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WORKING METHODS PAPER

Comparison of Derivatization Methods for the Determination of Butyl- and Phenyl-tin Compounds in Mussels by Gas Chromatography

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Two different derivatization methods, alkylation with Grignard reagents, and ethylation with sodium tetraethylborate, were compared for the determination of organotin compounds, viz. tributyltin, dibutyltin, monobutyltin, triphenyltin, diphenyltin and monophenyltin, in mussel samples. Temperature, reaction time and concentration of Grignard reagents were optimized in the former method; in the latter the effect of pH, concentration of sodium tetraethylborate and reaction time were studied. In the derivatization with Grignard reagents hexyl, pentyl, propyl, ethyl and methyl were used as alkyl Grignard groups. A critical evaluation of the different derivatization methods is presented.

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INTRODUCTION

Interest in the determination of organotin compounds in environmental compartments has increased in recent years due to their large use as plastic stabilizers, antifouling agents (mainly as tributyltin, TBT), and as biocides in agriculture (mainly as triphenyltin, TPhT).[1] Once those compounds are released into the environment they can undergo methylation and degradation processes,[2,3] forming compounds characterized by different bioavailability and toxicity. Most of the analytical methods developed for the separation and quantitation of the different organotin compounds are based on the use of hyphenated techniques using liquid or gas chromatography.[4,5] Gas chromatography has been mostly used, because of its high resolving power and an easy coupling to sensitive and selective detectors, e.g. AAS,[6] AES,[7] FPD[8] and MS.[9] Due to the low volatility of the organotins, a derivatization reaction is required when a technique based on gas chromatography is going to be used. The most common derivatization reactions are hydride generation with $NaBH_4$,[10] ethylation with sodium tetraethylborate[11] and alkylation with Grignard reagents.[6,12] Nevertheless, these derivatization procedures are far from being under control. A validation, or at least a careful study of the procedures, is necessary even if the substantial lack of suitable calibrants makes these tasks very difficult.[1]

In the present study optimization and comparison of different derivatization methods for the determination of organotin compounds in mussel samples have been performed. Mussels were chosen because after the direct introduction of organotins in the marine environment, mainly through their use as antifouling paints, they accumulate in mussels, being highly toxic for such organisms.[13] This study has been carried out

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EXPERIMENTAL

Reagents

The organic solvents (all pesticide analysis grade) were hexane (Baker), dichloromethane (Baker), iso-octane (Carlo Erba) and benzene (Carlo Erba).

A solution of 0.03% (w/v) tropolone (2-hydroxycycloheptatrienone) (Lancaster Synthesis) was prepared in methanol (Carlo Erba).

Hexylmagnesium bromide (2.0 mol l^{-1} in diethyl ether), pentylmagnesium bromide (2.0 mol $^{-1}$ l in diethyl ether), propylmagnesium chloride (2.0 mol $^{-1}$ l in diethyl ether), ethylmagnesium bromide (1.0 mol $^{-1}$ l in t-butyl ether) and methylmagnesium bromide (1.0 mol $^{-1}$ l in t-butyl ether) were purchased from Aldrich.

Standard solutions of 1000 μg ml^{-1} organotins (as tin) were prepared by dissolving appropriate amounts of tributyltin chloride (BDH), dibutyltin chloride (Aldrich), monobutyltin chloride (Aldrich), monophenyltin chloride (Aldrich), diphenyltin chloride (Aldrich), triphenyltin chloride (Merck) and tripropyltin chloride (Merck) in toluene; an intermediate mixed solution of 10 μg ml^{-1} of each compound was prepared in iso-octane; to prepare the standard working solutions 1 ml of this solution was derivatized with the different Grignard reagents and then the volume was made up to 50 ml with hexane so that the final concentration of each compound was 200 ng ml^{-1}.

Sodium chloride (Carlo Erba) solution (5% (w/v) was prepared in double-distilled water. The sodium chloride was heated at 450 °C for 6 h before use. Anhydrous sodium sulphate (Carlo Erba) was heated at 600 °C for 24 h before use.

A Florisil column for the clean-up step was prepared with 3 g of Florisil (60–100-mesh) (Merck) previously heated at 180 °C for 3 h.

Equipment

GC–FPD analyses, unless otherwise stated, were carried out on a Varian Vista 6000 chromatograph equipped with a flame photometric detector (FPD) operated without a filter on a megabore column (DB1 30 m × 0.53 mm × 1.5 μm). Gas flows were modified as follows: air (1) and hydrogen (1) were exchanged with respect to the standard instrumental configuration, and air (2) was exchanged with hydrogen. The flows and other chromatographic conditions are indicated in Table 1. Data were collected and integrated by a Hewlett–Packard HP 3396 A.

Table 1 Instrumental parameters used in the determination of organotin compounds by GC–FPD

Air (1)	200 cm^3 min^{-1}
Hydrogen (1)	120 cm^3 min^{-1}
Hydrogen (2)	80 cm^3 min^{-1}
Carrier gas flow	Helium 9 cm^3 min^{-1}
Injector	Hot-on-column, 240 °C
Detector temperature	240 °C
Temperature programme	
Initial temperature	80 °C (1 min)
Ramp	10 °C min^{-1}
Final temperature	280 °C (3 min)

GC–MS analyses were performed on a Hewlett–Packard HP 5890GC/HP 5970B MSD system on a capillary column (DB5 30 m × 0.25 mm × 0.25 μm) in the conditions indicated in Table 2.

Procedure

After approximately 0.1 g of freeze-dried mussels had been weighed out in quadruplicate, 50 ng as tin of tripropyltin (TPrT) was added as internal standard (in the propylation and methylation reactions, as discussed later, TPrT could not be used as internal standard, and calculations were made by reference to an external standard, carefully controlling the working volumes), followed by 15 ml of 0.03% (w/v) tropolone in methanol solution together with 1 ml of concentrated hydrochloric acid. The vials were ultrasonically shaken for 15 min and centrifuged for 10 min. The supernatant was transferred to a separation funnel and the extraction procedure was repeated; then 15 ml of dichloromethane and 100 ml of a 5% (w/v) NaCl water solution (to avoid the formation of an emulsion) were added to the collected extracts. The organotin compounds were extracted into the organic phase by shaking for 3 min, the two phases were allowed to separate and the organic phase was passed through sodium sulphate to eliminate traces of water. The extraction into 15 ml of dichloro-

Table 2 Instrumental parameters used in the determination of organotin compounds by GC–MS

Electron impact ionization mode	70 eV
Carrier gas	Helium, 120 kPa head pressure
Capillary column	DB5, 30 m × 0.25 mm × 0.25 μm
Injector	Splitless, 240 °C
Transfer line temperature	280 °C
Temperature programme	
Initial temperature	80 °C (2 min)
Ramp	10 °C min^{-1}
Final temperature	280 °C

SIM (for pentylated compounds) (dwell time 100 ms for all the ions)

Ion	Start time	*m/z*
TPrT	6.50	277, 275, 273
TBT	9.50	305, 303, 301
DBT	11.30	319, 317, 315
MBT	12.95	319, 317, 315
Sn(IV)	14.15	333, 331, 329
MPhT	15.80	339, 337, 335
DPhT	18.00	345, 343, 341
TPhT	19.50	351, 349, 347

methane was repeated. The organic phases were combined, evaporated with a rotavapor down to a final volume of 1 ml, transferred to a vial, and then evaporated almost to dryness. The different derivatization reactions were applied (ethylation with sodium tetraethylborate, and alkylation with the various Grignard reagents) as described below. A clean-up step was made on a Florisil column (3 g), eluting the retained derivatized compounds with 5 ml of hexane/benzene (1 : 1). The organic solvent was vented with a nitrogen flow down to a volume of about 0.3 ml. A 1 μl portion of the organic phase was injected in the gas chromatograph, using FPD or MS as detector.

Derivatization by alkylation with Grignard reagents

Concentrated Grignard reagent (1 ml) was added and the mixture was vigorously shaken for a few seconds. The excess of Grignard reagent was destroyed first by carefully adding a few drops of water and shaking gently and then 5 ml of a 1 mol^{-1} l sulphuric acid solution. The derivatized compounds were extracted twice into 1 ml of hexane. The clean-up step was applied as previously described.

Derivatization by ethylation with sodium tetraethylborate

Acetic/acetate buffer, pH 5 (50 ml) was added and the derivatization was carried out by adding to this solution 1 ml of a 1% (w/v) sodium tetraethylborate solution in double-distilled water. The ethylated organotin compounds were extracted into 5 ml of hexane for 5 min and the two phases were allowed to separate for another 5 min; the organic phase was taken and evaporated down to about 0.3 ml, then the clean-up step was applied.

RESULTS AND DISCUSSION

All the results discussed in this work were obtained with unspiked samples and represent environmental concentration levels. For all the recovery studies a set of results was randomly chosen as 100% recovery, and the rest of results

were referred to it. The concentration of diphenyltin (DPhT) in this sample was close to the quantification limit: it is for this reason that the results for DPhT are affected by a high irreproducibility and in some experiments the amount present in the final extract was not quantifiable.

Derivatization using Grignard reagents

The following parameters were studied and optimized for the pentylation reaction: temperature (25 and 50 °C), reaction (0, 15, 30 and 60 min), shaking or not shaking the sample during the reaction, and concentration of the Grignard reagent (0.5 and 2 mol l^{-1}).

The results obtained are shown in Fig. 1. Among all the parameters tested, only the concentration of the Grignard reagent produced a significant influence on the efficiency of derivatization (within the limits of the standard deviation of our method) (Fig. 1a). Lower recoveries were obtained when diluted (1:4) Grignard reagent was used. In this case it is necessary to use a longer reaction time (40 min) to obtain a good recovery for all the compounds, with the exception of monobutyltin (MBT).

The reaction time, temperature and shaking the sample during the reaction do not affect the derivatization yields (Fig. 1b–d) showing that the reaction is very fast even at room temperature.

Two common characteristics were observed in all the determinations: (1) the existence of a large chromatographic peak corresponding to inor-

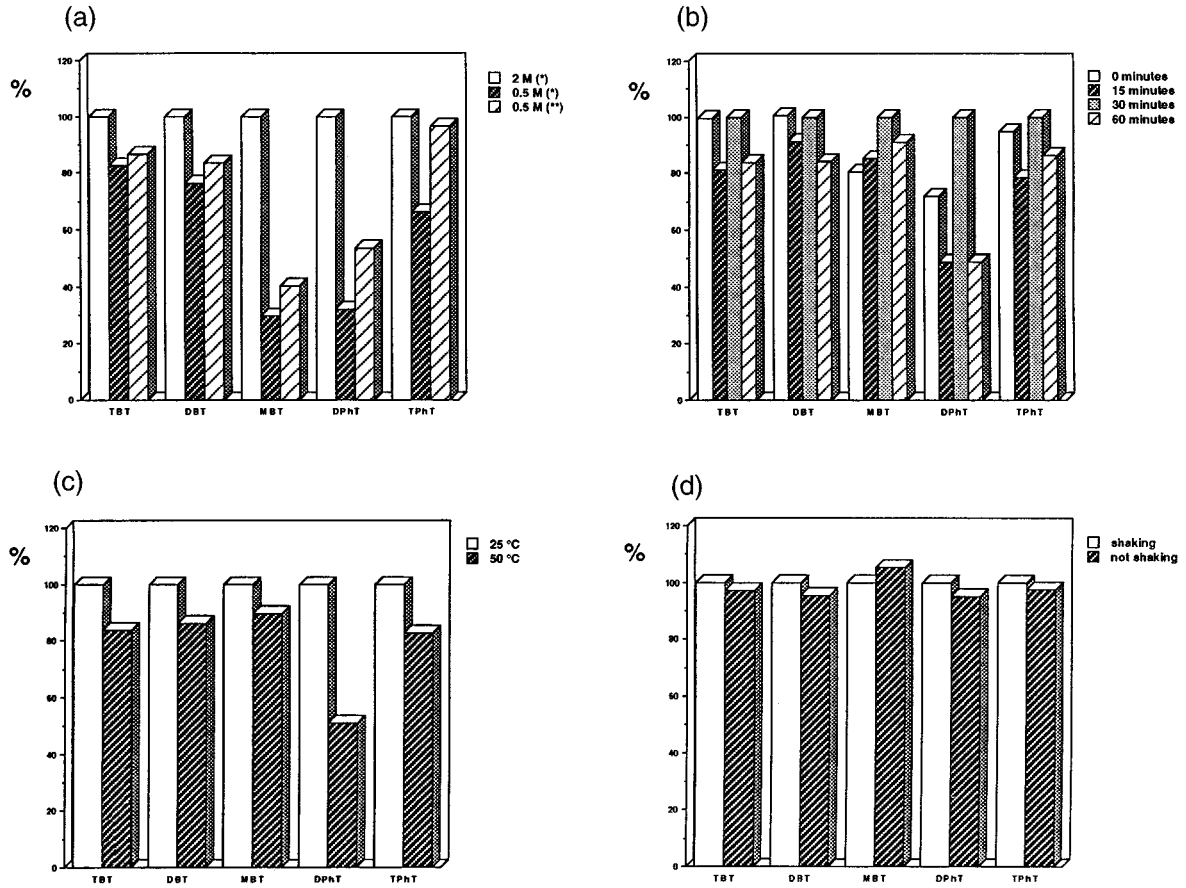

Figure 1 Optimization of the parameters affecting the derivatization of butyl- and phenyl-tin compounds using Grignard reagents: (a) concentration of Grignard reagent (∗ shaking for a few seconds; ∗∗ shaking for 40 min); (b) reaction time; (c) reaction temperature; (d) influence of shaking or not during the reaction.

ganic tin, and (2) very poor reproducibility for monophenyltin (MPhT) and, to a less extent, for MBT. Initially it was thought that this poor precision originated in integration errors due to the propinquity of the peaks of MPhT and MBT to the peak corresponding to inorganic tin (Fig. 2a). To check this possibility the same sample was analysed with a GC–MS in the single-ion monitoring mode (SIM). The same effect was observed even when, in this case, all the chromatographic peaks were perfectly resolved (Fig. 2b). These results suggested that the poor reproducibility achieved in the determination of MPhT and MBT was due to a problem during the extraction step rather than to an integration problem and/or to a problem in the derivatization reaction.

Influence of different alkyl groups in the derivatization reaction

Once the optimum conditions had been chosen for the pentylation reaction, the efficiency of the derivatization was checked for the different alkyl groups: hexyl, propyl, ethyl and methyl. It was observed that only for the less-volatile compounds, TPhT and DPhT, was the same recovery achieved for the different alkylation reactions tested (Fig. 3). Decreasing recoveries for the other organotin compounds were obtained on diminishing the length of the alkyl group. Therefore, it was thought that the decrease in the recovery could be due to volatilization occurring during the preconcentration steps under a nitrogen flow, rather than to a different efficiency in the derivatization depending on the alkyl group.

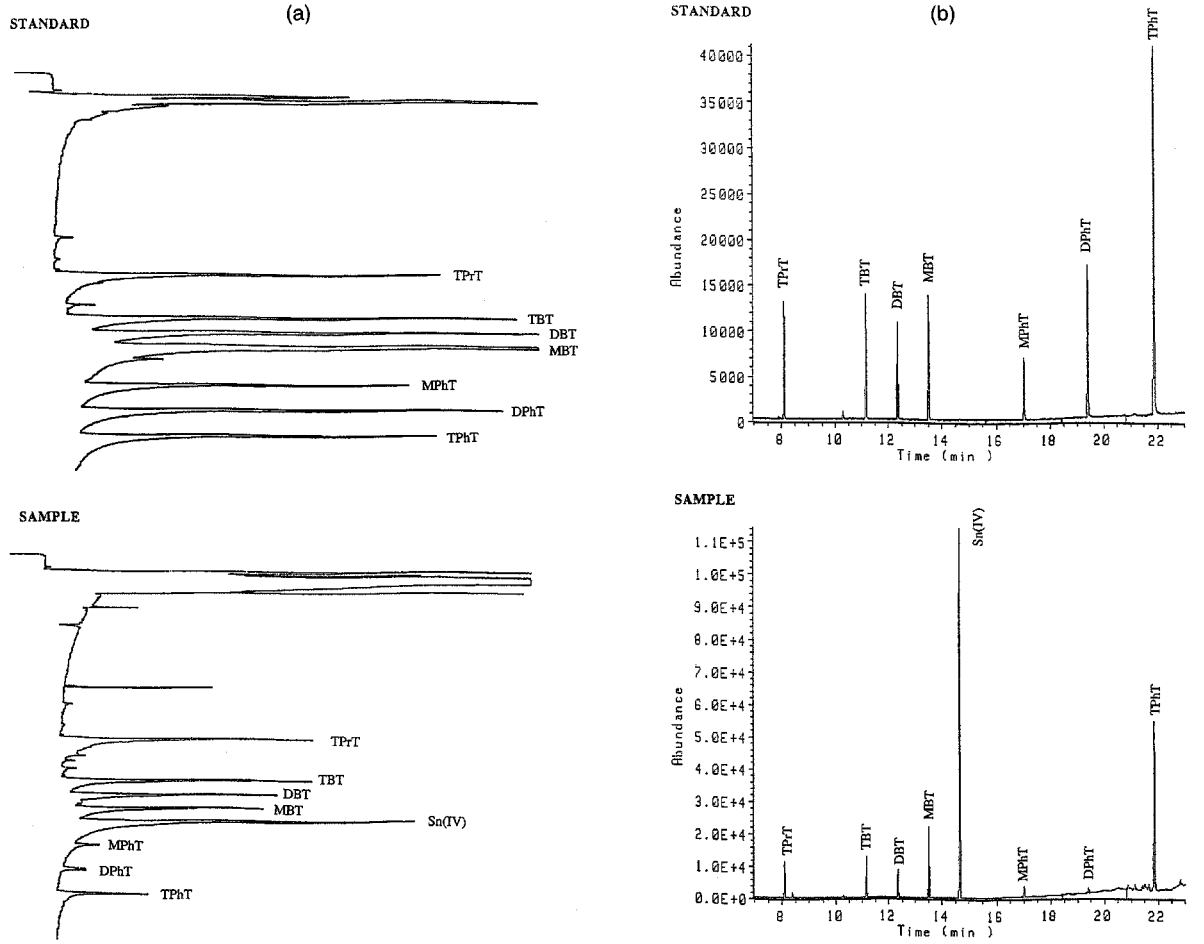

Figure 2 Chromatograms obtained for organotin compounds in mussel: (a) using FPD as detector; and (b) using MS as detector.

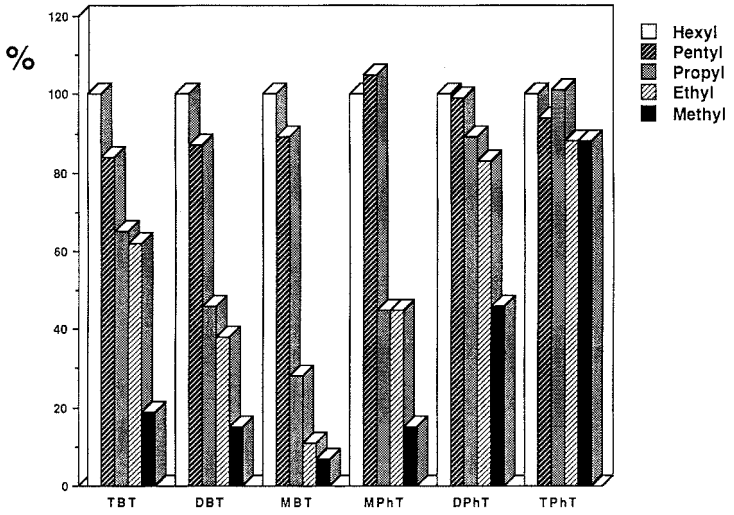

Figure 3 Influence of the use of different alkyl groups in the derivatization of butyl- and phenyl-tin compounds by means of the Grignard reaction.

To check this possibility, a study was performed on standard solutions derivatized with the different alkyl groups.

Two 5 ml aliquots of a 200 $\mu g\,l^{-1}$ standard solution were placed in two vials and evaporated under a nitrogen flow down to 0.3 ml and to dryness, respectively; then hexane was added to a final volume of 5 ml. The chromatographic signals obtained before and after the evaporation were compared. The results obtained are shown in Fig. 4. When the solvent was evaporated to dryness (Fig. 4a) it can be observed that, in the case of hexylation and pentylation, no volatilization losses were detected, even for the most volatile compounds. In contrast on alkylation with shorter alkyl groups, only the less volatile compound, TPhT, was not lost in the preconcentration step, even when methylation was carried out.

This effect is not so pronounced when the organic solvent is evaporated down to 0.3 ml (Fig. 4b). In this case no decrease in the signal of any of the compounds was observed when propylation was used.

In order to avoid losses by volatilization, the analyses of the lyophilized mussels were repeated for hexylation, propylation, ethylation and methylation, carefully controlling the solvent volume so as not to go below 1 ml in any of the preconcentration steps. The results are shown in Fig. 5. No significant difference was observed among the different alkylations for any of the organotin compounds (with the exception of the MPhT, as previously mentioned).

Derivatization with sodium tetraethylborate

Three parameters affecting the efficiency of this reaction were studied: pH, concentration of sodium tetraethylborate and reaction time.

Due to the low reproducibility obtained for MBT and MPhT, only tri- and di-substituted compounds were considered. The highest recoveries were obtained for all the compounds considered in the pH range 4.5–5.0; for TPhT a maximum analytical signal was obtained at pH 4; for higher and also lower pH values, a decrease in the recovery was observed (Fig. 6a).

The reaction time required to achieve a maximum analytical signal was 2 min for all the compounds with the exception of TPhT, which requires 3 min (Fig. 6b); 5 min reaction time was chosen for further experiments.

The highest recoveries were obtained for concentrations of sodium tetraethylborate equal to or higher than 0.3% (w/v) (Fig. 6c). Nevertheless, a significant increase in the recovery was obtained for TPhT when 1% (w/v) $NaBEt_4$ is

used (Table 3). Concentrations higher than 1% (w/v) were not tested because solutions were not completely clear and some interferences coming from carbonaceous compounds could take place. A comparison between ethylation carried out with the Grignard reagent (ethylmagnesium bromide) and sodium tetraethylborate showed that ethylation with the Grignard reagent provided slightly better results (Table 3). Working under the optimized conditions, the recovery obtained by ethylation with the Grignard reagent is comparable with that achieved by hexylation, also with Grignard reagent, which we have assumed to correspond to 100% recovery.

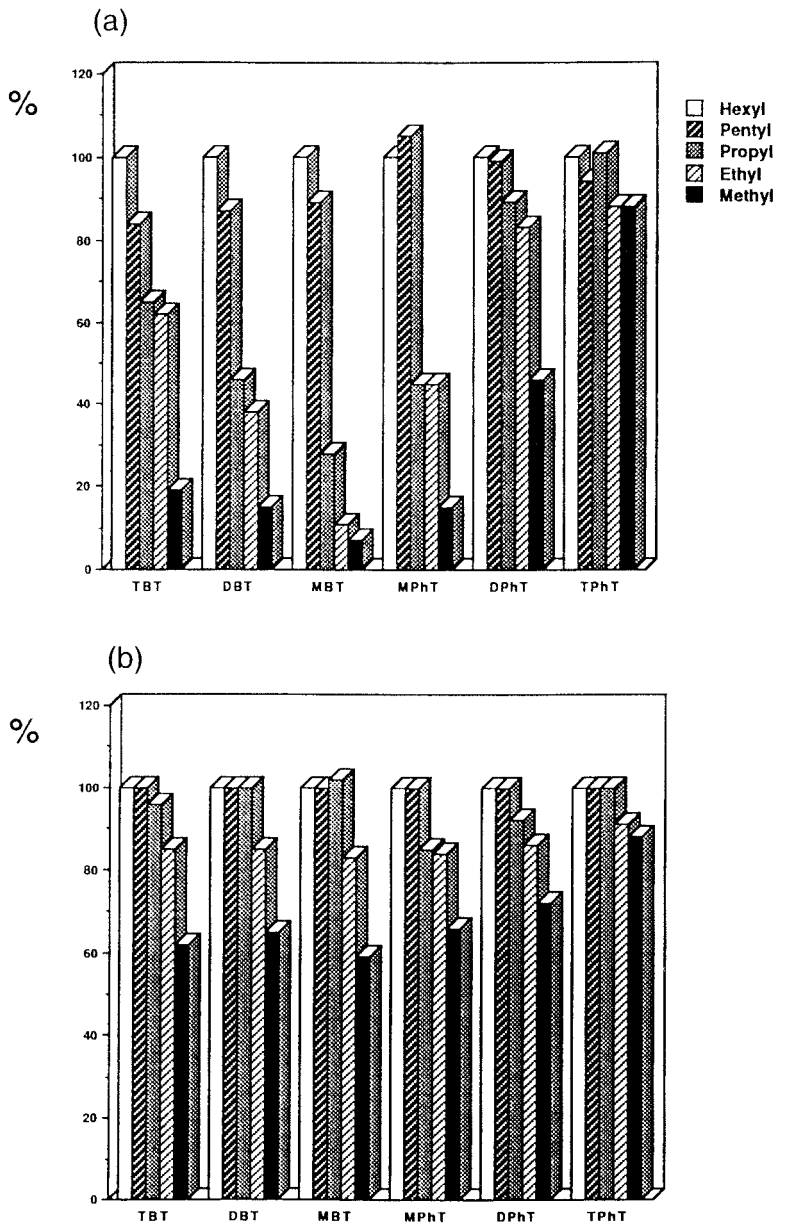

Figure 4 Study of losses due to volatility during the preconcentration steps: (a) evaporating the solvent to dryness and (b) down to 0.3 ml.

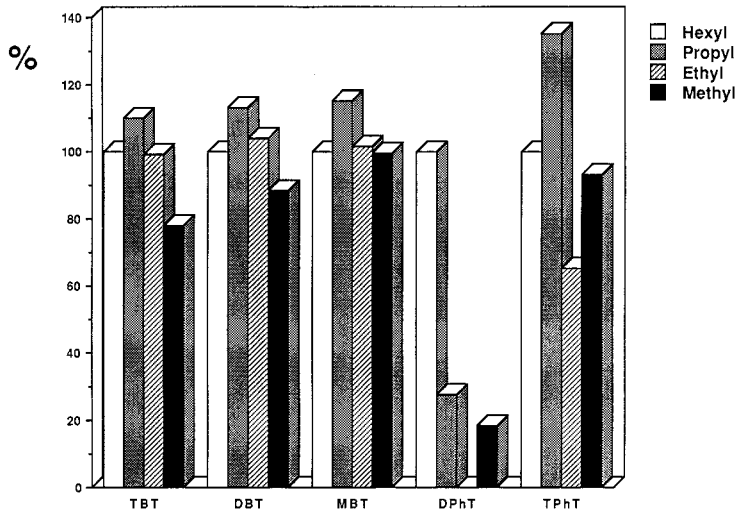

Figure 5 Concentration of butyl- and phenyl-tin compounds in mussels using different alkyl groups in the derivatization reaction with Grignard reagents, blowing the solvent down to 1 ml.

Table 3 Comparison of the recoveries obtained for phenylated and butylated organotin compounds in mussel samples

	Recovery (ng g^{-1}, as tin)					
Method[a]	TBT	DBT	MBT	MPhT	DPhT	TPhT
(1)	778±55	803±79	570±42	1090±300	81±12	592±58
(2)	639±42	637±62	440±60	592±20	—	449±65
(3)	627±42	593±29	522±74	—	—	183±56

[a] (1) Derivatization with ethylmagnesium bromide; (2) derivatization with 1% (w/v) sodium tetraethylborate; (3) derivatization with 0.3% (w/v) sodium tetraethylborate

Analytical characteristics of the method

The analytical characteristics of the method were calculated for pentylated TBT, following the IUPAC recommendations, and are summarized in Table 4. Very similar figures held for the other derivatization procedures and for the other compounds.

Table 4 Analytical characteristics of the method calculated for pentylated TBT, $n=4$

Detection limit (3σ)	1 pg
Quantification limit	5 pg
Sensitivity	22 height units/pg
Precision	10% for 50 pg

CONCLUSIONS

Hexylation, pentylation and ethylation derivatization with Grignard reagents may be successfully used for the determination of most of the organotin compounds present in the environment (e.g. methylated, butylated and phenylated). They also allow the use of tripropyltin as internal standard (being one of the most widely used). Among these three alkylation

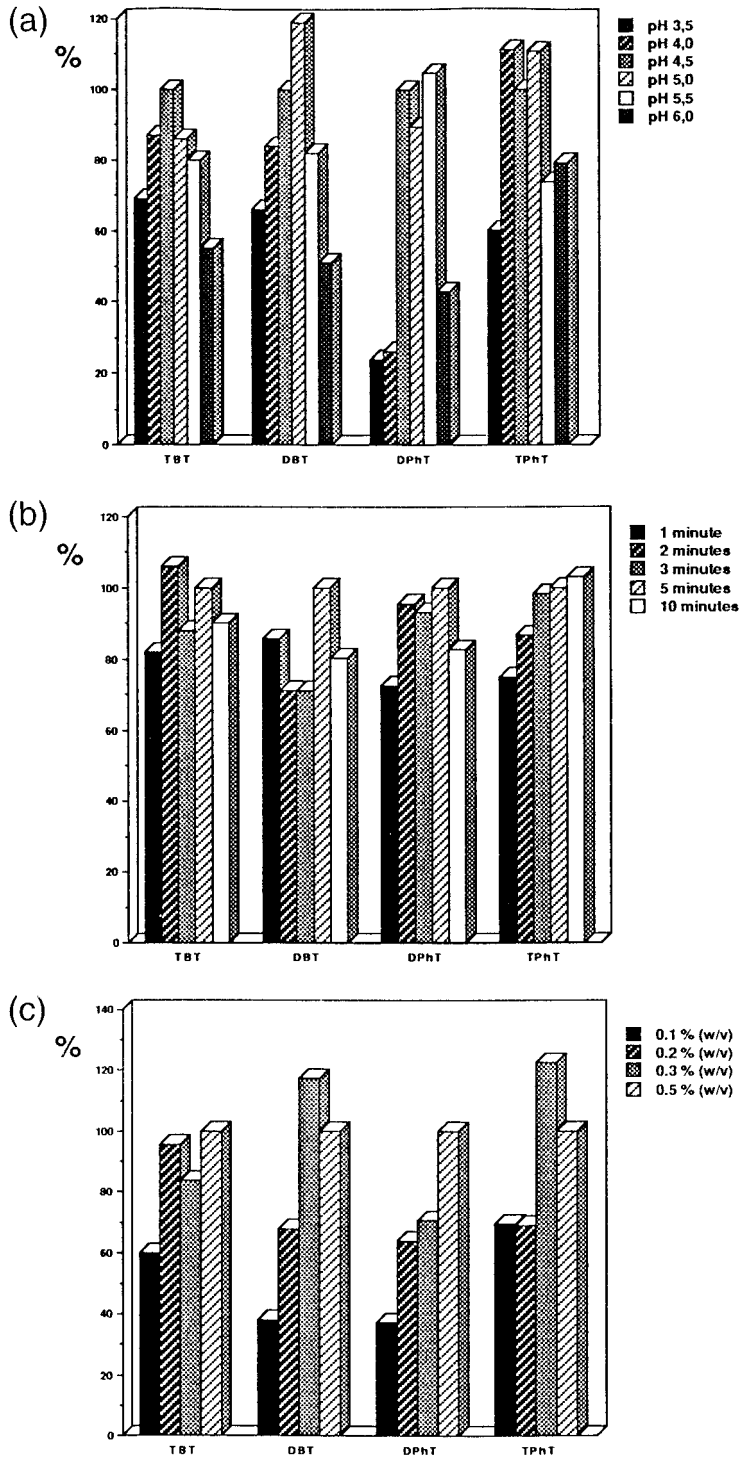

Figure 6 Optimization of the parameters affecting the derivatization of butyl- and phenyl-tin compounds using sodium tetraethylborate: (a) pH of the medium; (b) reaction time; and (c) concentration of derivatizing agent.

methods, hexylation and pentylation have the advantage of providing derivatized compounds with relatively low volatility which allows easy preconcentration steps in the sample pretreatment without taking special precautions; in contrast, due to the low volatility, condensation problems in the interface have been described when GC–AAS coupling is used.[1] Propylation, ethylation and methylation produce highly volatile compounds and partial losses during the preconcentration steps may occur; losses of dibutyltin (DBT) and TBT could be avoided by blowing the organic solvent down to a limit of 1 ml. Among these three alkylation reactions, ethylation is to be preferred because tripropyltin cannot be used as internal standard in propylation and methylation reactions. With propylation, inorganic tin yields the same compound as TPrT, i.e. tetrapropyltin; with methylation TPrT and DBT co-elute, so that TPrT interferes with the determination of DBT. Moreover, methylation has an additional disadvantage, in that it cannot be applied to methylation studies.

The ethylation reaction with sodium tetraethylborate showed slightly lower derivatization yields compared with hexylation and pentylation via Grignard derivatization. Losses by volatilization may occur, as previously discussed in ethylation with the Grignard reagent. Nevertheless, ethylation with sodium tetraethylborate has the advantage over the Grignard reaction that sodium tetraethylborate is stable in water and so the derivatization reaction can take place in aqueous media. However, this advantage is not exploitable when the very common acidic extraction procedures are performed, because in acid media sodium tetraethylborate is not stable and decomposes; therefore the organotins have to be extracted into organic solvent prior to the derivatization reaction.

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