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Environmental matrices effect in butyltin determinations by GC/MS

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Abstract

The present study evaluated the matrix effect associated to determination of butyltin compounds (tripropyltin (TPrT), tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT)) in sediment and mussel tissues using derivatization by Grignard reaction and quantification by gas chromatography coupled mass spectrometry (GC-MS). A non-negligible matrix effect was verified for sediments (54.2, 20.3, 13.6 and -53.6 %) and mussel tissues (-12.5, -32.0, 59.4 and 65.7 %) for TPrT, TBT, DBT and MBT respectively. However, this matrix effect was prevailed by preparing the analytical curves using standard addition techniques. Thus, an analytical method was optimized and validated for a more accurate and precise determination of butyltin compounds in sediment and mussel tissue samples.

Keywords: Validation; Butyltin; Sediment; Biota; Matrix effect

INTRODUCTION

Butyltin compounds (OTs), particularly tributyltin (TBT), were used as fluid in transformers and capacitors since 1920s, but its biocides properties were discovered by International Council of Researches on Paints only in 1950s. Thereafter began to be used commercially as fungicides, acaricids and other kinds of pesticides. However, the most well-known OTs application was as active agent in antifouling paints during the last four decades (Almeida *et al.,* 2004, Yebra *et al.,* 2004). Antifouling paints are used in solid surfaces exposed directly to seawater including, hulls of ships, aquaculture nets, off shore structures, and ducts, in order to avoid undesirable incrustation of marine organisms (Champ, 2000, Kotrikla, 2009).

The first TBT antifouling paints (used from 1970s) were soluble matrix type, which provided a very fast initial biocide liberation. However, this system use to lose its efficiency within approximately 12-15 months (Almeida *et al.,* 2007). Later, TBT was used in self-polishing-paints, compatible with steel and aluminum hulls. These paints were based on acrylic copolymer with TBT groups bonded to the main polymer chain by ester bonds (Godoi *et al.,* 2003). Due to the low polymer solubility in sea water, the paint dissolution could be controlled at molecular level with constant release rates of about 4 µg cm^{-2} per day, which keep the paint effective for up to 7 years (Swennen *et al.,* 1997). As a result, in 1999 almost 70% of all commercial shipping were using TBT-based self-polishing-paints, achieving direct savings of approximately US$ 2,400 million a year in fuel and other costs (Clark *et al.,* 1988; Almeida *et al.,* 2007). Thus, approximately 50,000 tons of organotin compounds were produced per year between 1990 and 2003 (Godoi *et al.,* 2003).

Due to the intensive use and high toxicity, several enviromental deleteriuos effects have been reported since 1980s in harbors, marinas and shipyards areas contaminated by TBT (Yebra *et al.,* 2004). The most commonly reported effects are

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oysters malformation (Alzieu, 2000), immunosuppression in dolphins (Yang *et al.,* 2006), immuntoxicity in fish (Nakayama *et al.,* 2009), imposex (Lima *et al.,* 2006), and decline of gastropod populations (Castro *et al.,* 2012). Hence, in the early 1980s many countries adopted restritive regulations to the use these marine coatings on ships. In 2001, a global ban against TBT based antifouling paints was proposed by International Maritime Organization (IMO) through the International Convention on the Control of Harmful Antifouling Systems on Ships (AFS convention). According to AFS convention, new applications of these products were banned since 1st January 2003 and its presence on ship surfaces as from 1st January 2008 (IMO, 2014). However, TBT-based antifouling paints are still being widely used in several developing countries, which makes its monitoring still relevant (Bigatti *et al.,* 2009; Paz-Villarraga *et al.,* 2015).

In aquatic environments, TBT is degraded by dealkylation in progressively less toxic dibutyltin (DBT) and monobutyltin (MBT). This cleavage of the tin–carbon bonds may occur photolytically by UV light, microbiologically by fungi or bacteria, or by chemical attack (Gadd, 2000). In order to appraise temporal environmental contamination and trends, DBT and MBT are also determined in addition to TBT (Díez and Bayona, 2009), requiring accurate and selective analytical methods. Currently, despite the derivatization requirement, Gas Chromatography (GC) coupled to flame photometric detector (FPD) (Oliveira et al., 2010), pulsed flame photometric detector (PFPD) (Fernandez *et al.,* 2005) or mass spectrometer detector (MS) (Thomaidis *et al.,* 2007) is most often used to analyze butyltin compounds (BTs) in environmental matrices.

Previous studies related TBT analyses in environmental samples have reported matrix effect (Pereiro *et al.,* 1996; Cardellicchio *et al.,* 2001; Gallego-Gallegos *et al.,* 2006; Tang and Wang, 2007). This problem was first described by Kebarle and Tang (1993) and frequently occur with environmental matrices such as seawater, sediment and biota and might bias, either by increasing or suppressing the analytical signal, the results (Gallego-Gallegos *et al.,* 2006). Standard addition method (Cardellicchio *et al.,* 2001), use of smaller sample mass (Tang and Wang, 2007), use of appropriate internal standards (Van Eeckhaut et al., 2009) and specific clean-up methods (Gallego-Gallegos *et al.,* 2006) have been proposed to reduce or avoid the matrix effect on pesticide analyses. Thus, as many research groups and environment agencies have analyzed BTs in environmental matrices to either investigate the effectiveness of international (IMO) or national restrictions on TBT use or simply appraise the environmental levels, the present study evaluated the matrix effect on the determination of BTs (TBT, DBT, MBT and TPrT) (Figure 1) in environmental samples (sediments and mussel tissues) by GC-MS and Grignard derivatization. Despite being the most widely used method to access TBT environmental contamination, no studies have appraised this bias factor properly yet. A method to reduce the matrix effect and some modifications in the sample preparation proposed by Morabito *et al.,* (1995) were presented.

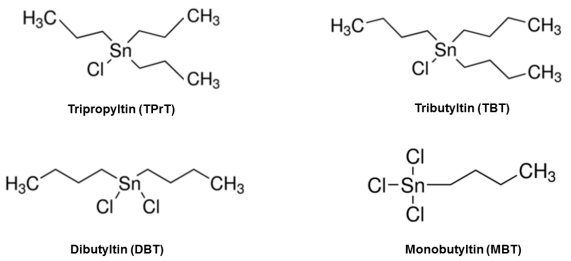

Figure 1: Molecular structures of tripropyltin (TPrT), tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT).

MATERIAL AND METHODS

Chemicals

Tributyltin chloride (TBT, 98.2%), dibutyltin dichloride (DBT, 96.0%), monobutyltin trichloride (MBT, 95.0%), tropolone (98.0%) and phenylmagnesium bromide in *dietylether solution 2M* (Grignard reagent) were purchased from Sigma-Aldrich (Bellefonte, PA, USA). Tipropyltin choride (TPrT, 98.9%, used as surrogate standard), tetrabutyltin (TeBT, 98.8%, used as internal standard), chloridric acid P.A. and NaCl were obtained from Merck (Darmstad, Germany). All butyltins concentrations are reported as Sn (ng Sn mL^{-1} for solutions or ng Sn g^{-1} for sediments and mussel tissue). The individual organotin stock standard solutions (1 mg Sn mL^{-1}) were prepared in hexane and stored in ambar vials at 4 °C. Working solutions were prepared daily by appropriate concentrations of the stock standard solutions. Hexane, methanol, dichloromethane and toluene of pesticide analysis grade and anhydrous sodium sulphate were purchased from JT Baker (Mexico). Water was purified with a Direct-Q UV3® (resistivity 18.2 mΩ cm) water purification system (Millipore, Bedford, MA, USA). All glassware was washed with Extran® solution (Merck, 5 % v/v) and submerged in the same solution for 24 hours. After, the glassware was submerged in nitric acid solution (5 % v/v) for 24 hours, washed under flow water and dry at 35 °C. Before the use, all material was rinsed with acetone (3x) and hexane (3x) of pesticide analysis grade.

Extraction

The extraction method for BTs followed Morabito et al., (1995), with modification in the final solvent exchange of isooctane to hexane. Exactly 5 g of sediments or 1 g of freeze dried mussel tissues were independently spiked with a mixture of TPrT, MBT, DBT and TBT in appropriate concentrations and left 30 minutes for equilibration. The samples were placed in 40 mL vials and 15 mL of tropolone solution 0.05 % (w/v) in methanol and 1 mL of concentrated HCl (37%) were sequentially added. The samples were sonicated for 15 min (water bath < 40 °C), and then centrifuged for 10 min at 3000 rpm. The supernatants were transferred to a 250 mL separatory funnel filled with 150 mL of a 10% NaCl solution. The extraction procedure was repeated twice. Later, the

initial extracts were extracted with 20 mL of dichlorometane (3x). The collected dichloromethane extract was eluted through anhydrous sodium sulphate and washed with 2 mL of dichloromethane. After, 5 mL of hexane were added and the volume reduced to 5 mL in a rotary evaporator (water bath < 40 °C and under moderate vacuum). The extracts were transferred to a 40 mL vials (screw cap with PTFE septa) and evaporated almost to dryness under moderate flow of nitrogen. The volume was then adjusted to 1 mL (using hexane) for derivatization.

Derivatization

The transformation of butyltin chlorides into more stable and volatile compounds to allow GC analysis was done by Grignard reaction. That alkylation is the most widely used derivatization technique for organotin determination and was performed by pentylation with pentylmagnesium bromide in dietylether solution (Morabito *et al.,* 2000).

Initially, the 40 mL vials containing the extract were closed and purged with nitrogen to obtain an inert atmosphere. For that step, it was used needles of stainless steel of 15 cm connected to the nitrogen line. Later, 2 mL of pentylmagnesium bromide in diethyl ether solution was injected inside the vials with a glass syringe. The vials were vigorously vortex mixed for 1 minute and allowed to stand for 30 min with occasional agitation. The excess of Grignard reagent was destroyed by adding 15 mL of ultrapure water and 1 mL of HCl (37%), both previously chilled down to 4 °C. That step was done in ice bath to minimize the evaporation losses.

After derivatization, the pentylated butyltins were liquid-liquid extracted with 5 mL of hexane (3x). Those extracts were evaporated to 0.5 mL under moderate nitrogen flow (≅ 1 L min^{-1}) and transferred to a silica column (3.5 g in a glass column 30 cm length and 15 mm as internal diameter) previously wet with hexane/toluene mixture (1:1). Hexane/toluene mixture (1:1) was passed through the column until 15 mL were collected in a vial. Finally, the extracts were concentrated to 0.9 mL under moderate nitrogen flow (≅ 1 L min^{-1}), 100 µL of tetrabutyltin solution (1000 ng Sn mL^{-1}) was added and then injected in the chromatographic system.

Instrument

All analyses were performed in a gas chromatography Perkin Elmer Clarus 500 coupled with a mass spectrometer and auto sampler. The GC was equipped with a Perkin Elmer column Elite-5MS (30 m x 0.25 mm I.D., 0.25 µm thickness) coated with 5 % diphenyl dimethilpolisiloxane. The carrier gas was high purity helium (99.999 %) with a constant flow of 1.7 mL min^{-1}. The GC oven temperature was programmed as follows: 2 min at 80 °C, to 300 °C at 11 °C min^{-1} and constant temperature until final analyses. The injector was kept at 240 °C in a split mode. The ion source and transfer line temperatures were set at 280 °C. The mass spectrometer was operated in electron ionization mode and electron impact was performed at electron energy of 70 eV. The mass spectra obtained at mass-to-change ratio scan range from 150 to 450 mu and the equipment run in the SIFI mode (simultaneous full scan and selected ion monitoring mode). The dwell time was set to 0.1 s for each ion. The confirmation was done against the spectra obtained for authentic standards.

Method validation

The validation process was carried out according to the parameters defined by method validation and quality control procedures for pesticide residues analysis in food and feed of the European Commission (SANCO, 2009), as described below:

Linearity and matrix effect study

In order to verify the linearity and matrix effect, three analytical curves were constructed for each analyte: in pure solvent, for matrix superposition (using sediments previously decontaminated by thermal treatment / 450 °C for 6 hours) and for standard addition (mussel tissues). The analytical curves concentrations were 2, 5, 10, 25, 50, 100, 250, 500 and 1000 ng Sn mL^{-1}. The method linearity was evaluated by the linear regression (y = ax + b) and its correlation coefficient (r^2). Afterwards, matrix effects were investigated by comparing the slopes in calibration solutions. The ratio Sm/Ss (Sm refers to matrix slope and Ss refers to solvent slope) were calculated and the intensity of the effects caused by matrix components was rated according to the % of signal enhancement or suppression (C%), which was calculated as % ME = 100 x (1 – Sm / Ss).

Limit of detection (LOD) and quantification (LOQ)

Limits of detection (LOD) and quantification (LOQ) of the method were calculated for a signal to noise (S/N) ratio ("peak to peak") obtained by the Turbo Mass software (S/N = 3 for LOD and S/N = 10 for LOQ, respectively) using the peak of the lowest concentration. The determination of the LOD was performed by preparing samples with known concentrations of the analyte and by establishing the level at which the analyte could be reliably detected.

Precision and Accuracy

The precision was evaluated in terms of repeatability (RSD_r) and intermediate precision (RSD_{ip}) and was expressed as relative standard deviation (RSD). Treatments with spiked sediment samples in four spiked concentrations (2, 4, 20 and 40 ng Sn g^{-1}) and in mussel tissues in three spiked concentrations (10, 20 and 100 ng Sn g^{-1}) were performed to evaluate repeatability. The intermediate precision was verified through of experiments intra-day and inter-day accomplished with three fortification levels and different operators, respectively.

The accuracy was evaluated in terms of recoveries from spiked samples and Certified Reference Material (CRM)

analyses. The analyses were carried out in four concentrations (2, 4, 20 e 40 ng Sn g^{-1}) of TPrT, TBT, DBT and MBT for sediment samples and three environmental relevant concentrations (10, 20 and 100 ng Sn g^{-1}) for mussel tissues. For TBT, was also tested the level 1.2 ng Sn g^{-1} for sediments and 5 ng Sn g^{-1} for mussel tissues. All spiked samples were analyzed in triplicate. Additionally the Certified Reference material (PACS-2 / National Research Council of Canada, Ottawa, Canada) was analyzed (n=5). All concentrations were reported as ng Sn g^{-1} (dry weight).

RESULTS AND DISCUSSION

The selected chromatography conditions showed a good resolution peak shape (tailing factors and asymmetry < 1.8 for the studied BTs) for the analytes (Figure 2).

The Table 1 display the retention times (t_R), relative retention times (t_{RR}) and monitored ions. The chromatographic conditions of the validated method provided a similar time of analysis (13.29 minutes) in comparison to other proposed methods for BT determinations (Chou and Lee, 2005). Additionally, the use of a versatile column (Elite-5MS - 30 m x 0.25 mm I.D., 0.25 µm thickness) facilitates the method implementation to BTs analysis in laboratories that have been using GC-MS.

Validation

Linearity, Matrix Effect(%ME), and LOD and LOQ

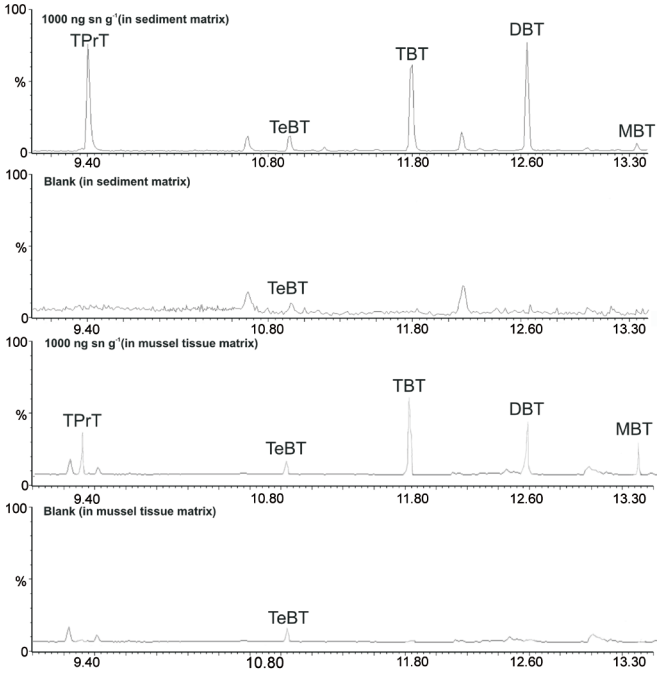

Figure 2: Typical chromatogram of Butyltin compounds in sediments and mussel tissues matrix (spiked and blank). TPrT, TeBT (internal standard), TBT, DBT and MBT. TeBT concentration = 100 ng Sn g^{-1}, other analites = 1000 ng Sn g^{-1}.

Table 1: Retention time (t_R), relative retention time (t_{RR}) and monitored ion.

Compounds	t_R (min)	t_{RR} (min)	Monitored Ion
TPrT	9.41	0.87	207, 275, 277
TeBT	10.88	-	177, 233,235, 291
TBT	11.71	1.07	177, 179, 305, 303
DBT	12.53	1.16	177, 249, 317, 319
MBT	13.31	1.22	193, 317, 319

The analytical curve equations, % ME, r^2, LOD and LOQ for TPrT, TBT, DBT and MBT in different tested matrices are summarized in Table 2. The square of regression coefficient (r^2) for the analytical curves of those four studied analytes was always higher than 0.99 for both matrices.

The comparison among the analytical curves equation showed either suppression or enhancement of the BTs signal related to the tested matrices. For sediments, the matrix effect ranged from -53 to 54 considering all analytes. Similarity, mussels tissues presented matrix effect between -12.5 and 65.7. In fact, several authors have reported the higher susceptibility of MS detectors to suffer from matrix effects in comparison to pulsed flame photometric detectors (PFPD) due to the metallic surface and absence of combustion step in the MS detector (Pinho *et al*., 2009). The matrix effect is caused by the interferents present in the environmental matrices which compete for the ionization with the analytes present in low concentrations. According to Kruve et al. (2008), signal changes higher than 20 % generated by matrix effect are extremely significant and may cause false negative or positive results at lower concentrations. Thus, some precautions such as the use of internal standards and implementation of clean-up methods have been recommended to minimize the matrix effects in pesticide analyses (Gallego-Gallegos *et al.,* 2006; Van Eeckhaut *et al.,* 2009). However, the present results showed that these procedures have not been able to efficiently avoid the matrix effect in BTs analysis by GC-MS. Therefore, it was necessary to apply methods of standard addition and superposition of the matrix for biota and sediments, respectively. This procedure had been used by Gallego-Gallegos *et al.,* (2006) for ethylated BT determinations in environmental matrices using GC-FPD, but never tested before for determinations of BTs using the derivatization by Grignard reaction and quantification by GC-MS.

In the analytical curve prepared in pure hexane, the LODs were 3.5, 2.5, 5.0 and 3.5 ng Sn mL^{-1} for TPrT, TBT, DBT and MBT, respectively. However, an improvement in LODs and, consequently, in LOQs, was obtained when the curves were constructed using matrix addition techniques. LODs for curves done into the sediment extracts were 1.5, 0.8, 1.0 and 0.7 ng Sn mL^{-1} for TPrT, TBT, DBT and MBT, respectively. Similarly, the LOD values for curves prepared using mussel extracts were, respectively, 0.5, 0.7, 0.7 and 0.5 ng Sn mL^{-1}. These results are in accordance with other studies where improvements in the LOD were obtained when the matrix effect was reduced (Iová and Zrostlíková, 2003; Carrasco *et al.,* 2007; Chambers *et al.,* 2007).

Table 2: Analytical curve equation, matrix effect (% ME), square of regression coefficient (R^2), instrumental limits of detection (LOD_i), instrumental limits of quantification (LOQ_i), sediment method limits of quantification (LOQ_{sed}) and biota method limits of quantification (LOQ_{bio}) for BTs prepared in pure $solvent_{(sol)}$, sediment $matrix_{(sed)}$ and mussel tissues $matrix_{(bio)}$.

Compound	Analytical curve equation	% ME	R^2	LOD_i (ng Sn mL^{-1})	LOQ_i (ng Sn mL^{-1})	LOQ_{sed} (ng Sn g^{-1})	LOQ_{bio} (ng Sn g^{-1})
$TPrT_{sol}$	y=0.002011660x + 0.0023393	-	0.9963	3.5	10		
$TPrT_{sed}$	y=0.000921047x – 0.0028651	54.2	0.9977	1.5	4.5	0.8	1.5
$TPrT_{bio}$	y=0.002276220x – 0.0204481	-12.5	0.9911	0.5	1.5		
TBT_{sol}	y=0.00570193x – 0.00728584	-	0.9983	2.5	7.5		
TBT_{sed}	y=0.00454291x – 0.01832051	20.3	0.9980	0.8	2.5	0.5	2.0
TBT_{bio}	y=0.00752929x – 0.03587190	-32.0	0.9950	0.7	2.0		
DBT_{sol}	y=0.00595851x – 0.00415379	-	0.9972	5.0	15		
DBT_{sed}	y=0.00514563x – 0.02247480	13.6	0.9960	1.0	2.0	0.5	2.0
DBT_{bio}	y=0.00241850x – 0.02520004	59.4	0.9958	0.7	2.0		
MBT_{sol}	y=0.00091140x + 0.00083390	-	0.9955	3.5	10		
MBT_{sed}	y=0.00140020x + 0.00417327	-53.6	0.9914	0.7	2.0	0.5	1.5
MBT_{bio}	y=0.00031220x + 0.00242932	65.7	0.9945	0.5	1.5		

Simply procedures of sample dilution and small sample injection are often used in pesticide analyses to reduce the amount of interference molecules injected into the chromatographic systems and, consequently, minimize the matrix effect (Hernando et al., 2007). However, the use of these techniques hampers the application of pre-concentration procedures, which are often necessary to quantify BTs in environmental levels. Considering the mass of sample used (5g to sediments and 1g to mussel tissues), the LOQ values obtained for sediment (0.8, 0.5, 0.5 and 0.5 ng Sn g^{-1} for TPrT, TBT, DBT and MBT, respectively) and for biota samples (1.5, 2.0, 2.0 and 1.5 ng Sn g^{-1} for TPrT, TBT, DBT and MBT, respectively) were consistent with environmental relevant concentrations (Castro *et al.,* 2012).

Precision and Accuracy

For sediments, the recovery values for all analyzed compounds and spiked levels varied from 92.7 to 117.1 %, and the RSD_r values ranged from 1.1 to 16.4%. In terms of intermediate precision, the recoveries varied between 78.5 and 110.0 % with RSD_{ip} ranging between 3.0 and 18.3 (Table 3). Similarly, these values ranged from 72.0 to 118.2 % with RSD_r between 0.8 and 12.2% for mussels. The recoveries for the intermediate precision varied between 78.3 and 119.4 % with RSD_{ip} from 0.7 to 11.4% (Table 4). These values are within the acceptable limits established for the method validation and quality control procedures for pesticide residues analysis in food and feed of the European Commission (SANCO, 2009).

The Certified Reference Material (for sediments) (PACS-2 / *n* =5) presented levels of TBT (871 ± 36 ng Sn g^{-1}) and , DBT (1012 ± 22 ng Sn g^{-1}) in good agreement with the certified concentrations for TBT (890 ± 105 ng Sn g^{-1}) and DBT (1047 ± 64 ng Sn g^{-1}). However, MBT (713 ± 42 ng Sn g^{-1}) levels were above the reported values (MBT = 600 ng Sn g^{-1}) for PACS-2. It is possible that the method of superposition of the matrix was not efficient to completely avoid the matrix interferents in the MBT analyses. In addition, the MBT values in PACS-2 are not certified. Similar results indicating worst analytical performance for MBT have been observed by several studies (Montigny *et al*., 1998, Aguerre *et al*., 2000, Santos *et al*., 2013). However, considering the small differences between

Table 3: Recovery, repeatability (RSD_r) and intermediate precision (RSD_{ip}) of the method used to analyze TPrT, TBT, DBT and MBT spiked at different levels in sediment extracts (n=9).

Compound	Spike level (ng Sn g^{-1})	Recovery (%)	RSD_r (%)	$Recovery_{pi}$ (%)	RSD_{pi} (%)
TPrT	2	97.7	10.8	78.5	18.3
	4	92.7	8.0	81.3	16.4
	20	106.3	16.4	89.3	14.5
	40	101.2	5.8	96.6	7.9
TBT	1.2	107.9	14.6	93.3	12.5
	2	104.3	6.3	97.6	11.4
	4	105.4	2.0	98.5	6.3
	20	101.3	1.1	98.3	7.1
	40	100.0	2.4	99.3	4.5
DBT	2	117.1	9.2	110.0	9.9
	4	97.5	7.6	88.2	8.6
	20	104.2	9.2	91.3	3.0
	40	99.6	3.8	98.6	4.5
MBT	2	97.2	9.1	94.5	16.3
	4	93.8	6.1	83.7	14.5
	20	95.7	6.2	90.3	9.1
	40	96.5	3.6	85.6	7.7

Table 4: Recovery, repeatability (RSD_r) and intermediate precision (RSD_{ip}) of the method used to analyze TPrT, TBT, DBT and MBT spiked at different levels in mussel tissue extracts (n=9).

Compound	Spike level (ng Sn g^{-1})	Recovery (%)	RSD_r (%)	$Recovery_{pi}$ (%)	RSD_{pi} (%)
TPrT	10	83.0	0.8	89.5	4.7
	20	72.0	1.9	78.3	3.1
	100	94.6	12.2	92.5	2.6
TBT	5	112.9	10.3	-	-
	10	101.1	2.1	100.7	2.5
	20	98.7	2.3	96.3	2.0
	100	101	1.6	98.2	0,7
DBT	10	112.0	1.2	108.0	4.6
	20	88.9	1.7	101.8	8.2
	100	93.2	2.5	98.4	2.1
MBT	10	118.2	4.2	119.4	10.2
	20	106.1	5.9	108.3	9.4
	100	105.5	10.4	110.6	11.4

CRM and detected levels, the present method using standard addition or matrix-matched techniques was more accurate for MBT quantifying.

CONCLUSIONS

Results confirmed that matrix effect on the determination of butyltins in sediments and mussel tissues samples by GC-MS and Grignard derivatization is relevant. However, this effect can be easily avoided by preparing the analytical curves using standard addition (mussel) or matrix-matched (sediment) methods. The analytical method proved to be effective for the determination of butyltin compounds in these environmental matrices, which are the most frequently used to access BTs contamination.

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