Available online at www.sciencedirect.com
ScienceDirect
C. R. Chimie 12 (2009) 831–840
COMPTES RENDUS CHIMIE
http://france.elsevier.com/direct/CRAS2C/

Full paper / Mémoire

Comparison of two analytical methods for the determination of organotin compounds in marine organisms

Camilla Liscio, Marina Di Carro, Emanuele Magi*

Department of Chemistry and Industrial Chemistry, University of Genoa, 16146 Genoa, Italy

Received 30 April 2008; accepted after revision 31 July 2008
Available online 27 December 2008

Abstract

Two analytical procedures were used for the determination of butyltin compounds in mussels.

Both methods include extraction with methanol containing tropolone, derivatization, purification on Florisil and GC–MS analysis. The main difference between the procedures is in the derivatization step: one employs a Grignard reagent (*n*-pentylmagnesium bromide) while the other uses sodium tetraethylborate (STEB).

Quantitative determinations were carried out in single ion monitoring using tripropyltin as internal standard. The accuracy of the procedures was verified on a certified reference material (ERM 477), providing good results for both methods.

All the considered compounds showed lower detection limits with STEB derivatization; in particular for tributyltin (TBT), the difference between the two methods overcame one order of magnitude.

An *in vivo* experiment was then performed, exposing mussels (*Mytilus galloprovincialis*) to known amount of TBT for 7 days; control and contaminated tissues were analyzed using the STEB derivatization method. Results showed the accumulation of TBT, especially in the gills. ***To cite this article: C. Liscio et al., C. R. Chimie 12 (2009).***

Keywords: GC–MS; Organotin compounds; Derivatization; *Mytilus galloprovincialis*

1. Introduction

Organotin compounds (OTCs) have been extensively studied in the last decades due to their toxicity and considerable impact on the aquatic environment [1–3]. These compounds have been used for industrial, agricultural and domestic applications as fungicide, biocides, wood preservatives and PVC stabilizers.

Among them, antifouling paints containing tributyltin (TBT) are the most important contributors of organotin compounds to the aquatic environment, where they are known to cause harmful effects on marine organisms even at extremely low concentrations [3]. In the marine environment TBT degrades into more polar compounds, dibutyltin (DBT) and monobutyltin (MBT), less toxic to aquatic organisms. Butyltins have been shown to interfere with the biological processes of several species, causing disorders in the hormonal system. In particular, different biological effects on target and non-target organisms have been documented, such as larval mortality [4], reduction of growth [5] and imposex (imposition of male sexual attributes in the females) in snails and gastropods [4,6,7].

* Correspondence at: Department of Chemistry and Industrial Chemistry, Via Dodecaneso 31, 16146 Genoa, Italy.
E-mail address: magie@chimica.unige.it (E. Magi).

1631-0748/$ - see front matter
doi:10.1016/j.crci.2008.07.006

For these reasons the use of TBT in antifouling paints has been partially restricted from early 1980s in several countries; later the International Maritime Organization (IMO) adopted an International Convention that has prohibited for the application of organotin compounds to any vessel of the member states from 1st July 2003, and provides for the removing of any antifouling system containing organotin compounds from 1st January 2008. Nevertheless, the IMO convention has failed to be transposed into domestic legislation in several countries, including non-EU Mediterranean nations, and TBT-based antifouling paints are still used. Due to these restrictions, different studies worldwide have shown a slow decline in TBT contamination [2]. However, in several areas OTC concentrations are still relevant and can be found in marine water, sediments and biota at levels being chronically toxic for most susceptible organisms [8].

TBT is considered one of the priority hazardous substances in the priority pollutant lists of the European Union [9] and United States Environmental Protection Agency. TBT is also included in the endocrine disruptor groups, because of its effects on the hormonal system.

In order to verify the effectiveness of the legal provisions and to evaluate the distribution and fate of OTCs in the marine environment, several analytical methods have been developed, including different steps such as extraction, clean-up, derivatization and analysis.

The most commonly used techniques for the determination of organotin compounds are liquid chromatography (LC) and especially gas chromatography (GC) coupled with selective and sensitive detectors: mass spectrometry (MS), atomic emission spectrometry (AES), flame photometry (FPD) and inductively coupled plasma mass spectrometry (ICP-MS). In recent years, several papers have reviewed analytical methods for organotin determination [10–15].

Among them, GC–MS has been widely used due to its low detection limit and high selectivity, in spite of the need to derivatize the analytes to form volatile compounds.

Derivatization of butyltin species is one of the crucial steps of GC-based methods [13]. The derivatization reactions most commonly used for OTCs are alkylation with Grignard reagents, ethylation with sodium tetraethylborate ($NaBEt_4$) and hydride generation with sodium borohydride.

Until a few years ago alkylation with Grignard was the most widely used [14]; this method is time consuming and involves a multi-step procedure with anhydrous conditions and non-protic solvents. A solvent exchange is required before derivatization if polar solvents are used as extracting agents and a liquid–liquid extraction is needed to isolate the derivatized OTCs.

In recent years, ethylation using $NaBEt_4$ followed by liquid–liquid extraction has provided an alternative to Grignard alkylation, especially in aqueous samples, for which the in situ derivatization is possible, with a consequent reduction in the analytical steps [16,17]. Moreover, with sodium tetraethylborate ethylation and extraction of organotin compounds are carried out simultaneously, simplifying the analytical procedure still further.

In our laboratories, an HPLC-hydride generation-ICP-AES analytical procedure was developed [18] and used [19] for the certification of CRM 477 (a lyophilized marine mussel tissue produced by BCR of the European Community). A GC–MS method, including extraction with tropolone and methanol followed by Grignard pentylation, was then developed and applied to the determination of TBT, DBT and MBT in the Antarctic bivalve *Adamussium colbecki* [20] and in *Mytilus galloprovincialis* exposed in a Ligurian harbour [21].

In this work a GC–MS method with derivatization with sodium tetraethylborate was developed and compared to the one using derivatization with Grignard. The former was then used for the determination of butyltins in mussels subjected to an *in vivo* experiment.

2. Experimental

2.1. Instrumentation

Analyses were performed on an Agilent 7890 Series Gas Chromatograph coupled to an Agilent 5975N MSD quadrupole (Agilent Technologies, Little Falls, DE, USA). The GC was equipped with a Phenomenex ZB5 capillary column (30 m × 0.25 mm I.D. × 0.25 μm) coated with 5% phenylpolysiloxane. Automated injection was carried out with a Multi-Purpose Sampler (MPS-2) from Gerstel GmbH (Mülheim an der Ruhr, Germany) using a split/splitless injector. Injection was performed in split mode 10:1. Helium was employed as carrier gas with a constant flow of 1.2 ml/min. The oven temperature for the analysis of the ethylated analytes was programmed as follows: 50 °C for 1 min, from 50 °C to 200 °C at 20 °C/min, from 200 °C to 300 °C at 40 °C/min and then held at 300 °C for 3 min. As concerns the pentylated analytes, the oven temperature was held at 90 °C for the first min, then ramped at 30 °C/min to

140 °C, then at 12 °C/min to 250 °C and finally at 30 °C/min to the final temperature of 290 °C, which was maintained for 10 min. The injector temperature was 300 °C. Transfer line, ion source and quadrupole temperatures were 300 °C, 230 °C and 150 °C, respectively. Electron impact ionization was performed at an electron energy of 70 eV.

2.2. Standards and reagents

Dichloromethane, hexane and isooctane were purchased from Merck (Darmstadt, Germany). Methanol was obtained from Riedel-de-Haen (Seelze, Germany). All organic solvents were of analytical or chromatographic grade. Monobutyltin trichloride (95%), dibutyltin dichloride (96%), tributyltin chloride (96%) were purchased from Sigma–Aldrich (Milan, Italy).

Tripropyltin chloride (98%), used as internal standard, was obtained from Merck (Darmstadt, Germany). Stock organotin solutions (1000 mg/l as cations) were prepared in methanol and stored at +4 °C in the dark. Working standards containing 100 mg/l were prepared weekly in methanol; solutions containing 10 mg/l were prepared daily by dilution in methanol.

Tropolone (2-hydroxy-2,4,6-cycloheptatrienone, 98%), sodium tetraethylborate and *n*-pentylmagnesium bromide were purchased from Sigma–Aldrich (Milan, Italy). The working solution of sodium tetraethylborate was prepared daily in Milli-Q water.

Glacial acetic acid (99.8%) and sodium acetate (99%) were obtained from Merck (Darmstadt, Germany). The CH_3COOH/CH_3COONa buffer was prepared by adding an appropriate amount of CH_3COONa in Milli-Q water followed by pH adjustment with CH_3COOH.

Water was purified by a Milli-Q system (Millipore, Watford, Hertfordshire, UK). Lichrolut Florisil SPE cartridges (1000 mg, 6 ml, 125-150 μm) were obtained from Merck (Darmstadt, Germany). Freeze-dried mussel tissue (ERM 477) certified for butyltin concentrations was obtained from IRMM (Institute for Reference Material and Measurement, Geel, Belgium). All glasswares were soaked overnight in 4 M HNO_3 to remove sorbed organotin compounds, rinsed with Milli-Q water and then with acetone.

2.3. Sampling and in vivo experiment

Mussels (*M. galloprovincialis*) were collected from a mussel farm in La Spezia (Italy). Live organisms were transported to the laboratory where they were held one day in aerated artificial seawater to eliminate possible contamination. Some individuals were then used as the control sample and some others were exposed for a week to TBT (5 ng/L/day). The water was changed every day and TBT was added immediately after. Twenty individuals, control and polluted mussels, were dissected to separate the gills, the mantle and the digestive gland; the tissues obtained were then pooled, freeze-dried for 48 h, ground and stored at −80 °C before GC–MS analysis.

2.4. Organotin compound extraction

The extraction procedure of organotin compounds from the tissues is based on the method employed by Caricchia et al. [22] and modified in our laboratories. Approximately 0.5 g of freeze-dried mussel tissue (precisely weighed) were introduced in a 50 ml glass tube and then spiked by the addition of a minimal volume (50 μl) of a methanolic solution of tripropyltin (TPrT) chloride as internal standard. A rehumidification step was carried out adding 1 ml of methanol and sonicating for 10 min. Tubes were stored at −20 °C overnight. The resulting slurry was then extracted twice with 15 ml of methanol containing tropolone (0.05% w/v) in a ultrasonic bath for 20 min, then the suspension was centrifuged at 3500 rpm for 10 min. The surnatant was then collected and treated differently depending on the derivatization technique used.

2.5. Derivatization with Grignard reagent

The organotin compounds were extracted with 30 ml dichloromethane by shaking vigorously for 5 min in a separating funnel. The dichloromethane phase, containing the organotins, was collected and evaporated to dryness by a vacuum rotary evaporator. The samples were redissolved in 3 ml of dichloromethane. Derivatization was carried out by the addition of 1.6 ml of $n\text{-}C_5H_{11}MgBr$ as Grignard reagent. The excess of Grignard reagent was destroyed by adding 2 ml of 2 N hydrochloric acid. The organic phase was collected and re-extracted by a liquid/liquid procedure. The dichloromethane phase, containing the organotins, was collected and evaporated to dryness by a vacuum rotary evaporator. The sample was redissolved in 1 ml of *n*-hexane. A clean-up step was performed by solid-phase extraction (SPE), using Florisil. After a preliminary washing with dichloromethane, the column was conditioned with *n*-hexane (3 ml) and the elution was performed with a 1:1 *n*-hexane:dichloromethane mixture (2 ml). The eluted sample was evaporated under a gentle stream of N_2 and redissolved in 1 ml

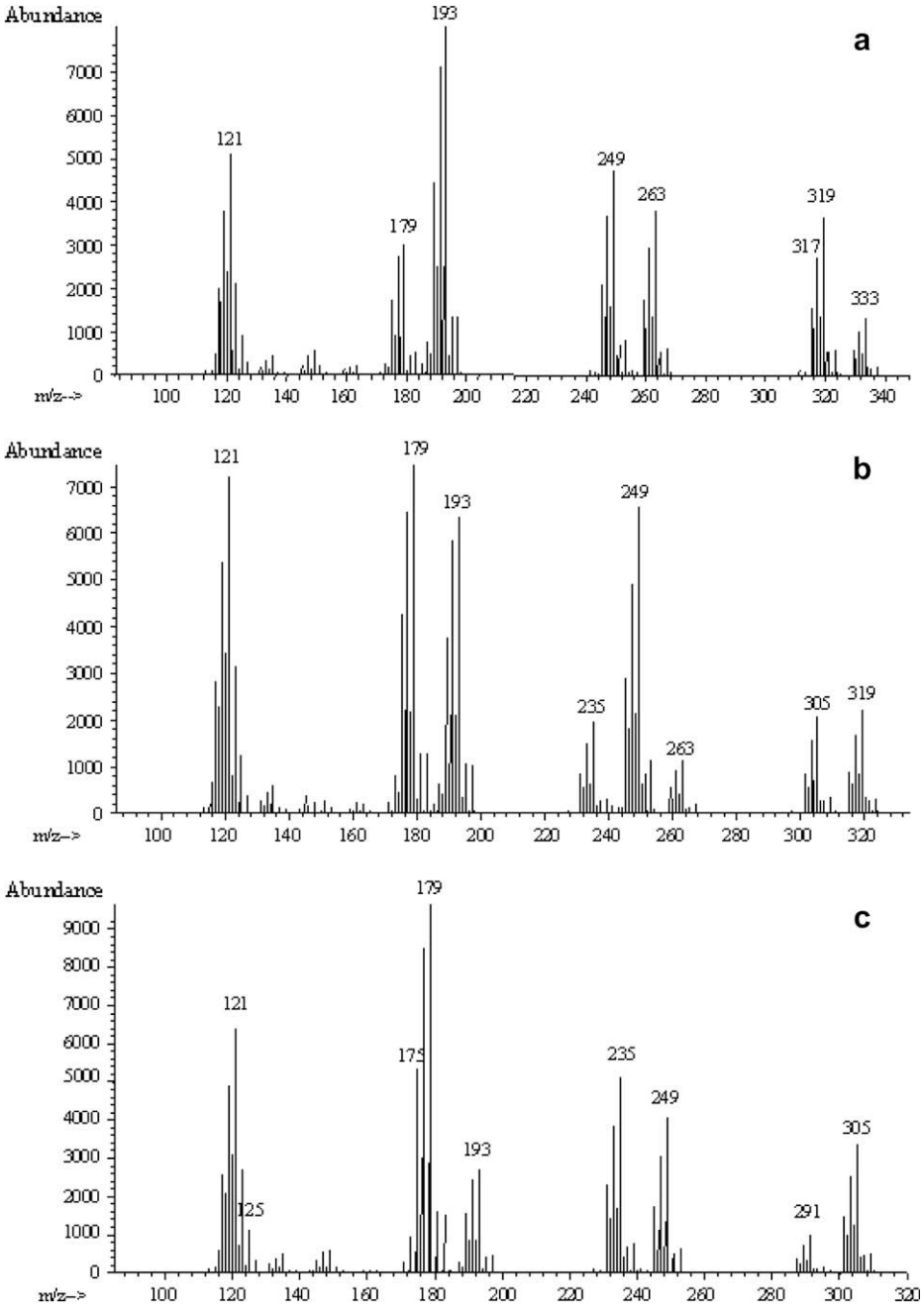

Fig. 1. EI mass spectra of pentylated compounds: (a) MBT ($BuSnPe_3$), (b) DBT (Bu_2SnPe_2), (c) TBT (Bu_3SnPe).

isooctane. The analysis was carried out injecting 1 µl of this solution in the GC–MS system.

2.6. Derivatization with sodium tetraethylborate (STEB)

Ethylation of the butyltin species was carried out in 30 ml clear polycarbonate centrifuge tubes with polypropylene screw caps. Extracts were rotavaporated to dryness, reconstituted with 2 ml of methanol and directly introduced in the centrifuge tubes; pH was adjusted to 4 with 10 ml of 0.6 M acetic acid/sodium acetate buffer. Ethylation was performed adding 500 µl of a 6% w/v $NaBEt_4$ solution in MQ water; 500 µl of hexane was then added for the extraction of the derivatized compounds. After 30 min of vigorous shaking, samples were

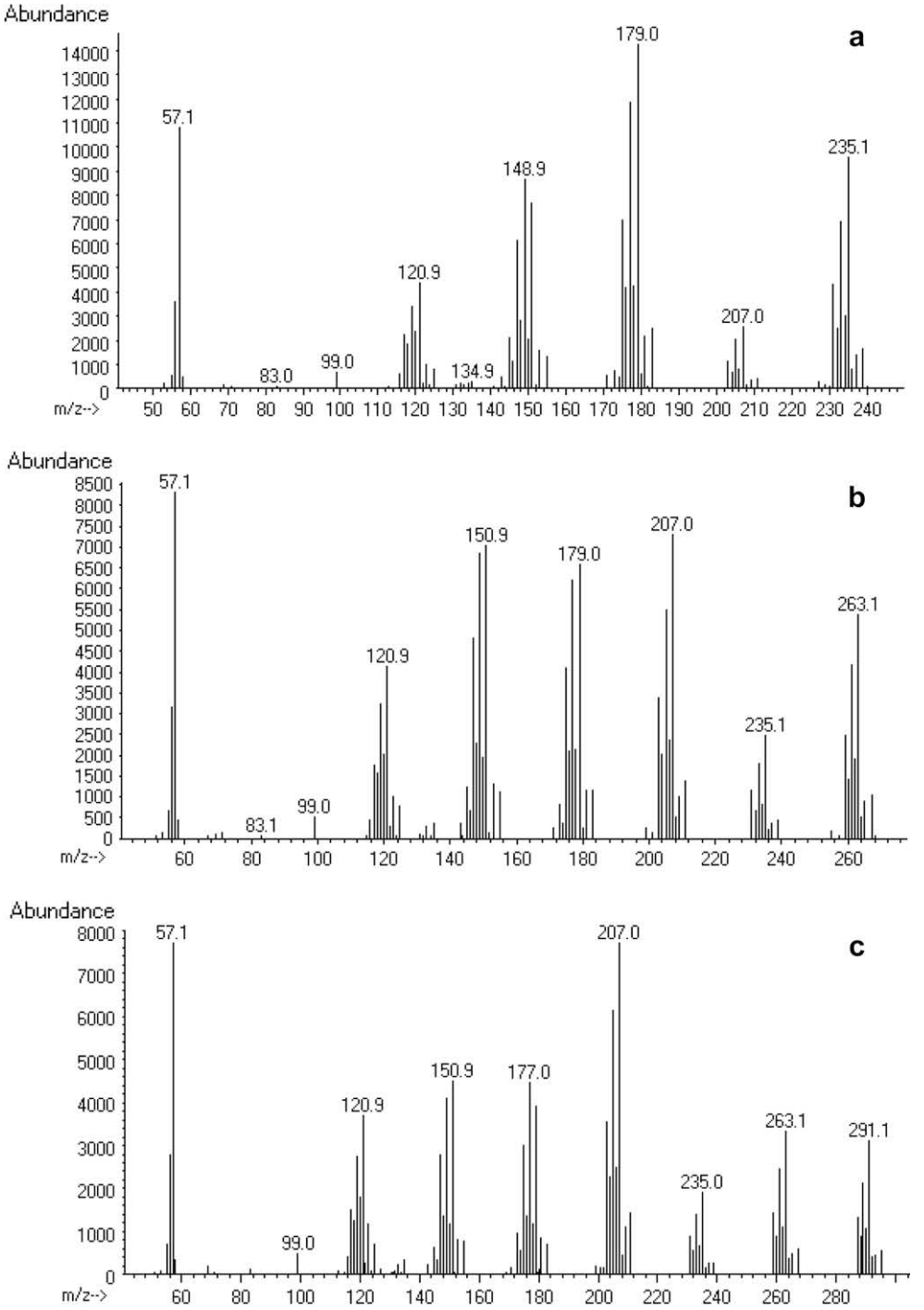

Fig. 2. EI mass spectra of ethylated compounds: (a) MBT ($BuSnEt_3$), (b) DBT (Bu_2SnEt_2), (c) TBT (Bu_3SnEt).

centrifuged at 4000 rpm for 30 min to facilitate phase separation. The organic layer was transferred to a glass vial before clean-up. The clean-up procedure involved solid-phase extraction using Florisil as stationary phase. The SPE cartridges were first conditioned with 6 ml of 1:1 dichloromethane–hexane, then 8 ml of hexane. After loading the sample into the column, the analytes were eluted with 3 ml hexane in a glass vial and concentrated to 0.5 ml under a gentle nitrogen stream. The analysis was carried out injecting 1 μl of this solution into the GC–MS.

3. Results and discussion

3.1. GC–MS analysis

Standard mixtures of pentylated and ethylated analytes were prepared in our laboratories, according to the procedures described in Section 2, and were used to obtain the optimum chromatographic conditions. The EI mass spectra of the three pentylated and ethylated analytes are shown in Figs. 1 and 2, respectively. The typical fragmentation pattern of organotin compounds, due to the isotopic distribution of tin, can be recognized.

Analysis of real samples, including the certified reference material, was performed in selected ion monitoring (SIM) mode. For each compound, three ions not affected by interferences were monitored to provide good specificity, using the most abundant for the quantitation. The use of an internal standard is necessary as the sample preparation procedure consists of various steps that can cause analyte losses. TPrT was used as internal standard, since it is chemically similar to butyltins and is not present in the marine environment. Retention times and selected ions of considered compounds are reported in Table 1.

Table 1
Retention times and selected ions of pentylated (Grignard) and ethylated (STEB) analytes. Ions used for the quantification are typed in bold font.

	Retention time [min]		*Ions [m/z]*	
	Grignard	**STEB**	**Grignard**	**STEB**
MBT	10.74	6.52	301-303-**305**	175-177-**179**
DBT	10.05	7.64	315-317-**319**	259-261-**263**
TBT	9.34	8.59	315-317-**319**	259-261-**263**
TPrT [a]	7.37	6.99	273-275-**277**	189-191-**193**

[a] Internal Standard

In Fig. 3, SIM chromatograms of the certified reference material (ERM 477) obtained with the two methods of derivatization are shown. The pentylated analytes present higher retention times than the ethylated derivatives due to their higher molecular weight and consequently lower volatility.

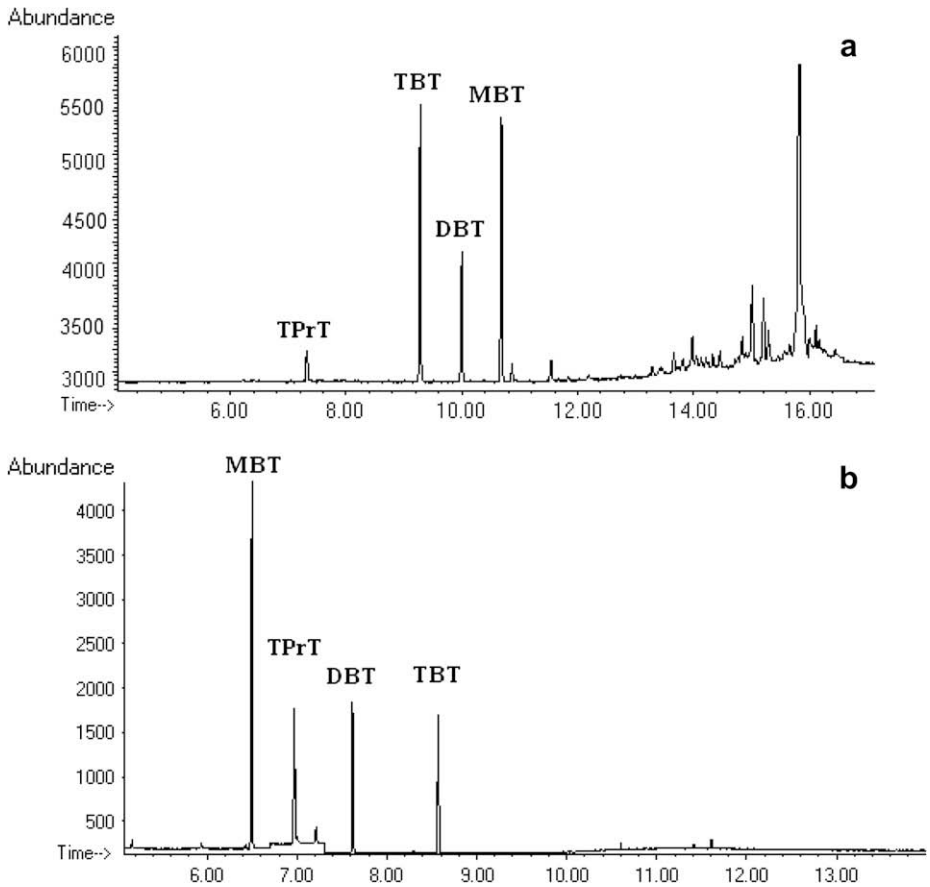

Fig. 3. SIM chromatograms of the certified reference material (ERM 477) spiked with the internal standard (1 μg/ml) derivatized with: (a) pentylmagnesium bromide, (b) sodium tetraethylborate.

3.2. Blank levels of the two procedures

Environmental samples usually present low concentrations of the monitored pollutants; therefore, a careful blank evaluation should be performed to evaluate possible contamination due to the sample handling. The number of analytical steps required to prepare the sample before the GC–MS analysis increases the risk of contamination.

As discussed in Section 1, the derivatization of organotin compounds is necessary to obtain volatile compounds suitable for GC analysis; this step is then particularly critical. Alkylation with Grignard reagents is an efficacious derivatization technique although it needs several analytical steps, since Grignard reagents require the use of non-protic solvents. After the extraction of the analytes from the matrix, normally performed using a polar solvent added with a complexing agent, a liquid–liquid extraction is necessary to change the medium of reaction.

In the derivatization with sodium tetraethylborate, ethylation can occur directly in situ, together with the liquid–liquid extraction of the derivatives; therefore, analytical steps and, as a consequence, the possible sources of contamination are drastically reduced.

Furthermore, most Grignard reagents are commercialized at relatively low purity grade [23]; in particular pentylmagnesium bromide was found to be contaminated with TBT [24]; this contamination can significantly affect the detection limits of the analytical method.

Our results confirm this observation since the blank of the procedure with Grignard reagent presents not negligible levels of butyltin compounds, as shown in Fig. 4a. On the contrary, the blank of the STEB derivatization (Fig. 4b) does not present peaks at the retention time of the analytes (even if a quite intense signal is observed) and shows a very low background noise; as a result, this procedure leads to definitely lower detection limits (Table 2). Moreover, the comparison of the

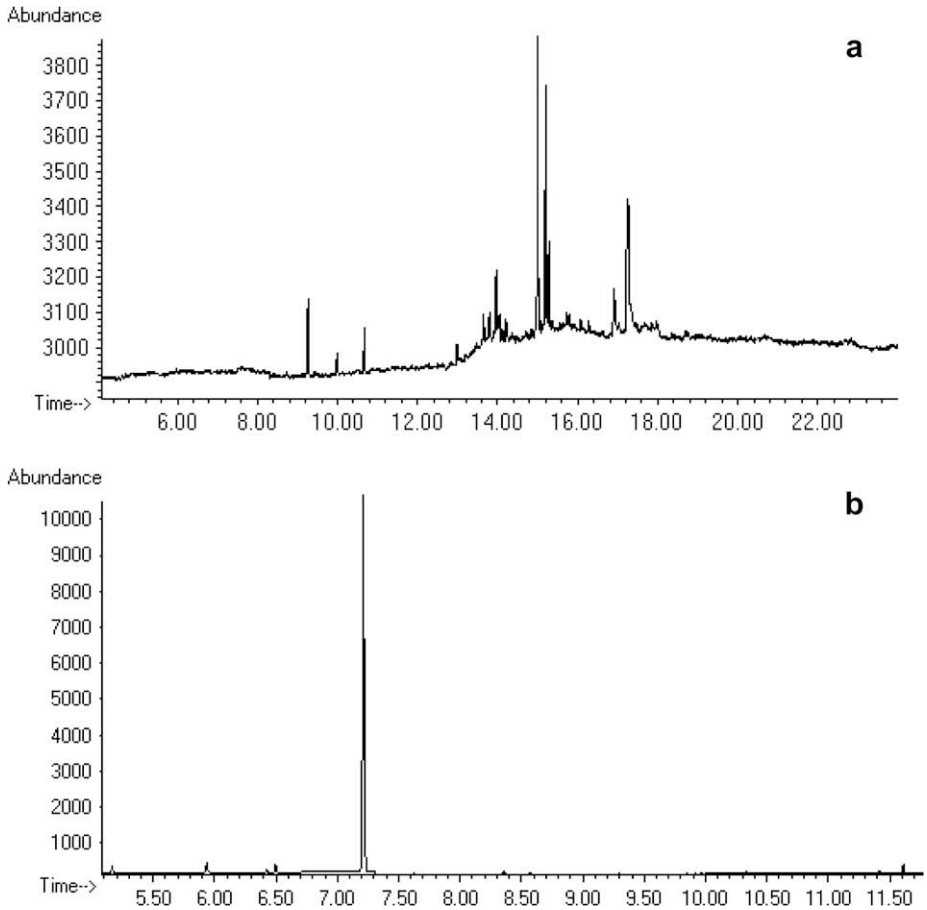

Fig. 4. SIM chromatograms of a blank for the two derivatization procedures: (a) pentylmagnesium bromide, (b) sodium tetraethylborate.

Table 2
Comparison of the detection limits of the two derivatization techniques, expressed as nanograms of organotin cation per gram of dry weight.

	Grignard LOD [ng/g]	STEB LOD [ng/g]
TBT	377	25
DBT	148	13
MBT	99	19

blank chromatograms in Fig. 4 highlights that the Grignard method produces quite a dirty sample, which will contaminate the GC injector and column more quickly compared to the STEB procedure.

3.3. Analytical performance comparison

Quantitative analysis was performed using tripropyltin chloride as internal standard throughout the whole analytical procedures, at a concentration of 1 μg/ml. Calibration curves were drawn by plotting the ratio between the analyte peak area and the internal standard peak area versus the analyte concentration. Five different concentration levels were taken into account. Each point of these curves was the mean of three replicates. Both derivatization methods provided good linearity for all the analytes with correlation coefficients ranging from 0.9980 to 0.9997.

The limits of detection (LOD) were calculated as the signal of the blank plus three times the standard deviation of 10 independent measurements of a procedure blank sample. The results obtained for both the analytical procedures are reported in Table 2. All the considered compounds show lower detection limits with STEB procedure; in particular for TBT, the most important analyte, the LOD is decreased more than one order of magnitude.

Precision and accuracy of the two methods were evaluated by analyzing the certified reference material ERM 477 and repeating the whole procedure three times. The mean values and the standard deviation obtained from the three replicates are shown in Table 3. Precision is satisfactory for both procedures and the standard deviation is comparable to that of the reference material. Accuracy is also satisfactory in both cases, although the STEB derivatization provides a TBT concentration closer to the certified value.

Table 3
ERM 477 certified values compared to the mean of three replicates. Values are expressed as micrograms of organotin cation per gram of dry weight plus/minus the standard deviation of the whole procedure.

	Certified values [μg/g]	Grignard	STEB
		Obtained values [μg/g]	Obtained values [μg/g]
TBT	2.20 ± 0.19	2.06 ± 0.15	2.29 ± 0.18
DBT	1.54 ± 0.12	1.47 ± 0.19	1.38 ± 0.14
MBT	1.50 ± 0.27	1.74 ± 0.13	1.16 ± 0.28

Table 4
Concentration of butyltins in *Mytilus galloprovincialis* exposed to TBT, expressed as micrograms of organotin cation per gram of dry weight.

Sample	Organ	Concentrations [μg/g]		
		MBT	DBT	TBT
Control 0	Mantle	0.18 ± 0.03	0.43 ± 0.08	0.59 ± 0.09
	Digestive gland	0.16 ± 0.02	0.43 ± 0.08	0.96 ± 0.15
	Gills	0.28 ± 0.04	0.64 ± 0.12	0.77 ± 0.12
Control 7	Mantle	0.17 ± 0.03	0.43 ± 0.08	0.60 ± 0.10
	Digestive gland	0.15 ± 0.02	0.48 ± 0.09	0.59 ± 0.09
	Gills	0.15 ± 0.02	0.40 ± 0.08	0.56 ± 0.09
Exposed	Mantle	0.43 ± 0.07	2.21 ± 0.42	9.60 ± 1.54
	Digestive gland	0.17 ± 0.03	2.07 ± 0.39	6.22 ± 0.99
	Gills	0.26 ± 0.04	3.44 ± 0.65	29.99 ± 4.80

In short, the STEB procedure appears preferable to Grignard derivatization from many points of view: quickness, sensitivity, accuracy and precision. Therefore, we have applied the STEB procedure to an *in vivo* experiment.

3.4. Application to mussels

Among bivalve molluscs, mussels are largely employed as pollution indicators since they are filter feeders and sessile; therefore, they are exposed to large volume of seawater and local contamination. Mussels are worldwide used as sentinels to rapidly assess the level of contamination of the marine environment for several pollutants. In our laboratory an *in vivo*

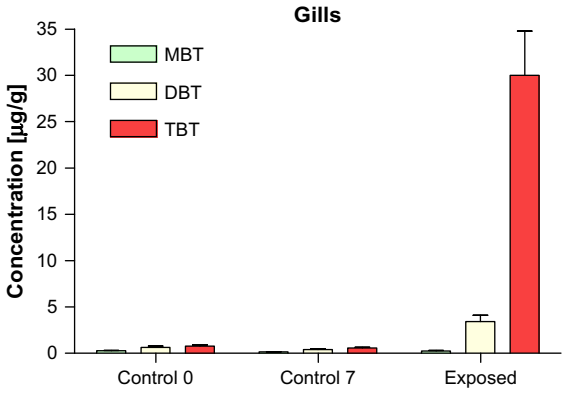

Fig. 5. Butyltin concentration (micrograms of organotin cation per gram of dry weight) in control and exposed mussel gills.

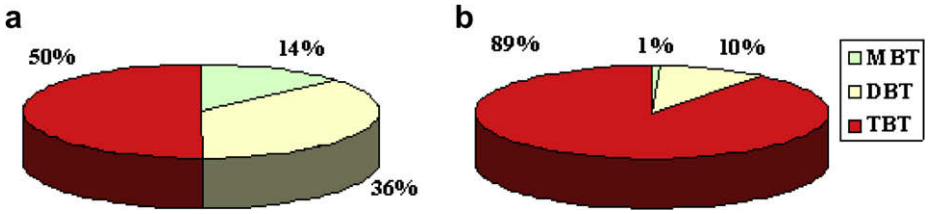

Fig. 6. Percentage of butyltin compounds in the gill tissue of (a) control and (b) exposed mussels.

experiment was performed to evaluate the bioaccumulation of TBT in mussels and the main tissues involved in this process. Mussels were exposed to the toxicant for 7 days and control mussels were also considered. The concentration of TBT and its metabolites measured in mussel tissues (mantle, digestive gland and gills) are shown in Table 4. The data represent the mean plus/minus the standard deviation of three replicates of the whole procedure.

The range of linearity considered was 0.5–4 μg/ml with the correlation coefficients of $R^2 = 0.999$ for each of the three analytes (MBT, DBT and TBT). The equations obtained for the three regression curves are $y = 0.4701x - 0.07056$ for TBT, $y = 0.8232x + 0.1085$ for DBT and $y = 2.254x - 0.1335$ for MBT. For the exposed mussels, whose concentrations were out of the range of linearity considered, a new regression curve was plotted in the range 10–60 μg/ml; the results obtained with the two curves showed no significant differences, highlighting a very good linearity of the method.

The mussels exposed for a week showed a substantial accumulation of TBT in all the tissues considered; in particular about 30 μg/g were found in the gills. This result was partially expected because, as mentioned above, mussels are filter-feeding animals and then gills are considered a target tissue for most chemicals [25,26].

DBT was found in all the tissues while MBT concentrations are not significantly different from those of the control samples. Considering that DBT and MBT are degradation products of the metabolism of tributyltin, the observed concentration trend is in agreement with the low metabolism efficiency of mussels, well described in the literature [27].

In Fig. 5 the analyte concentrations in the gills is represented as a histogram; the levels of the two controls are similar, suggesting that during the 7 days of the experiment the animals were not able to discharge the toxicants present in the tissues as a natural background. TBT and DBT are definitely lower than in the exposed samples; anyway their concentration values are not negligible, indicating that, in spite of the legal restrictions, tributyltin is still an environmental problem in the Mediterranean Sea.

The organisms from the mussel farm in La Spezia are subjected to a low and constant level of TBT; in natural conditions the uptake of the toxicant is partially counterbalanced by the metabolism of the animals. In fact, calculating the ratio between each analyte concentration and the sum of MBT, DBT and TBT measured in the gills, the control sample shows relevant percentages of all the three analytes (Fig. 6a).

On the contrary, when organisms are exposed to high doses of TBT as in the present experiment, metabolizing rate is too low and accumulation of the toxicant is observed (Fig. 6b).

4. Conclusion

The two considered methods proved to be effective for the determination of butyltin compounds in biota samples; in fact both procedures allow to obtain accurate results for the analysis of a certified reference material (ERM 477).

The comparison between the procedure involving derivatization with Grignard reagent and that with sodium tetraethylborate (STEB) highlighted that the latter is preferable for several reasons: sensitivity, accuracy and precision. In this method derivatization and extraction of organotin compounds are carried out simultaneously, simplifying and quickening the analytical procedure. Furthermore, derivatization with STEB allowed to obtain significantly lower background levels that led to lower detection limits for all the considered compounds.

This procedure was used for the determination of TBT and its metabolites in mussels that were exposed to known amounts of TBT for a week. Results showed the accumulation of TBT in the different tissues (mantle, gills and digestive gland) after 7 days of exposure; a particularly high concentration value of TBT was measured in gills (29.99 μg/g dry weight).

References

[1] K. Fent, Crit. Rev. Toxicol. 26 (1996) 1.
[2] M. Hoch, Appl. Geochem. 16 (2001) 719.
[3] B. Antizar-Ladislao, Environ. Int. 34 (2008) 292.
[4] Q.H. Zhou, Z.H. Li, G.B. Jiang, R.Q. Yang, Environ. Pollut. 125 (2003) 301.
[5] M.H. Salazar, S.M. Salazar, Mar. Environ. Res. 32 (1991) 131.
[6] P.E. Gibbs, G.W. Bryan, in: M.A. Champ, P.F. Seligman (Eds.), Organotin-Environmental Fate and Effects, Chapman & Hall, London, 1996, p. 259.
[7] M.H. Depledge, Z. Billinghurst, Mar. Pollut. Bull. 39 (1999) 32.
[8] S. Diez, S. Lacorte, P. Viana, D. Barcelo, J. Bayona, Environ. Pollut. 136 (2005) 525.
[9] Decision no. 2455/2001/EC of the European Parliament, <http://ec.europa.eu/environment/water/water-framework/priority_substances.htm>.
[10] C. Dietz, J. Sanz, E. Sanz, R. Muñoz-Olivas, C. Cámara, J. Chromatogr. A 1153 (2007) 114.
[11] E. González-Toledo, R. Compañó, M. Granados, M.D. Prat, Trends Anal. Chem. 22 (2003) 26.
[12] J.L. Gómez-Ariza, E. Morales, I. Giráldez, D. Sánchez-Rodas, A. Velasco, J. Chromatogr. A 938 (2001) 211.
[13] R. Morabito, P. Massanisso, P. Quevauviller, Trends Anal. Chem. 19 (2000) 113.
[14] M. Abalos, J.M. Bayona, R. Compaño, M. Granados, C. Leal, M.D. Prat, J. Chromatogr. A 788 (1997) 1.
[15] G.M.B. de la Calle-Guntinàs, R. Scerbo, S. Chiavarini, P. Quevauviller, R. Morabito, Appl. Organomet. Chem. 11 (1997) 693.
[16] K.C. Bowles, M.D. Tiltman, S.C. Apte, L.T. Hales, J. Kalman, Anal. Chim. Acta 509 (2004) 127.
[17] C. Devos, M. Vliegen, B. Willaert, F. David, L. Moens, P. Sandra, J. Chromatogr. A 1079 (2005) 408.
[18] P. Rivaro, L. Zaratin, R. Frache, A. Mazzucotelli, Analyst 120 (1995) 1937.
[19] R. Morabito, H. Muntau, W. Cofino, P. Quevauviller, J. Environ. Monit. 1 (1999) 75.
[20] E. Magi, M. Di Carro, P. Rivaro, Appl. Organometal. Chem. 18 (2004) 646.
[21] E. Magi, C. Liscio, E. Pistarino, B. Santamaria, M. Di Carro, M. Tiso, A. Scaloni, G. Renzone, M.E. Cosulich, Anal. Bioanal. Chem. 391 (2008) 671.
[22] A.M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito, R. Scerbo, Anal. Sci. 7 (1991) 1193.
[23] T. De Smaele, L. Moens, R. Dams, P. Sandra, J. Van der Eycken, J. Vandyck, J. Chromatogr. A 793 (1998) 99.
[24] M. Takeuchi, K. Mizuishi, T. Hobo, Anal. Sci. 16 (2000) 349.
[25] H. Harino, S.C.M. O'Hara, G.R. Burt, B.S. Chesman, W.J. Langston, Chemosphere 58 (2005) 877.
[26] J.L. López, J. Chromatogr. B 815 (2005) 261.
[27] T. Horiguchi, Z. Li, S. Uno, M. Shimizu, H. Shiraishi, M. Morita, J.A.J. Thompson, C.D. Levings, Mar. Environ. Res. 57 (2004) 75.