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Short communication

Determination of tributyltin in environmental water matrices using stir bar sorptive extraction with *in-situ* derivatisation and large volume injection-gas chromatography–mass spectrometry

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ABSTRACT

Stir bar sorptive extraction with *in-situ* derivatization using sodium tetrahydridoborate ($NaBH_4$) followed by liquid desorption and large volume injection-gas chromatography–mass spectrometry detection under the selected ion monitoring mode (SBSE($NaBH_4$)$_{in\text{-}situ}$–LD/LVI-GC–MS(SIM)) was successfully developed for the determination of tributyltin (TBT) in environmental water matrices. $NaBH_4$ proved to be an effective and easy *in-situ* speciation agent for TBT in aqueous media, allowing the formation of adducts with enough stability and suitable polarity for SBSE analysis. Assays performed on water samples spiked at the 10.0 μg/L, yielded convenient recoveries (68.2 ± 3.0%), showed good accuracy, suitable precision (RSD < 9.0%), low detection limits (23 ng/L) and excellent linear dynamic range (r^2 = 0.9999) from 0.1 to 170.0 μg/L, under optimized experimental conditions. By using the standard addition method, the application of the present methodology to real surface water samples allowed very good performance at the trace level. The proposed methodology proved to be a feasible alternative for routine quality control analysis, easy to implement, reliable and sensitive to monitor TBT in environmental water matrices.

1. Introduction

Over the past three decades, organotin compounds have been widely used in several industrial and agricultural applications. Organotin compounds are mainly used as fungicides, biocides, wood preservatives and polyvinyl chloride stabilizers. Among them, tributyltin (TBT) has been extensively used as a biocide agent in antifouling paints of ship hulls, harbor structures and aquaculture nets [1]. Owing to their numerous applications, these compounds are continuously released into marine and fresh water environment leading to its contamination. Toxic effects of organotin compounds on aquatic organisms and mammals are well known, such as induction of the imposex effect, *e.g.*, superimposition of male sexual characteristics on female organisms [2,3]. Due to the fact that organotin compounds have high bioaccumulation potential, control of contamination levels in environmental samples is necessary [4]. As a consequence, several water quality criteria and legislative restrictions have been adopted in order to control the usage of these compounds. The Marine Environmental Protection Committee proposed a global prohibition on the application of organotins as biocides in antifouling systems on ships by January 2008 [5].

For many years, researchers have been focused on the determination of organotin compounds from many different types of matrices. Nevertheless, the quantification of these compounds in environmental samples has been considered a very difficult task, since they had shown instability and usually occur at the trace level [6]. Therefore, new sensitive and cost-effective methodologies are still demanded for organotin speciation in particular using gas chromatography–mass spectrometry (GC–MS), once it is the hyphenated system commonly used in many laboratories and allows the unequivocal identification through the mass spectral features. Among the analytical schemes usually proposed, liquid–liquid extraction, solid-phase extraction and solid-phase micro extraction (SPME) with derivatization followed by GC–MS have been currently used for the determination of organotin compounds in environmental and biological matrices [7–9]. More recently, stir bar sorptive extraction (SBSE) has been successfully employed as a novel sample preparation technique, based on the same principles as those of SPME, particularly for enrichment and sensitive determination of priority organic pollutants, including organotins, in water samples but also in many other matrices [10–16]. Besides the noteworthy performance demonstrated by SBSE for TBT speciation, the analytical approaches claim also to be an

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in-situ derivatization step compatible with the hot injection by GC analysis [16]. Even so, most of those derivatisation agents such as some borate derivatives (*e.g.* sodium tetraethylborate) are neither stable nor easy to manipulate. So far, sodium tetrahydridoborate ($NaBH_4$) has been accepted as a much more simple and effective chemical agent to produce organotin adducts prior to GC–MS analysis [17]. Furthermore, $NaBH_4$ exhibits a remarkable stability and an easier practical approach in comparison to other derivatization agents.

The present contribution aims the development of a novel and easy analytical strategy for TBT speciation by combining SBSE with *in-situ* derivatization using $NaBH_4$, followed by liquid desorption and large volume injection-gas chromatography–mass spectrometry under the selected ion monitoring mode (SBSE $(NaBH_4)_{in\text{-}situ}$–LD/LVI-GC–MS(SIM)). The performance of the proposed methodology was evaluated in terms of accuracy, precision, linearity and detection limits. The application to environmental water matrices is also addressed.

2. Materials and methods

2.1. Chemicals and standards preparation

All reagents and solvents were of analytical grade and used with no further purification. HPLC-grade methanol (MeOH, 99.9%), acetonitrile (ACN, 99.9%), acetone (DMK, 99.5%) and ethyl acetate (EtOAc, 99.9%) were purchased from Panreac (Spain). The tributyltin chloride (TBT, $Sn(C_4H_9)_3Cl$; 96%) was purchased from Sigma-Aldrich (Germany). Sodium chloride (NaCl, 99.9%) was obtained from AnalaR (England). $NaBH_4$ and *n*-pentane (*n*-C_5, 99%) were purchased from Riedel-de-Haën (Germany). Ultra-pure water was obtained from milli-Q water purification systems (USA). A stock solution of TBT (1108.0 mg/L) was prepared by dissolving 27.7 mg of TBT in 25 mL of MeOH. To study the derivatization step and instrumental evaluation, a TBT hydride (TBTH) working standard solution was daily prepared through the pre-derivatization by adding 300 μL of 4% (w/v) $NaBH_4$ followed by incubation at room temperature and diluted to the desired concentration. For *in-situ* derivatization, method optimization, validation and real matrix assays, derivatization solutions (40.0 mg/L) were prepared by mixing 0.12 mg of $NaBH_4$ to a final volume of 3 mL with ultrapure water. Environmental water samples were collected with PVC bottles from the Tagus river in three naval docks (Santa Apolónia, Parque das Nações and Alcântara) in the metropolitan area of Lisbon (Portugal). All the samples were filtrated (Whatman No1 filters, USA) and stored at −4 °C before used.

2.2. Experimental set-up

The stir bars (Gerstel, Germany) coated with 10 mm in length and 0.5 mm film thickness of PDMS (24 μL) were pre-conditioned before use by treating them with ACN during 20 min. In a typical assay, 30 mL of ultrapure water with 40% MeOH spiked with 200 μL TBT working standard at different concentrations (0.1–170.0 μg/L) and 300 μL of the derivatization solution were introduced into glass flasks already contained the stir bar. The assays were performed with 4 h extraction time and 1250 rpm agitation speed. For back-extraction, the stir bars were removed from the samples, placed into a 2 mL vial with insert containing 200 μL of the desorption solvent (*n*-pentane), ensuring their total immersion prior to ultrasonic treatment (15 min) at a constant temperature (25 °C). After back-extraction, the stir bars were removed and the vials were resealed and placed on the autosampler prior to GC–MS analysis. For real sample assays, 30 mL with 40% MeOH were used, for which 300 μL of derivatization solution and spiked TBT working standard were used, following by the same procedure described before in triplicate. Control and blank assays were also performed in triplicate using the same procedure without spiking.

2.3. Instrumentation settings

Large volume injection GC–MS analysis were carried out on an Agilent 6890 Series gas chromatograph equipped with a 5973 N mass selective detector (USA). A programmed temperature vaporization (PTV) injector with a septumless sampling head (Gerstel, Germany) operating in the solvent vent mode (vent time 0.30; flow 150 mL/min; pressure 0 psi; purge 60 mL/min at 2 min), for which the inlet was programmed from −20 °C (0.35 min) to 300 °C at a rate of 600 °C/min and subsequently reduced to 200 °C at a rate of 50 °C/min. The injection volume was 5 μL. 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 oven temperature was programmed from 70 °C (2 min) at 20 °C/min to 250 °C (held for 5 min) [16].

The transfer line, ion source and quadrupole temperatures were maintained at 280, 230 and 150 °C, respectively and a solvent delay of 5 min was selected. Electron ionization was performed at 70 eV and mass spectra in the full scan acquisition mode were recorded in the range 35–550 Da. In the selected ion-monitoring acquisition (SIM) mode, target ions (*m/z* 121, 179 and 235) were selected according to the mass spectra characteristic features obtained in the full-scan mode and by comparison with Wiley's library reference spectral bank (G1035B; RevD.02.00). Data recording and instrument control were performed by the MSD ChemStation software (G1701 CA; ver.C.00.00; Agilent Technologies).

3. Results and discussion

3.1. In-situ derivatization and instrumental conditions

Since the very beginning, the derivatization step, involving the reaction of TBT with $NaBH_4$, was carefully examined in MeOH media. According to literature [16,18], preliminary experiments showed that $NaBH_4$ provided good specificity for TBT derivatization, as depicted in Fig. 1a. Even so, we also tested the peak areas against the incubation time to foresee the TBT derivatization progress with $NaBH_4$. Fig. 1b depicts the response obtained showing that after 60 min the TBTH ($Sn(C_4H_9)_3H$) is completely

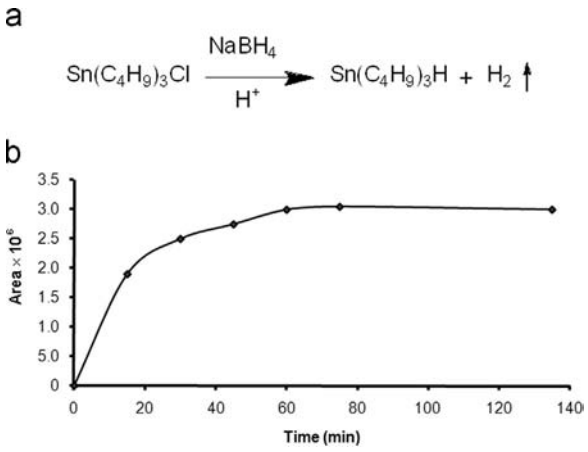

Fig. 1. Scheme of the derivatization involving TBT with $NaBH_4$ (a) and reaction progress plot obtained by LVI-GC–MS showing the peak areas (TBTH) against incubation time (b).

formed without any advantages for longer incubation periods. The stability of TBTH was also checked through GC–MS analysis two days after the derivatization procedure, in which the abundance of TBTH remained in the same order of magnitude. It must be emphasized that the derivatization step plays an important role in the present methodology, due to conferring volatile characteristics for TBT prior to GC–MS analysis. Once the derivatization step established, we start to study the best instrumental conditions for TBTH analysis using pre-derivatized working standards.

For sensitivity and selectivity enhancement at the trace level, MS recording in SIM mode acquisition was used, choosing m/z of 235, 179 and 121 target ions, in agreement with the mass spectra characteristic features obtained in the full-scan mode [17]. The PTV inlet configuration has a great effect on the analytical performance of the present methodology, and therefore, the instrumental set-up and optimization were based on the previous work [17]. The instrumental limits of detection (LOD) and quantification (LOQ) for TBTH were calculated with signal-to-noise ratios (S/N) of 3/1 and 10/1, where values of 2.3 μg/L and 7.6 μg/L were achieved, respectively. Subsequently, the instrumental calibration was performed with four standard solutions having concentrations ranging from 14.0 to 1450.0 μg/L from which excellent linear dynamic responses were obtained with convenient determination coefficients ($r^2=0.9985$). The precision was also evaluated, resulting in relative standard deviations (RSD) below 4.0% and no carry-over was observed by series of replicate injections ($n=5$) since the background was always below the LOD achieved.

3.2. Optimization and validation of $SBSE(NaBH_4)_{in\text{-}situ}$–LD/LVI-GC–MS(SIM)

After establishing the derivatization and instrumental conditions, we proceed to evaluate SBSE assays using *in-situ* derivatization and parameter optimization that are known to influence the analytical process [10,19]. In the first approach, we start to evaluate the best liquid desorption (LD) conditions, which ensure the complete back-extraction of TBTH from the SBSE polymeric phase. Solvents such as MeOH, ACN, DMK, EtOAc and n-C_5 were chosen in order to survey the stripping performance, using standard experimental conditions; extraction: 1 h (1000 rpm), 10% MeOH; back-extraction: 10 min under sonification treatment. From the data obtained (Fig. 2a), n-C_5 was chosen as back-extraction solvent due to the higher ability to remove the TBTH from the stir bars polymeric phase. On the other hand this solvent is one of the most favorable for the solvent-vent LVI operation due the low boiling point exhibited. After the selection of the most effective solvent for back-extraction, the desorption time was evaluated (5, 10 and 15 min) using n-C_5, where a slightly increment on the chromatographic signal was obtained for 15 min (data not shown). Consequently, a period of 15 min was established for the back-extraction step with n-C_5.

According to SBSE theory [11,20], the equilibrium time, agitation speed and characteristics of the aqueous media (ionic strength and polarity) evaluation are very important parameters to better control the extraction conditions. Starting with the stirring rate, this parameter can influence the mass transfer of the analytes towards the polymeric phase during the equilibrium process. For the present study 1250 rpm showed much higher performance on the recovery yields of TBTH. Subsequently, the equilibrium time was also evaluated by performing experiments within 1 and 17 h which proves that no advantages were observed for extractions above 4 h (data not shown). Nevertheless and since TBTH presents slightly non-polar characteristics ($\log K_{O/W}=4.10$), and the "wall-effect" phenomena can occur, *i.e.*, the adsorption of the analytes onto the glass walls of the sampling flasks. Therefore, assays performed with the addition of MeOH have in many cases

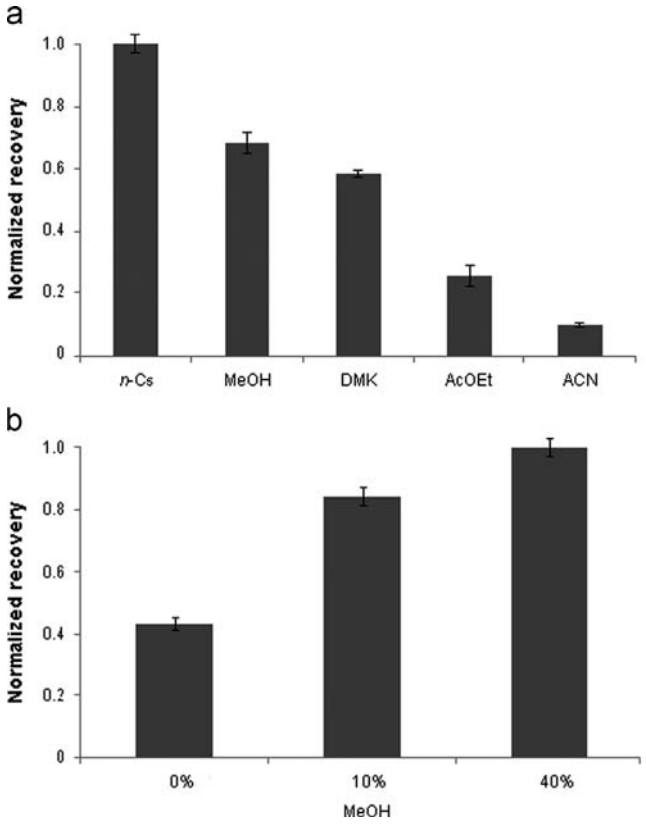

Fig. 2. Effect of the back-extraction solvent (a) and organic modifier (b) on the recovery efficiency of TBTH by the present methodology.

a positive influence on the efficiency, eliminating the adsorption phenomena of the analytes onto the glass wall of the sampling flask [21]. In order to study the polarity effect on the recovery yields, addition of MeOH (up to 40%, v/v) onto aqueous media was performed. As it is clearly observed in Fig. 2b the progressive addition of MeOH increase significantly the recovery yields of the TBTH. Nevertheless, higher MeOH content has a negative effect on the extraction process. Furthermore, the evaluation of the ionic strength was also evaluated through the addition of NaCl (up to 10%, w/v) onto matrix media, from which any advantage was observed (data not shown).

After establishing the best experimental conditions, assays performed on spiked (10.0 μg/L) water samples, yielded average efficiencies of $68.2 \pm 3.0\%$ for TBTH. This average recovery is expected since it is compatible with the low hydrophobic characteristics of the TBT adduct. Subsequently and for validation purposes, we also evaluate the linear dynamic range of the present methodology with five calibration standards having TBTH concentrations ranging from 0.1 to 170.0 μg/L, under optimized experimental conditions. From the data obtained, excellent linearity was attained with remarkable determination coefficients ($r^2=0.9999$). It is also noteworthy that the precision achieved for the present methodology, using within- and between-day repeatability assays calculated as RSD on three replicates, gave rise to variations lower than 8.3%. It must be noted that according to the requirements of Directive 98/83/EC for trace level analysis of priority organic compounds, the proposed methodology may be considered acceptable since an overall precision lower than 25% is reached. The sensitivity of the actual methodology was also checked through the LOD and LOQ achieved for TBTH and measured with a S/N of 3/1 and 10/1, respectively. The values achieved were 23 ng/L for LOD and 77 ng/L for LOQ, presenting the same order of magnitude previously reported by using different methodologies but the

Table 1
Average contents (C_0) and regression parameters obtained through SAM for TBT determined in real water matrices by SBSE($NaBH_4$)$_{in\text{-}situ}$–LD/LVI-GC–MS(SIM), under optimized experimental conditions.

Water matrices/sites[a]	C_0 (μg/L)	r^2
Alcântara	< LOD	0.9945
Santa Apolónia	25.3 ± 1.7	0.9931
Parque da Nações	7.9 ± 1.7	0.9990

[a] Lisbon (Portugal).

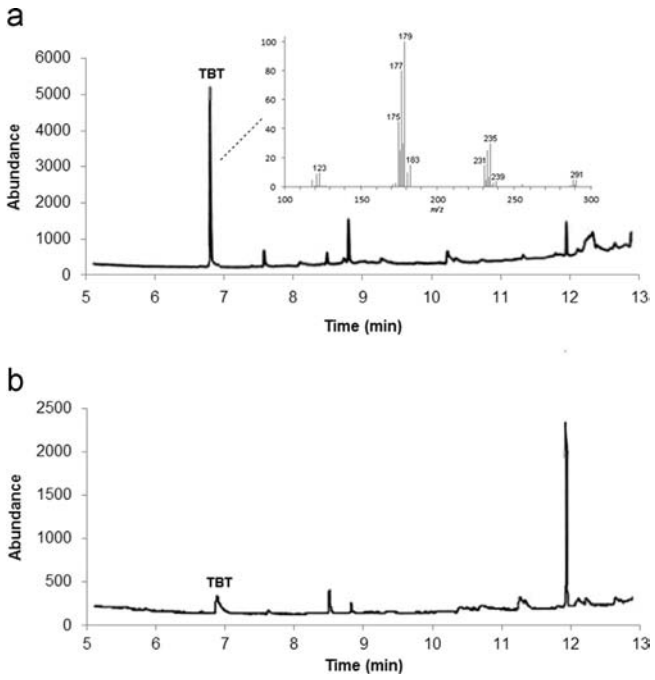

Fig. 3. Mass fragmentograms and mass spectral confirmation ("full-scan" recording) obtained in environmental water samples from Parque das Nações (a) and Santa Apolónia (b) sites (Lisbon, Portugal) by SBSE($NaBH_4$)$_{in\text{-}situ}$–LD/LVI-GC–MS (SIM), under optimized experimental conditions.

same derivatizing agent [15,16]. Furthermore, no carry-over was observed by series of replicates, for which the background was always below the LODs achieved.

3.3. Application to real matrices

To demonstrate the analytical ability of the present methodology, assays were performed on environmental water matrices. Therefore, three water samples were obtained in different shipyard docks from Tagus river close to Lisbon (Portugal) and studied. Thereby, to account for intrinsic contamination and particular pronounced matrix effects, the standard addition method (SAM) was used. In a first approach, the matrices were fortified with four working standards to produce the corresponding spiking levels for the compound under study and blank assays were also performed without spiking to assure maximum control of the methodology. Table 1 summarizes the content detected for the TBT in the three different water matrices, as well as the regression parameters obtained from the assays performed by the SAM using the proposed methodology, under optimized experimental conditions. Beyond the remarkable linear dynamic ranges attained ($r^2 > 0.9931$), levels of TBT were just detected in matrices from Santa Apolónia and Parque das Nações. Fig. 3 depicts mass fragmentograms of those environmental matrices, including the mass spectra confirmation, which presenting the same order of magnitude (Table 1) of those contents reported in previous studies [3,16,17,22].

4. Conclusions

The present methodology (SBSE($NaBH_4$)$_{in\text{-}situ}$–LD/LVI-GC–MS (SIM)) was successfully applied to determine TBT in environmental water matrices. The derivatization agent $NaBH_4$ demonstrated good specificity for TBT in aqueous media, allowing the formation of stable adducts with suitable polarity for SBSE analysis. Under optimized experimental conditions, good accuracy, suitable precision, low detection limits and excellent linear dynamic range were achieved. By using the SAM, the application of the present methodology to real water matrices provided very good performance at the trace level. The proposed method demonstrated to be easy to work-up, reliable, sensitive, and with low sample volume requirements, to monitor TBT in environmental water matrices proving to be a suitable alternative for organotin speciation.

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References

[1] D. Cao, G. Jiang, Q. Zhou, R. Yang, J. Environ. Manag. 90 (2009) S16–S24.
[2] M.V. Laitano, J.D. Nuñez, M. Cledón, Ecol. Indic. 34 (2013) 345–351.
[3] S. Díez, S. Lacorte, P. Viana, D. Barceló, J.M. Bayona, Environ. Pollut. 136 (2005) 525–536.
[4] B. Antizar-Ladislao, Environ 34 (2008) 292–308.
[5] M.A. Champ, Mar. Pollut. Bull. 46 (2003) 935–940.
[6] Q. Xiao, B. Hu, M. He, J. Chromatogr. A 1211 (2008) 135–141.
[7] G.A. Zachariadis, E. Rosenberg, J. Chromatogr. B 877 (2009) 1140–1144.
[8] J.M.F. Nogueira, P. Teixeira, M.H. Florêncio, J. Microcolumn Sep. 13 (2001) 48–53.
[9] J.M.F. Nogueira, Anal. Chim. Acta 757 (2012) 1–10.
[10] N.R. Neng, C.A.A. Cordeiro, A.P. Freire, J.M.F. Nogueira, J. Chromatogr. A 1169 (2007) 47–52.
[11] E. Baltussen, C. Cramers, P. Sandra, Anal. Bioanal. Chem. 373 (2002) 3–22.
[12] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernández, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1261 (2012) 151–157.
[13] C. Devos, F. David, P. Sandra, J. Chromatogr. A 1261 (2012) 151.
[14] F.J. Camino-Sánchez, A. Zafra-Gómez, B. Oliver-Rodríguez, I. Ruiz-Naranjo, J. Ruiz-García, J.L. Vílchez, J. Chromatogr. A 1263 (2012) 14.
[15] J. Vercauteren, C. Pérès, C. Devos, P. Sandra, F. Vanhaecke, L. Moens, Anal. Chem. 73 (2001) 1509–1514.
[16] C. Almeida, P. Serodio, M.H. Florencio, J.M.F. Nogueira, Anal. Bioanal. Chem. 387 (2007) 2569–2583.
[17] H. Serra, J.M.F. Nogueira, J. Chromatogr. A 1094 (2005) 130–137.
[18] G.A. Zachariadis, J. Chromatogr. A 1296 (2013) 47–69.
[19] A.R.M. Silva, J.M.F. Nogueira, Talanta 74 (2008) 1498–1504.
[20] E. Coelho, R. Perestrelo, N.R. Neng, J.S. Câmara, M.A. Coimbra, J.M.F. Nogueira, S.M. Rocha, Anal. Chim. Acta 624 (2008) 79–89.
[21] P. Serodio, M.S. Cabral, J.M.F. Nogueira, J. Chromatogr. A 1141 (2007) 259–270.
[22] J.M.F. Nogueira, B. Simplício, M.H. Florêncio, A.M.M. Bettencourt, Estuaries 26 (2003) 798–802.