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Organotin speciation in environmental matrices by automated *on-line* hydride generation-programmed temperature vaporization-capillary gas chromatography–mass spectrometry detection

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Abstract

In the present contribution, a new automated *on-line* hydride generation methodology was developed for dibutyltin and tributyltin speciation at the trace level, using a programmable temperature-vaporizing inlet followed by capillary gas chromatography coupled to mass spectrometry in the selected ion-monitoring mode acquisition (PTV-GC/MS(SIM)). The methodology involves a sequence defined by two running methods, the first one configured for hydride generation with sodium tetrahydroborate as derivatising agent and the second configured for speciation purposes, using a conventional autosampler and data acquisition controlled by the instrument's software. From the method-development experiments, it had been established that injector configuration has a great effect on the speciation of the actual methodology, particularly, the initial inlet temperature (−20 °C; He: 150 ml/min), injection volume (2 μl) and solvent characteristics using the solvent venting mode. Under optimized conditions, a remarkable instrumental performance including very good precision (RSD < 4%), excellent linear dynamic range (up to 50 μg/ml) and limits of detection of 0.12 μg/ml and 9 ng/ml, were obtained for dibutyltin and tributyltin, respectively. The feasibility of the present methodology was validated through assays upon in-house spiked water (2 ng/ml) and a certified reference sediment matrix (Community Bureau of Reference, CRM 462, Nr. 330 dibutyltin: 68 ± 12 ng/g; tributyltin: 54 ± 15 ng/g on dry mass basis), using liquid–liquid extraction (LLE) and solid-phase extraction (SPE) sample enrichment and multiple injections (2 × 5 μl) for sensitivity enhancement. The methodology evidenced high reproducibility, is easy to work-up, sensitive and showed to be a suitable alternative to replace the currently dedicated analytical systems for organotin speciation in environmental matrices at the trace level.

Keywords: *On-line* hydride generation; Sodium tetrahydroborate; PTV-GC/MS(SIM); Speciation; Organotins; Dibutyltin; Tributyltin; CRM; Environmental matrices

1. Introduction

Organotin compounds have been extensively studied in the last years due to their high toxicity and remarkable impact in the aquatic environment. These compounds have been used worldwide in several areas of the human activity, particularly as components in anti-fouling paints, herbicides, fungicides and wood preservatives [1].

Tributyltin (TBT), appears as a top hazard substance in the priority pollutant lists of the European Union and United States Environmental Protection Agency, and has been included in the xenoestrogens or endocrine disrupters group, since the biocide effects observed in natural ecosystems of which the onset of *imposex* to a great number of gastropod species is a shocking example [2,3]. Shipyard docks and naval activities are in general the main sources of TBT in the estuaries and coastal waters, owing to the frequent use of anti-fouling paints, which must be carefully monitored to control the contamination level of those harmful substances. In environment, TBT can decompose into less

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substituted compounds (e.g. dibutyltin) and usually accumulates in sediments, which act as a long-term repository and help to maintain its persistence into aquatic ecosystems [4,5].

State-of-the-art analytical methodologies currently available to monitor organotin compounds are based in liquid–liquid extraction (LLE) and solid-phase extraction (SPE) sample preparation methods for enrichment, prior to Grignard alkylation followed by injection into hot vaporization inlets of gas chromatographs having selective detectors or hyphenated to particular techniques, such as mass spectrometry [6–10]. However, the modern approaches on organotin sample preparation are more dedicated to solventless methods, such as headspace solid phase micro-extraction and stir bar sorptive extraction after in situ derivatization using sodium tetrahydroborate or tetraethylborate, followed by introduction into hot vaporization inlets and thermal desorption devices coupled to gas chromatographs, respectively [11–22]. Besides those approaches have been proved to decrease the detection limits at the ultra-trace level, they are still a bit expensive, time consuming particularly to reach optimized equilibrium conditions and for the very non-polar volatile organotins, adsorption onto sampling flask glass walls and other surfaces, is a very important phenomenon causing sometimes analyte loss and reducing sensitivity. On the other hand, sodium tetraethylborate commonly used as ethylation reagent, is very sensitive to air and moisture and must be handled under nitrogen atmosphere, which is not practical for routine analysis. Therefore, sodium tetrahydroborate is much more suitable as hydridization reagent for organotin derivatization during the experimental work, since exhibit higher stability.

So far, hydride generation has been widely accepted as a simple and effective method to produce organotin hydrides prior to *on-line* cryogenic trapping followed gas chromatographic separation and quartz furnace atomic absorption spectrophotometric detection [23–25]. In spite of organotin derivatives being significantly more volatile and peak shapes been greatly improved with reduced tailing in the course of chromatographic analysis, low yields during in situ hydride generation can occur, particularly when organic and inorganic interferences are present in environmental matrices [26].

Sullivan et al. [27], proposed the *on-line* hydride generation using a sodium tetrahydroborate packed reactor inside the hot injector port of a gas chromatograph, which showed good performance for organotin speciation in biota matrices. By incorporating the derivatization step into the inlet of the analytical instrument, much time can be saved and previous sample preparation enrichment can be conventional (e.g. SPE) and simplified, experimental errors such as loss of volatiles and low derivatization yields can be avoided, the amounts of derivatising agent and analytical steps reduced and the analytical performance increased. Furthermore, automation is another potential benefit for *on-line* hydride generation through an autosampler and if a programmable temperature-vaporizing inlet is used, the sensitivity can be greatly improved by large volume injection [28–31].

The aim of the present study is to assess whether automated *on-line* hydride generation using a programmable temperature-vaporizing inlet followed by capillary gas chromatography coupled to mass spectrometry (PTV-GC/MS), is a suitable method for organotin speciation in environmental matrices at the trace level. The methodology is evaluated in terms of the *on-line* hydridization yields and speciation using sodium tetrahydroborate as derivatising agent, an automatic liquid sampler, a configured multi-method sequence and data acquisition controlled by the instrument's software. Optimization studies were carried out in order to foresee the most important parameters that could affect the analytical performance, including precision, linearity and limits of detection. Finally, the analytical validation of the methodology to monitor environmental matrices is tested through in-house standards and a certified reference material (CRM), including assays on water and sediment matrices, respectively.

2. Experimental

2.1. Reagents and standards

All solvents and reagents used had analytical grade. Ultra grade HCl (37%, Merck), methanol, ethyl acetate and hexane (for HPLC, Merck) were used. Sodium tetrahydroborate ($NaBH_4$; 95%) was obtained from Riedel-de Haën, for which freshly 4% aqueous solutions were daily prepared. Tropolone (99%), dibutyltin dichloride (DBT; 96%) and tributyltin chloride (TBT; 96%) were supplied from Sigma–Aldrich. A stock standard methanolic solution of DBT (0.8 mg/ml) and TBT (0.8 mg/ml) was used for method development and calibration studies, as well as for in-house spiked water assays. A standard mixture of DBT and TBT hydrides was prepared into a vial by dilution of 10 μl of the stock solution in 0.5 ml of hexane, following the addition of $NaBH_4$ powder and closed with a seal using a hand crimper prior to agitation by vortex (Velp Scientifica, Zx^3).

A certified reference material for DBT and TBT in coastal sediments (Community Bureau of Reference, CRM 462, Nr. 330; Certified value—DBT: 68 ± 12 ng/g and TBT: 54 ± 15 ng/g on dry mass basis) kept at $-30\,^{\circ}C$ was used for the sediment assays [32]. Deionised water was obtained from Milli-Q water purification systems.

2.2. Experimental set-up

In sediment assays, to each accurate weight (1 g; Mettler Toledo AG135) of CRM after a previous preparation according with the instructions for use (the material was re-homogenized manually for 5 min and each sample analyzed was dried in an oven at $100\,^{\circ}C$ for 4 h) [32], 30 ml of deionised water having 5% of HCl were added and the

mixture sonicated for 10 min (Branson 3510; 42 kHz). A centrifugation step (Hermle Z300) at 4000 rpm (2750 × *g*) for 10 min was used to recover the solution. For in-house spiked water assays, the stock solution was added to 30 ml of deionised water having 5% of HCl followed by sonification (10 min) to a final concentration of 2 ng/ml.

Solid-phase extraction enrichment was performed on a vacuum manifold (Visiprep SPE™; Supelco) and Supelclean™ ENVI™-18 cartridges (3 ml, 500 mg; Supelco) were used, according to previous studies [9]. The cartridges were preconditioned with 3 ml of ethyl acetate (−20 kPa) followed by vacuum (KnF Laboport, Neuberger) drying for 5 min (−90 kPa). The conditioning was performed with 3 ml of methanol, 3 ml of deionised water, 3 ml of HCl (5%) and slowly aspirated (−40 kPa). After loaded with the aqueous samples (−80 kPa), the column was washed with 3 ml of HCl 5% and 3 ml of deionised water followed by vacuum drying for 10 min. Subsequently, the elution took place with three times of 0.5 ml of ethyl acetate, followed by evaporation under a gentle stream of purified nitrogen.

For liquid–liquid extraction enrichment, aqueous samples were extracted with 25 ml of hexane having 0.05% of tropolone, and after phase separation the organic layer was removed followed by evaporation under a gentle stream of purified nitrogen.

The dry residues obtained were redissolved with 100 μl of ethyl acetate into vials and closed with seals using a hand crimper and after agitation by vortex, were placed into the automatic liquid sampler tray for speciation and GC/MS analysis. For *on-line* hydride generation, vials having fresh aqueous solutions of $NaBH_4$ (4%) were placed into the automatic liquid sampler tray.

2.3. Instrumentation

Capillary GC/MS analysis were performed on an Agilent 6890 Series gas chromatograph equipped with an Agilent 7683 automatic liquid sampler (ALS) coupled to an Agilent 5973N mass selective detector (Agilent Technologies, Little Falls, DE, USA). A programmed temperature vaporization (PTV) injector with a septumless sampling head (SLH; Gerstel) operating in the solvent vent mode, a silanized glass wool packed liner, liquid nitrogen as inlet cooling and a 10 μl syringe (sampling depth: −2 mm) were used. GC analysis were performed on a TRB-5MS (30 m × 0.25 mm I.D., 0.25 μm film thickness) capillary column (5% diphenyl, 95% dimethylpolysiloxane; Teknokroma, Spain), using helium as carrier gas maintained in the constant pressure mode (19.6 psi) at an average velocity of 54 cm/s.

The transfer line, ion source and quadrupole temperatures were maintained at 280, 230 and 150 °C, respectively. Electron ionization was performed at 70 eV electron energy and mass spectra in the full-scan mode were recorded in the range 35–550 Da. In the selected ion-monitoring (SIM) mode, two groups having target ions of DBT and TBT were monitored at different time windows defined by the corresponding retention times, maintaining a dwell time of 100 ms. The ions were chosen according to the mass spectra characteristic features obtained in the full-scan mode and by comparison with the Wiley's library reference spectral bank (G1035B; Rev D.02.00; Agilent Technologies). Data recording and instrument control were performed by the MSD ChemStation software (G1701CA; version C.00.00; Agilent Technologies).

Table 1
PTV, GC and MS instrumental operating parameters for the two methods defined in the running sequence; method #1: configuration for *on-line* hydride generation, method #2: configuration for organotin speciation

Parameters	Method #1	Method #2
PTV		
Injection mode	Solvent vent	Solvent vent
Injection speed	Fast	Fast
Injection volume (μl)	2; 5[a]	2; ×5[a]
Pre- and post-injection needle rinsing solvent	Methanol	Ethyl acetate
Vent gas flow rate (ml/min)	150	150
Vent pressure (psi)	0	0
Vent time (min)	0.95	1.45
Inlet initial temperature (°C) (hold time/min)	−20 (1.00)	−20 (1.50)
Temperature ramp rate (°C/min)	–	720
Inlet final temperature (°C) (hold time/min)	–	300 (5.00)
He purge flow rate (ml/min)	–	50
GC		
Oven initial temperature (°C) (hold time/min)	70 (1.00)	70 (2.00)
Temperature ramp rate (°C/min)	–	20
Oven final temperature (°C) (hold time/min)	–	250 (5.00)
MS		
Solvent delay (min)	0.99	5.00
SIM ions (*m*/*z*) (DBT)	–	120, 177
SIM ions (*m*/*z*) (TBT)		179, 235

[a] Used for multiple injections.

Each analysis was performed using a sequence constituted by two configured running methods. For *on-line* hydride generation (method #1) and speciation purposes (method #2), PTV, GC and MS instrumental operating parameters are summarized in Table 1. Method development and calibration studies were performed in triplicate by injecting 2 μl of $NaBH_4$ aqueous solution (method #1) followed by 2 μl of a organotin standard mixture in ethyl acetate (method #2). Pre- and post-injection needle rinsing to eliminate possible sample carryover was set using adequate solvents (Table 1) and blank runs were carried out by injecting pure ethyl acetate in method #2. Calibration studies were performed through the external standard methodology, using several diluted standard mixtures in ethyl acetate. In-house spiked water and sediment assays were performed in triplicate, by injecting 5 μl of $NaBH_4$ aqueous solution for hydride generation (method #1) followed by multiple injections (×5 μl) for speciation (method #2).

3. Results and discussion

The present study focused on the development of a new approach for organotin speciation using an automated *on-line* hydride generation PTV-GC/MS methodology. During our experiments, DBT and TBT were selected as model compounds once the significative occurrence in a large number of environmental reports.

For automation purposes, simultaneous *on-line* hydride generation and speciation injections into the PTV-inlet are required during a single run analysis. Nevertheless, owing to the fact that conventional instrument's software does not allow for multiple injections from different vials in a single run, this limitation could be overcome by using a multi-method sequence.

Since the beginning, we start to establish the best analytical strategy for *on-line* hydride generation followed organotin speciation using an ALS, a running sequence and data acquisition controlled exclusively by the instrument's software. Thus, a sequence table was previously defined for two configured running methods (Table 1), including the corresponding vials location in the ALS tray, for the derivatising reagent and samples under study.

The first method (#1) of the running sequence was configured for *on-line* hydride generation purposes, in which a vial having an aqueous solution of $NaBH_4$ is automatically selected from the ALS tray, to inject fresh derivatising reagent into the inlet. During all running time (1 min) required for the injection operation, the PTV and GC operating conditions stay unaltered and no MS data was acquired, for which the solvent delay was set at 0.99 min (Table 1). Subsequently, a second method (#2) configured for speciation purposes, start with the same PTV and GC conditions of the former method, in which a vial having the sample is automatically selected from the ALS tray, to inject the organotins into the inlet. After injection and simultaneous solvent venting, the PTV inlet temperature starts to ramp up following GC oven temperature and consecutively, MS data acquisition starts to record (Table 1) after a pre-defined solvent delay period (5.00 min).

Therefore, the optimization of the instrumental operating conditions including the calibration parameters, as well as the analytical validation for organotin speciation in environmental matrices, will be discussed in detail.

3.1. Optimization of the instrumental operating conditions

Before starting the method development studies, the main GC/MS operating conditions were previously established to attain the best instrumental performance (Table 1). Since method #1 was configured exclusively for *on-line* hydride generation, method #2 was configured with standard GC/MS conditions for organotin high-resolution separation and detection, according to literature [6,7,9].

The direct injection of a standard mixture having both organotin hydrides, allowed a suitable analytical time to monitor DBT (5.35 min) and TBT (7.75 min), by using the operating GC/MS conditions of method #2 in the full-scan mode acquisition (Table 1). Nevertheless, for sensitivity and selectivity enhancement at the trace level, MS recording in SIM mode acquisition was used, by choosing two groups having target ions for DBT (m/z 120 and 177) and TBT (m/z 179 and 235), according to the mass spectra characteristic features obtained in the full-scan mode. Each group was monitored at time windows from 5.00 to 6.50 min and 7.00 to 8.50 min, defined by the corresponding DBT and TBT retention times, respectively.

Taking into consideration that PTV inlet configuration has a great effect on the analytical performance of the present methodology, a series of experiments were designed, including parameters such as the type of the solvents and volume used for the injection operation, as well as vent temperature, He flow rate and vent time, whether to foresee the optimized instrumental conditions under the solvent venting mode.

In a first approach, the effect of the solvent characteristics on the injection operation for *on-line* hydride generation showed to be crucial for organotin speciation, according to literature [27]. During the injection stage of method #1, aqueous solutions of $NaBH_4$ (4%) proved that water is the most suitable solvent to introduce the derivatization reagent into the PTV-liner, providing effective hydridization. Moreover, water exhibits better solvation medium for $NaBH_4$ than other low boiling point solvents, which plays an important role on the speciation yields of DBT and TBT [27].

In the course of the optimization for *on-line* hydride generation (method #1), the vent temperature and He flow rate parameters were kept constant at $-20\,^{\circ}C$ and 150 ml/min (0 psi), respectively. Because part of the water becomes trapped with $NaBH_4$ into the liner, this occurrence greatly enhances hydridization and consequently organotin speciation. Lower $NaBH_4$ concentrations or other solvents (e.g. methanol) were avoided, since they proved to decrease organotin abundances, promoting substantial background instability.

Throughout the injection stage of method #2, solvents that have non-polar characteristics such as ethyl acetate, showed to be the most suitable for an efficient venting and simultaneously not interfering with organotin hydrides formation. Therefore, the effect of the inlet temperature and He flow rate on the abundances of the highly volatile organotin hydrides are important parameters, particularly throughout the venting process, which were studied individually in the range from -40 to $40\,^{\circ}C$ and 25 to 150 ml/min, respectively.

Fig. 1a and b depicts the effect of both parameters on the average abundances of DBT and TBT hydrides during method #2, where it is noteworthy that TBT present a much higher abundance than DBT for the same operating conditions, which shows that the derivatization efficiency can be assumed to be greater for the former. For vent temperatures above $-20\,^{\circ}C$, the average abundance of both compounds decreases drastically (Fig. 1a), owing to the very high volatility exhibited, which promotes substantial losses during the

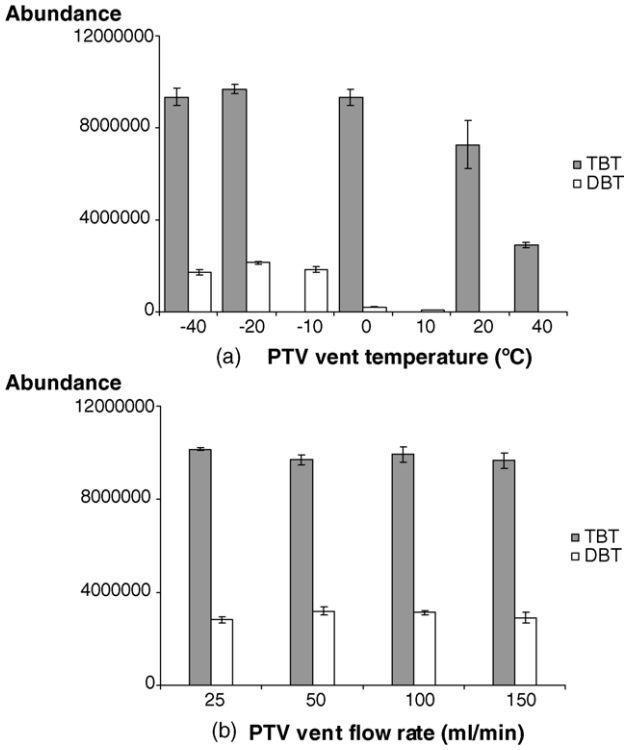

Fig. 1. Effect of the PTV vent temperature at 150 ml/min (a) and vent flow rate at −20 °C (b) on the average abundance of DBT and TBT (25 μg/ml) during method #2 of the present methodology.

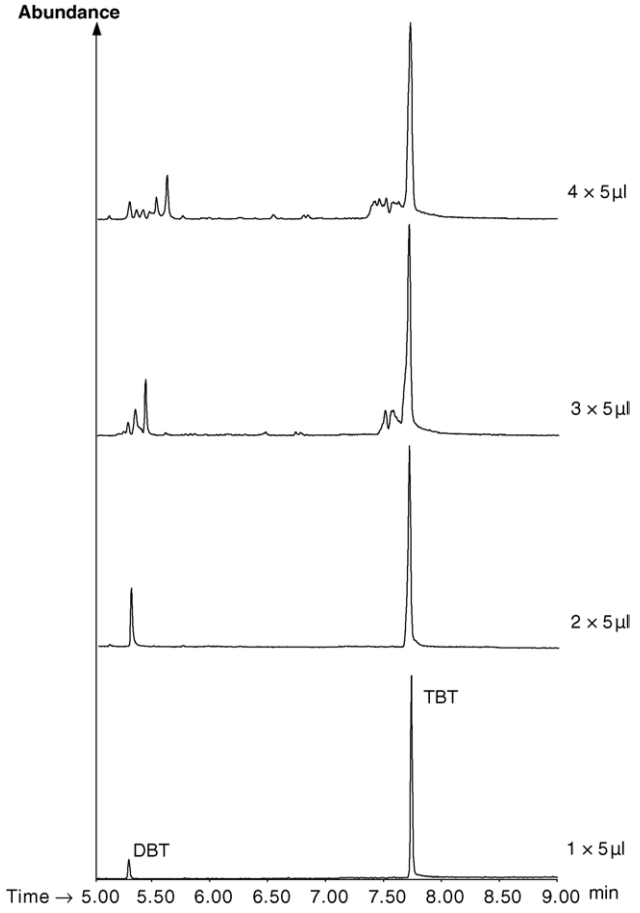

Fig. 2. Total ion chromatograms from a standard mixture of DBT and TBT (25 μg/ml) showing the effect of multiple injections (×5 μl) on the peak shape and chromatographic data during method #2 of the present methodology.

venting process at 150 ml/min, particularly for DBT. However, for vent flow rates ranging from 25 to 150 ml/min (−20 °C), negligible differences occurred in the average abundances of both target compounds (Fig. 1b). These results are in agreement with several reports [28–30], which pointed out that vent temperature has the greatest effect for trapping organotin derivatives into the PTV inlet. Consequently, it could be stated that temperature is the most important parameter on the effective trapping of DBT and TBT hydrides, even for higher venting flow rates. Furthermore, by using the same starting vent temperature in both methods (−20 °C), analytical time could be also saved in the running sequence, particularly for inlet temperature equilibration during the pre-run of method #2. Therefore, initial PTV vent temperature and He flow rate of method #2 were keep at −20 °C and 150 ml/min for further studies, respectively, providing suitable solvent elimination during organotin speciation.

Regarding the effect of the vent time on the ethyl acetate evaporation into the inlet, several experiments were performed during the injection operation of method #2, which proved that no advantages were attained for venting periods longer than 1.45 min.

Throughout the method development studies, 2 μl of 4% $NaBH_4$ aqueous solution (method #1) followed 2 μl of an ethyl acetate standard mixture of DBT and TBT (method #2) were chosen, since good analytical performance was reached. However, organotin speciation from environmental matrices at the ultra-trace level demands for large volume injection to enhance sensitivity, particularly during the injection operation of method #2. Previous tests had showed that syringe system configuration, such as size and plunger speed parameters, had a great influence on the analytical performance. By using 100 μl syringes with low speed plunger, the absence of chromatographic peaks and very poor analytical data was achieved. To overcome this limitation, 10 μl syringes using the injection system in the fast plunger mode, allowed good performance during all the running sequence studies and subsequently, multiple injections could be selected for sensitivity enhancement in the course of method #2.

Fig. 2 depicts total ion chromatograms from a standard calibration mixture of DBT and TBT injected one to four times (×5 μl) during method #2, after the injection of 5 μl of $NaBH_4$ aqueous solution for hydride generation (method #1), under optimized instrumental conditions. It could be observed that when more than two consecutive injections are set, poor chromatographic data is obtained, particularly the occurrence of multiple peaks presenting a shift in the elution for DBT and poor peak shape for TBT. This evidence suggests that in spite of the hydridization yield may decrease, especially due the occurrence of the solvolysis

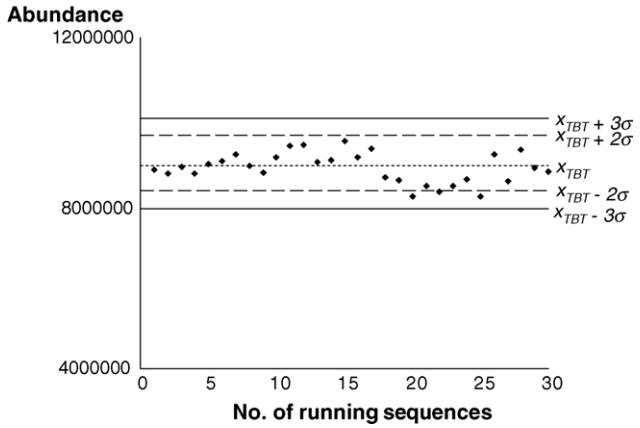

Fig. 3. Shewart chart of the average abundance of TBT hydride (x_{TBT}; (25 μg/ml)) and corresponding confidence bands ($x_{TBT} \pm 2\sigma$; $x_{TBT} \pm 3\sigma$), showing the reproducibility after 30 consecutive running sequences by *on-line* hydride generation PTV-GC/MS(SIM) analysis, under optimized instrumental conditions.

reaction ($NaBH_4 + 4H_2O \rightarrow 4H_2 + NaB(OH)_4$), other effects such as the adsorption phenomenon promoted by the solvolysis products, seems to assume a great influence when more than two consecutive injections are set. Therefore, 2×5 μl was chosen as maximum sample volume during multiple injection operation of method #2 for all subsequent experiments, involving the analytical validation of the present methodology for organotin speciation in environmental matrices.

By using this injection volume configuration (2×5 μl) in method #2, the initial vent time and oven temperature hold time were set at 2.0 and 2.5 min, respectively, which provide enough dwell time for adequate venting and injection operation prior to GC/MS analysis.

3.2. Calibration parameters

To evaluate the linear dynamic range of the present methodology, several diluted calibration mixtures of both organotins were studied in triplicate, under optimized instrumental conditions. By measuring the abundance of the corresponding target ions in SIM mode acquisition (method #2) through the external standard method, the results obtained showed good linearity, ranging from 0.5 to 50 μg/ml and 0.1 to 50 μg/ml, with good correlation coefficients for DBT ($r^2 = 0.9980$; *m/z* 120 plus 177) and TBT ($r^2 = 0.9988$; *m/z* 179 plus 235), respectively.

The instrumental precision was carried out by performing six replicates of a standard calibration mixture at the 10 μg/ml level under optimized instrumental conditions, which enable relative standard deviations (RSD) lower than 4%. The shewart chart of Fig. 3, exemplifies the average abundance of TBT hydride (x_{TBT}) after 30 running sequences, showing the remarkable reproducibility of the present methodology, in which $x_{TBT} \pm 3\sigma$ confidence bands would exactly include 99.74% of Gaussian data.

Furthermore, the instrumental limit of detection (LOD) was also evaluated through the injection of diluted standard mixtures having both organotins and calculated with a signal-to-noise ratio of three (method #2). For the present methodology, it was found an instrumental LOD of 0.12 μg/ml for DBT and 9 ng/ml for TBT, under optimized instrumental conditions.

During method development and calibration experiments, pre- and post-injection needle rinsing were set using methanol and ethyl acetate, respectively (Table 1), eliminating possible contamination between both methods of the running sequence. Besides no carryover was observed during several blank runs, it is desirable change the liner after 30 running sequences to avoid for solvolysis products overloading ($NaB(OH)_4$), which could give rise to poor analytical data.

3.3. Analytical validation to monitor environmental matrices

To validate the present methodology for organotin speciation, several assays were carrying out, including in-house spiked water and CRM sediment samples.

In general, to attain the vestigial content of organotins usually found in environmental matrices, previous sample preparation is a must to decrease instrumental LODs. By using an enrichment factor of 300 (from 30 to 0.1 ml), LODs in samples can drop easily to the ultra-trace level. Thus, our analytical strategy was also to demonstrate the suitability of the present methodology for the well-established sample preparation techniques, i.e. LLE and SPE, which had been proved very good recovery yields for organotins from environmental matrices [6–10].

Fig. 4a and b shows mass fragmentograms of DBT and TBT hydrides, obtained after SPE and LLE enrichment from CRM sediment and in-house spiked water matrices, respectively, followed by *on-line* hydride generation PTV-GC/MS(SIM) analysis under optimized instrumental conditions. It could be observed that excellent performance is attained by the present methodology, in particular a remarkable selectivity and sensitivity. The average contents achieved for DBT and TBT from several assays are summarized in Table 2.

The use of CRM's seems to be the best way to validate the present methodology for organotin speciation in environmental sediments. The results obtained for both organotins, showed consistent data in relation with the CRM calibration certificate reported by five reference European laboratories (DBT: 68 ± 12 ng/g and TBT: 54 ± 15 ng/g), which used other analytical methodologies [32].

According to the European Co-operation for Accreditation of Laboratories guidance (EA2/03) [9,33], a suitable way for judge the quality of measurement results during inter-laboratory comparisons, consists in calculating the deviation normalized (E_n) with respect to the stated uncertainty, i.e. $E_n = |x_{lab} - x_{ref}|/|\sigma_{lab}^2 + \sigma_{ref}^2|^{1/2}$. In this work, x_{lab} represents the average content for organotins measured in the CRM

Table 2
Summary of the average contents obtained for DBT and TBT from in-house spiked water and CRM sediment matrices by LLE and SPE sample enrichment followed *on-line* hydride generation PTV-GC/MS(SIM) analysis, under optimized instrumental conditions

Sample preparation method ($n = 3$)	Organotin	CRM sediment matrices	In-house spiked water matrices[a]
		Mass fraction ($x_{lab} \pm \sigma_{lab}$; ng/g)	Recovery (percent ± RSD)
LLE	DBT	47 ± 19	87 ± 8
	TBT	33 ± 22	93 ± 18
SPE	DBT	48 ± 19	85 ± 7
	TBT	34 ± 27	80 ± 11

[a] Spiked at the 2 ng/ml level.

sediment obtained by the present methodology after LLE or SPE sample preparation enrichments, x_{ref} is the reference value given by the CRM calibration certificate (see Section 2), σ_{lab} and σ_{ref} are the corresponding uncertainties, respectively. As established in the same guidance, absolute values of deviation normalized less than unity ($E_n < 1$) should be expected as a guideline for the measurement to be acceptable. By using the experimental data obtained (Table 2), values of E_n lower than one are attained for all assays performed, showing that either LLE or SPE sample preparation enrichment followed by *on-line* hydride generation PTV-GC/MS(SIM) analysis is well validated for DBT and TBT speciation in sediment matrices.

Because the chemical stability of organotin species in water matrices is still of great concern even in CRM's

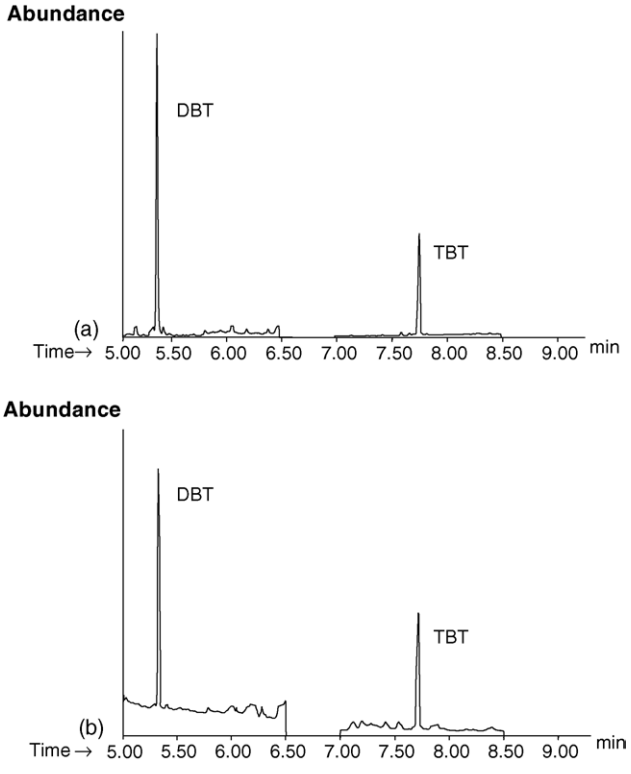

Fig. 4. Mass fragmentograms of DBT (m/z 120 plus 177) and TBT (m/z 179 plus 235) obtained from CRM sediment (DBT: 68 ± 12 ng/g and TBT: 54 ± 15 ng/g) (a) and in-house spiked water (2 ng/ml) (b) matrices by SPE and LLE, respectively, prior to *on-line* hydride generation PTV-GC/MS(SIM) analysis under optimized instrumental conditions.

[34], recovery tests (percent = $x_{lab}/\mu_{std} \times 100$) using in-house spiked water assays were rather performed, whether to judge the quality of the present methodology for aqueous samples, where x_{lab} is the average measurements obtained for organotins and μ_{std} is the expected value (2 ng/ml). From the results obtained, it could be observed that recoveries higher than 80% were obtained for all assays performed (Table 2), showing that either LLE or SPE followed *on-line* hydride generation PTV-GC/MS(SIM) analysis are also well validated for DBT and TBT speciation in water matrices.

Taking into consideration the analytical data obtained, it could be stated that LLE or SPE prior to *on-line* hydride generation PTV-GC/MS(SIM) methodology shows high robustness for organotin speciation in environmental matrices at the trace level, due to the remarkable accuracy obtained in all experiments performed. However, it must be emphasized that the deviations observed in the validation assays, may be partly attributed to losses during the previous sample preparation procedures. Unlike other dedicated analytical systems, several advantages can be found by the present methodology, particularly, no expensive devices or robotic systems for automated derivatization purposes are required, currently sample preparation procedures can be implemented with minimal sample requirement avoiding other tedious and costly enrichment techniques, the derivatization reagent may be prepared easily without special apparatus or time consuming sample handling and *on-line* automated derivatization brings competitiveness and productivity for routine work. In opposition to organotin speciation using solventless methodologies, sample reanalysis is always possible, interferences or possible losses during in situ organotin derivatization are avoided, and sample enrichment could be performed without a prompt analysis.

At last, some other benefits of using sodium tetrahydroborate is the least expensive hydridization derivatization agent, safety, ease to work-up and versatility, since the present methodology may have application for other organometallic compounds (e.g. organic arsenicals) or environmental pollutants that can undergo hydridization.

4. Conclusions

In this work, a new automated *on-line* hydride generation PTV-GC/MS(SIM) method was successfully developed for

DBT and TBT speciation at the trace level. The methodology was evaluated in terms of hydride generation and speciation using sodium tetrahydroborate as derivatising agent, an autosampler, a sequence defined by two configured run methods and data acquisition controlled by the instrument's software.

From method-developed studies, it has been established that PTV configuration in the solvent venting mode, particularly, the initial inlet temperature, injection volume and solvent characteristics are critical parameters to obtained suitable analytical data. Under optimized conditions, a remarkable analytical performance including very good precision, excellent linear dynamic range and limits of detection at the ultra-trace level were attained for DBT and TBT speciation.

The present methodology was validated through assays upon in-house spiked water and CRM sediment matrices at the trace level, allowing a remarkable accuracy using previous LLE and SPE sample preparation techniques and multiple injections for sensitivity enhancement. The cost-effectiveness and the reliability of the *on-line* hydride generation PTV-GC/MS(SIM) methodology should undoubtedly make it a valuable tool to monitor organotins from environmental matrices at the trace level.

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