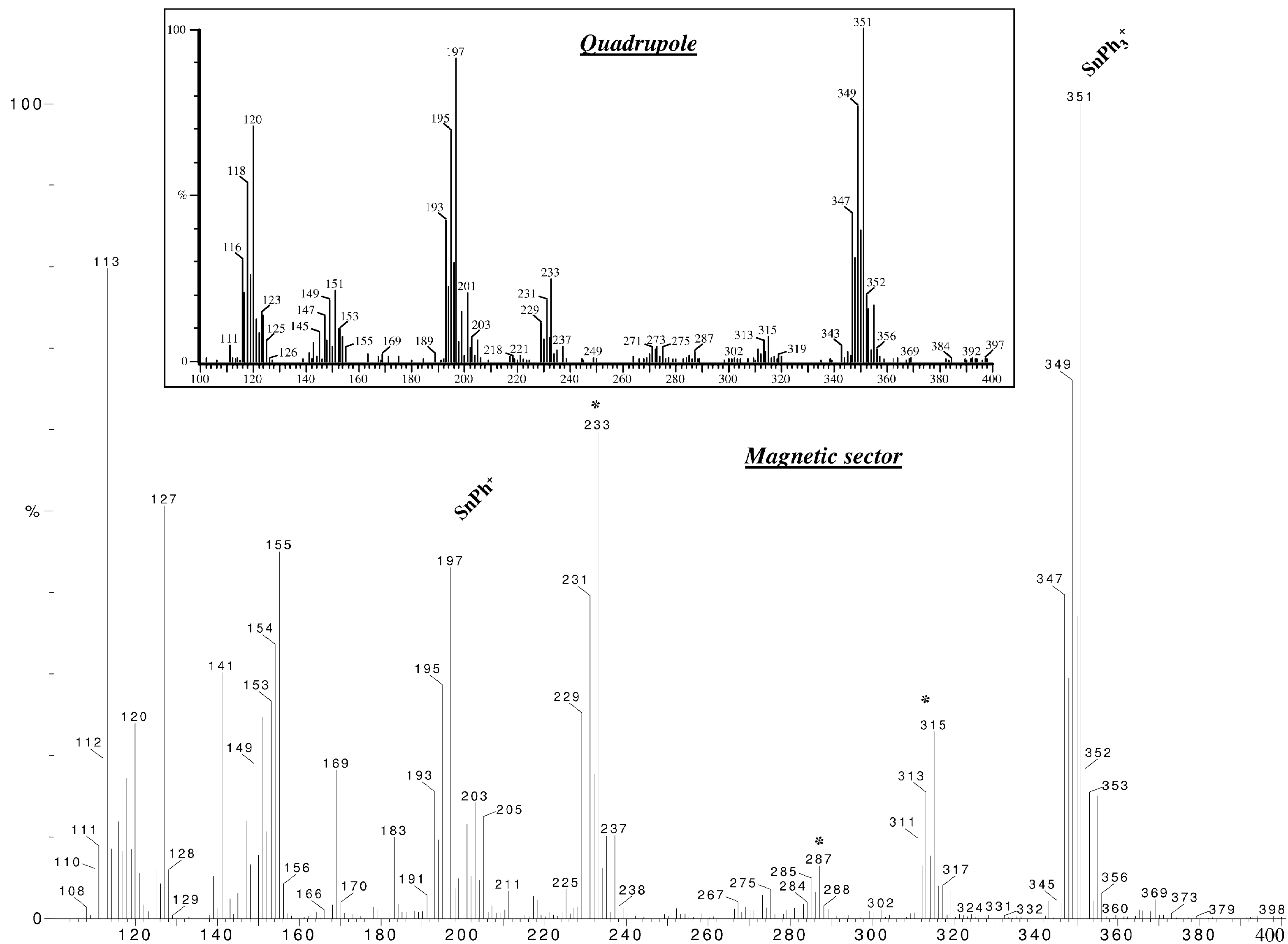

Fig. 2. EI mass spectra of triphenylethyltin/tricyclohexylethyltin (prepared as 50 µl OT standard mixture). Main spectrum is magnetic sector MS in full scan mode at 5000 U resolution; inserted spectrum is low resolution quadrupole MS. *Peaks due to fragment ions of tricyclohexylethyltin.

Table 3
Mass ions and their relative intensities for various ethylated organotin analytes by high resolution magnetic sector (Mag) and low resolution quadrupole (QP)

Compound	$Sn(Et)H_2$	$Sn(Et)_2H$	$SnRH_2$	Sn(Et)RH	$Sn(Et)_2R$	SnR_2H	$Sn(Et)R_2$	SnR_3
MBT								
Mag	151(41)		179(100)	207(14)	235(54)			
QP	151(55)		179(100)	207(25)	235(47)			
DBT								
Mag	151(86)		179(91)	207(100)	235(30)		263(60)	
QP	151(89)		179(83)	207(100)	235(21)		263(38)	
TBT								
Mag	151(54)		179(46)	207(100)	235(26)		263(36)	291(28)
QP	151(59)		179(63)	207(100)	235(25)		263(29)	291(24)
TeBT								
Mag			179(100)		235(78)			291(43)
QP			179(100)		235(70)			291(33)
MPhT								
Mag			N/A	N/A	N/A			
QP			197(100)[a]	227(53)	255(67)			
DPhT								
Mag			197(93)[a]			275(65)	303(100)	
QP			197(100)[a]			275(65)	303(89)	
TPhT								
Mag			197(40)[a]					351(100)
QP			197(91)[a]					351(100)
DCyT								
Mag	151(64)	179(100)	205(5)	233(57)	261(43)		315(30)	
QP	151(90)	179(100)	205(6)	233(44)	261(29)		315(22)	
TCyT								
Mag	151(49)		205(20)	233(100)		287(10)	315(40)	369(4)
QP	151(85)		205(24)	233(100)		287(10)	315(29)	369(4)

[a] Unique ion cluster confirmed from isotope patterns of SnR.

tested. TPhT and TCyT showed R^2 less than this along with higher SEE values than most other analytes. This was especially true for TCyT which suffered from a large amount of scatter from linearity ($SEE = 1.94 \times 10^{-1}$) which would indicate that there would be larger errors associated with the quantitation of this analyte. Method LoDs were estimated at 7–29 ppt in water based on a 100-ml sample and 0.35–1.45 ppb in tissue or sediments based on a 2-g sample.

3.3. Sample preparation considerations and method validation

As implied previously, organometallic compounds such as organotins are known to form associations with glass surfaces which leads to high background levels in environmental analysis. As a preventative measure, a 5% (w/v) DCDMS solution was used to silanize all glassware and thus deactivate the siloxane surface. Prior to this silanization, the OT analysis

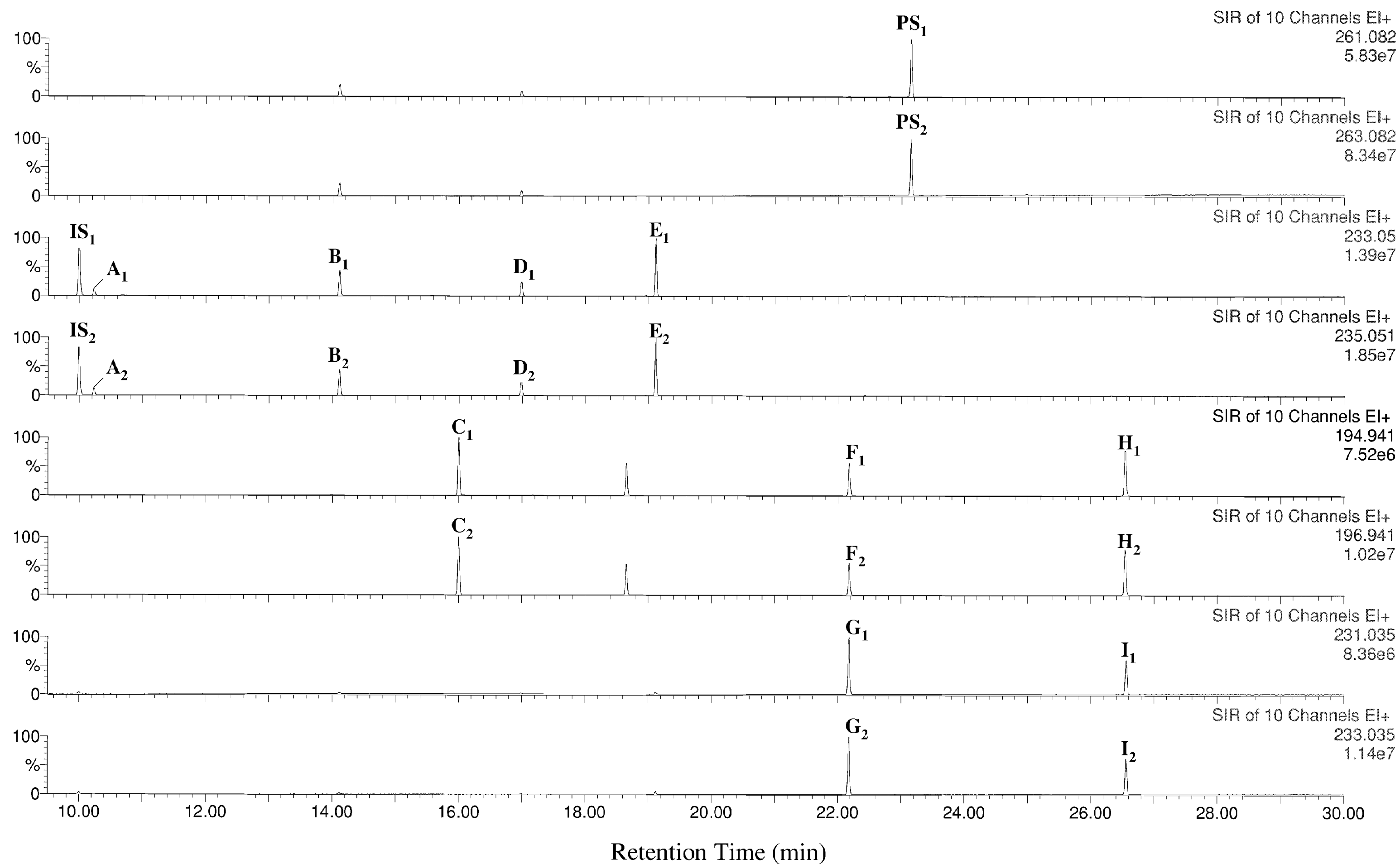

Fig. 3. SIM profiles of OT standard mixture run by GC–HRMS. I.S. = TPrT at 10.00 min; P.S. = TePeT at 23.15 min; A = MBT at 10.24 min; B = DBT at 14.11 min; C = MPhT at 16.00 min; D = TBT at 17.00 min; E = TeBT at 19.12 min; F = DPhT and G = DCyT at 22.18 min; H = TPhT and I = TCyT at 26.55.

Table 4
Calibration curves and MDLs for the nine target OT analytes

Compound	Total amount of each analyte tested (ng)	$R^2((R_{critical})^2=0.197)$	SEE[a]	MDL (pg)
MBT	0, 0.99, 4.95, 14.9, 39.6, 49.5, 79.2, 149	0.986	1.77×10^{-2}	2.4
DBT	0, 2.00, 10.0, 30.0, 80.0, 100, 160, 300	0.998	2.56×10^{-2}	1.8
TBT	0, 0.91, 4.55, 13.7, 36.4, 45.5, 72.8, 137	0.996	1.72×10^{-2}	2.4
TeBT	0, 10.0, 50.0, 150, 400, 500, 800, 1.50×10^{3}	0.992	9.64×10^{-2}	5.9
MPhT	0, 1.04, 5.20, 15.6, 41.6, 52.0, 83.2, 156	0.998	2.97×10^{-2}	0.13
DPhT	0, 1.17, 5.85, 17.6, 46.8, 58.5, 93.6, 176	0.991	3.51×10^{-2}	0.53
TPhT	0, 1.00, 5.00, 15.0, 40.0, 50.0, 80.0, 150	0.976	9.24×10^{-2}	0.50
DCyT	0, 1.06, 5.30, 15.9, 42.4, 53.0, 84.8, 159	0.985	8.64×10^{-2}	1.4
TCyT	0, 0.95, 4.76, 14.3, 38.1, 47.6, 76.2, 143	0.809	1.94×10^{-1}	0.87

[a] SEE, standard error of the estimate.

suffered from background levels of MBT, DBT, TBT, TeBT, and MPhT. After silanization was adopted, the background contamination was significantly reduced, however, MBT and DBT were still present in procedural blanks at ~4 and 0.5 ng, respectively. As the levels of these two analytes are quite consistent in the procedural blanks, the samples may be background corrected.

The in situ ethylation with $NaB(Et)_4$ used by Michel and Averty [16] for tributyltin analysis in water was used in this work to overcome the difficulties associated with extracting ionic organotin analytes from an aqueous matrix. Fig. 4 illustrates the loss incurred when performing a separate liquid–liquid extraction with hexane and then derivatization, as opposed to the in situ derivatization and simultaneous extraction. It is apparent from Fig. 4 that monobutyl and monophenyl compounds suffer the largest recovery loss when separate extraction and derivatization procedures are employed, especially when the less polar 2:8 Et_2O:hex extraction solvent was used. This solvent also was less efficient in

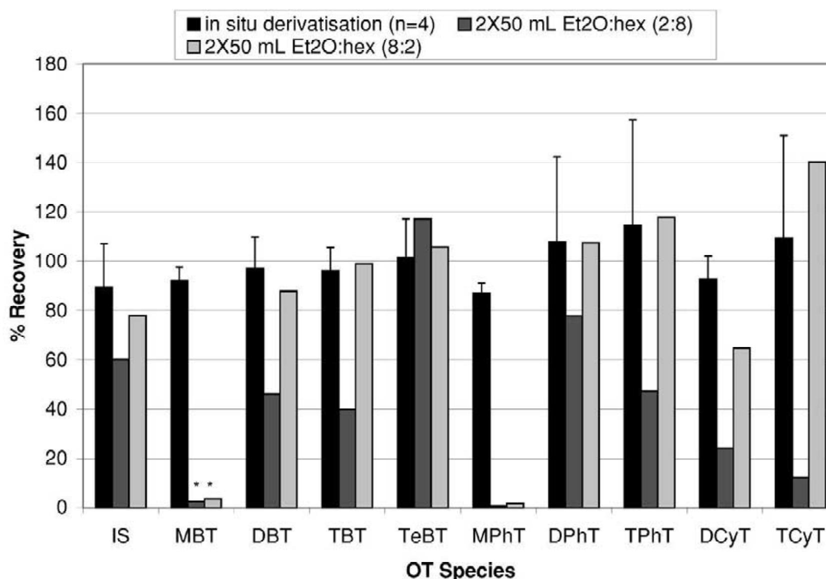

Fig. 4. Absolute recovery of OT analytes from water by in situ derivatization with $NaB(Et)_4$ ($n=4$ and error bars represent SD) compared to separate extractions (with Et_2O:hex combinations) and derivatization. *NDR value reported.

extracting all other ionic analytes compared to the more polar 8:2 Et_2O:hex illustrating the enhanced solubility of ionic OT compounds in solvents of greater polarity. However, in situ extraction/derivatization with $NaB(Et)_4$ provided, by far, the most quantitative extraction and derivatization of organotins.

The effect of buffer pH on derivatization efficiency of organotins with $NaB(Et)_4$ has been extensively studied elsewhere [19]. It has been empirically determined that a pH of 4.5 be used for the derivatizations in this work which was well in agreement with de la Calle-Guntiñas et al. [19]. Furthermore, shaking the separatory funnel for at least 1 min directly after the addition of $NaB(Et)_4$ in MeOH, and slow and careful evaporation of solvents during concentration steps have been identified as important factors affecting recovery in this work. Fig. 4 (black bars) shows the results of 50 μl OT standard mixture spiked water samples using the developed method. The average recovery for each of the nine target analytes is well between 80 and 120% and the total method standard deviation is <20% for the I.S., first five target OTs and DCyT. However, TCyT, DPhT and TPhT appear to suffer from high variability which is also evident in the RRF variation (interbatch) reported in Table 2. This high variability could most likely be improved by the incorporation of a second internal standard which elutes closer to these analytes.

With more complex matrices such as sediments or mussel tissue, in situ derivatization leads to poor selectivity for OTs and a large amount of co-extracted components. Thus, for such matrices extraction and derivatization must be performed in separate stages. There exists an optimum between choosing an extraction solvent system polar enough to quantitatively extract polar OT compounds from an aqueous tissue matrix (or conversely the tissue may have been freeze dried) and not too polar such that a large amount of matrix components are co-extracted and reduce the efficiency of the subsequent derivatization with $NaB(Et)_4$ and/or interfere with the detection of target OTs by GC–HRMS by suppression of ion formation. A way of increasing the recovery of mono-substituted organotin analytes without drastically increasing solvent polarity involves using a complexing agent such as tropolone dissolved in a non-polar extraction solvent [12].

Fig. 5 shows the efficiency gained particularly for

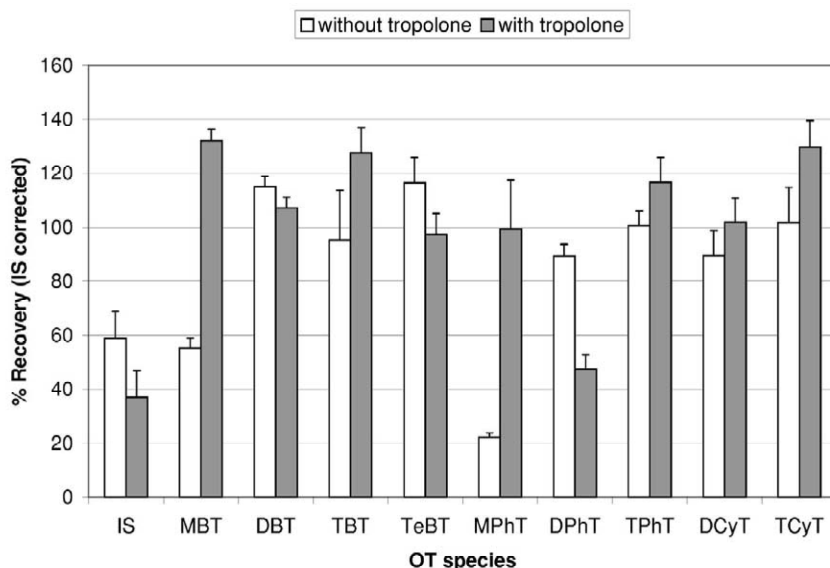

Fig. 5. Recovery (I.S. corrected) of OT analytes from sediments using developed method without (open bars) and with (closed bars) 0.02% (w/v) tropolone as a complexing agent dissolved in 8:2 Et_2O:hex ($n=5$ for without tropolone and $n=6$ for with tropolone; error bars represent SD). Note: the recoveries shown here for target OTs are corrected for losses incurred by the I.S. since the I.S. recovery was much lower (~40–60%) than that in the water analyses.

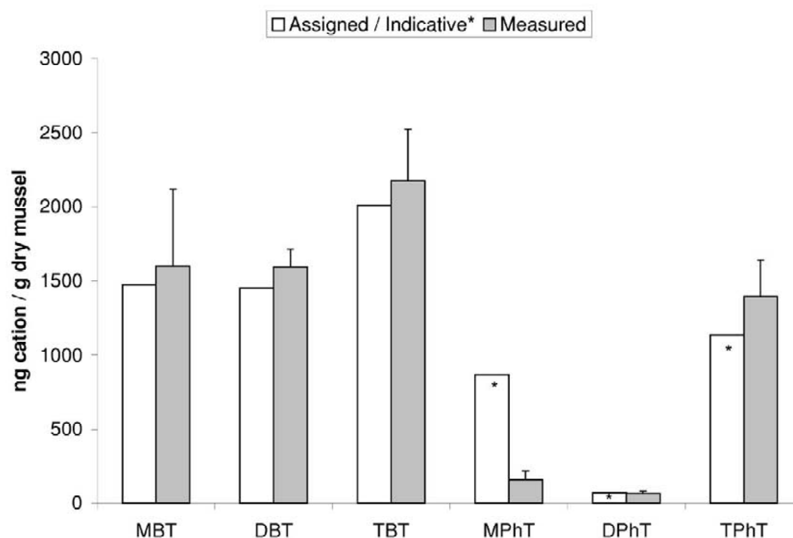

Fig. 6. Assigned or indicative values versus measured ($n = 4$; error bars are 95% confidence intervals) of six OT compounds in spiked dry mussel tissue CRM from a Quasimeme inter-calibration study. *The data obtained by expert laboratories for the phenyl derivatives were quite variable (>44% RSD) such that the assigned values actually only correspond to indicative values.

monobutyl- and monophenyl-tin trivalent ions, in extraction of the nine target OT analytes from the standard mixture spiked sediment samples by incorporation of 0.02% (w/v) of tropolone in the 8:2 Et_2O:hex extraction solvent. However, the cause of reduced recovery seen for the I.S. and DPhT in the trials with tropolone is unknown, but was reproducible for this particular matrix.

The method used for tissue in this work was almost identical to that for sediments except an initial TMAH digestion was incorporated to increase the exposure of contaminated tissue to the extraction solvent for a more efficient extraction. Our laboratory participated in a Quasimeme Intercalibration Study using spiked mussel tissue (dry). The results of our analysis are shown in Fig. 6 together with the assigned or indicative values determined from the robust mean of the values submitted from several participating laboratories ($n = 12$–18) after the exclusion of extreme values ($|Z| > 6$). The RSD of the robust mean from the inter-calibration results was 17–44% for the butyltins, however, for the phenyltins RSD was >44% and thus the values for these species were listed as only ''indicative'' by Quasimeme. In most cases, the 95% confidence interval of our measured values encompassed the assigned or indicative value. However, for MPhT the measured value was several times lower than the indicative value. In agreement with our results, Quasimeme reported that the inter-laboratory variance for phenyltins was high (>44%), and monosubstituted tins in particular were difficult to keep stable.

4. Conclusions

A comprehensive GC–HRMS based method for the determination of nine organotin compounds in water, tissue and sediment matrices was developed. The high resolution method allows specific determination of well resolved OT compounds with additional confirmation based on Sn isotope ratios. Multiple matrices can be extracted and determined to achieve comparable results due to the high selectivity of the analysis and low susceptibility to matrix specific interferences.

In-situ ethylation provided a quick sample preparation for water samples with good recovery of all nine organotin analytes. Tissue and sediment samples required a separate extraction and ethylation step followed by sample clean-up. The extraction

efficiency of monosubstituted OT analytes (i.e. MBT and MPhT) was greatly increased by the incorporation of 0.02% of tropolone in the extraction solvent. Overall method accuracy was assessed for butyl- and phenyl-tin analytes in mussel tissue using a certified reference material (Quasimeme dry mussel). Measured values agreed well with those assigned to the material except in the case of MPhT.

5. Nomenclature

DBT	Dibutyltin
DCDMS	Dichlorodimethylsilane
DPhT	Diphenyltin
DPrT	Di(*n*-propyl)tin
EI	Electron impact
eV	Electron volts
FPD	Flame photometric detection
GC	Gas chromatography
HRMS	High resolution mass spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
I.S.	Internal standard
LoD	Limit-of-detection
Mag	Magnetic sector
MBT	Monobutyltin
MDL	Minimum detectable limit
MIP-AES	Microwave induced plasma-atomic emission spectrometry
MPhT	Monophenyltin
MS	Mass spectrometry
NDR	Not detected due to incorrect isotopic ratio
OT	Organotin
PFPD	Pulsed flame photometric detection
ppb	Part-per-billion (i.e. ng/g)
ppt	Part-per-trillion (i.e. pg/g)
P.S.	Performance standard
QA/QC	Quality assurance/quality control
QP	Quadrupole
RRF	Relative response factor
RSD	Relative standard deviation
RT	Retention time
SEE	Standard error of the estimate
SIM	Selected ion monitoring
TBT	Tributyltin
TCyT	Tricyclohexyltin
TePeT	Tetra(*n*-pentyl)tin
TMAH	Tetramethyl ammonium hydroxide
TPhT	Triphenyltin

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We thank M. Fischer and T. Fraser from DFO's Regional Dioxin Lab (RDL) for help with analytical work, G. Stern from DFO's Freshwater Institute for his comments and revisions on earlier drafts of this manuscript and Fisheries and Oceans Canada and Environment Canada for financial support.

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