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Organotin analysis by gas chromatography–pulsed flame-photometric detection (GC–PFPD)

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Abstract Monobutyltin (MBuT), dibutyltin (DBuT), and tributyltin (TBuT) mixtures have been separated and quantified by gas chromatography with pulsed flame-photometric detection (GC–PFPD). The compounds were first derivatized with $NaBEt_4$, then extracted with hexane and injected into the GC in splitless mode. Optimum GC and detector conditions were established. For GC, various injector temperatures and oven temperature programs were tested. For the PFPD detector, gate settings (gate delay and gate width) and detector temperature were optimized. A very good linearity was obtained up to 100–150 ppb for all organotin compounds. The detection limits obtained were: MBuT (0.7 ppb), DBuT (0.8 ppb), and TBuT (0.6 ppb). RSD for repeatability and reproducibility were well below 20% when the instrument was in routine operation. A biological sample (CRM 477) was also analyzed for organotins. Extraction from the biological matrix was performed with TMAH. Besides the increased risk of contamination, the derivatization step seemed to be critical. pH and amount of derivatizing agent were tested. When using an internal standard (TPrT) between 90% and 110% of the certified amounts of organotin were recovered.

Keywords Organotin · Analysis · Gas chromatography · Pulsed flame-photometric detection · Water · Biota

Introduction

Tin is the element with the largest number of organometallic derivatives in commercial use. Mono, di, and triorganotin compounds have large scale applications, mainly as stabilizers, catalysts, biocides, or pesticides and tetraorganotin compounds are important intermediates in the production of other organotin compounds. Global organotin production has been estimated to exceed 50,000 tons per year [1]. Approximately 25% of this amount consists of trisubstituted biocides, tributyl, triphenyl, and tricyclohexyl tin compounds (TBT, TPhT, and TcHT, respectively) of which 4,000 tons are TBT. A list of possible applications can be found in Ref. [2]. The high toxicity of the trisubstituted species to a variety of organisms was quickly established, and the materials were immediately used in a large variety of biocidal applications. The most successful of these was tributyltin (TBuT) employed as an anti-fouling paint on major sea-going vessels, but also leisure boats adopted this most efficient paint, unwittingly contributing to the contamination of coastlines throughout the world. Nowadays its use is strongly regulated.

The properties of mono and tri-substituted organotin compounds differ widely and depend directly on the number (*n*) and nature of the organic groups (R) covalently bound to the tin atom. In general, the anionic group, or counter-ion, does not have any particular effect on the organotin properties, other than its own properties. However, it may affect the octanol–water partition coefficient and its adsorption potential, but relatively little information is available on these issues.

Although tin in its inorganic form is considered to be non-toxic, the toxicology of the organotin compounds is very complex—it largely depends on the nature and number of alkyl groups but also varies from organism to organism. In general we can say that the alkyltin toxicity increases with the number of organic groups (tetraorganotin compounds are an exception). So the trisubstituted compounds, for example TBuT, have the highest toxicity. Mono and tetraorganotins are much less toxic, the first because they are too polar, the latter because they are practically nonpolar. Polarity plays an important role in the rates of uptake and accumulation of a compound by an organism and therefore strongly

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determines the toxicity of the compound. Tetraorganotin compounds often have delayed toxic activity, because of degradation to (much more toxic) trisubstituted compounds. In fact, for most organisms di- and triorganotin compounds are most dangerous. They are regarded as hormone disruptors and the phenomenon of imposex, the appearance of male characters in females, is well known in gastropods exposed to organotin compounds [3].

Most analytical methods for organotin determination employ extraction of the compounds from the matrix, then derivatization, if chromatographic separation is being used, and a purification step. Sample treatment can be regarded as one of the most critical steps, being prone to errors from many sources. Contamination of samples can arise from glassware, reagents, the laboratory, and even the operator. In organotin analysis plastic labware, and in particular PVC (which contains di and monobutyltin), but also plastic gloves (sometimes containing trioctyltin), must be avoided. However, the crucial point is in the choice of the most suitable extraction and derivatization reagents. It is trivial to mention that low extraction efficiencies and/or low derivatization yields lead directly to an underestimation of the real concentration.

Furthermore, changes in speciation during treatment, because of degradation, must be taken into account—the most suitable reagents must be chosen and the experimental conditions during the various steps, for example temperature, pH, light, time of processing, etc., must all be optimized.

Many instruments can be used for speciation and detection of organotin compounds. Nowadays gas chromatography (GC) and high performance liquid chromatography (HPLC) are the most important speciation techniques for organotin compounds [4]. Although flame photometric detection (FPD) and inductively coupled plasma-mass spectroscopy (ICPMS) are often used for detection of tin, many different detectors have been, and still are, in use for organotin analysis [5]. In this work the instrumental analysis was performed with a pulsed flame-photometric detector (PFPD) coupled to a GC. The PFPD is a new generation of FPD in which the continuous flame is replaced by a pulsed flame. Although this has many advantages, especially with regard to sensitivity, its performance for organotin speciation is not very well documented.

Materials and methods

All materials coming into contact with samples or solutions containing organotin compounds or with reagents used for analysis of those compounds were decontaminated by immersion in strong nitric acid solution, usually for 48 h, and thoroughly rinsed with ultra-pure Milli-Q water. For some materials (especially those coming in contact with contaminated samples) the procedure was repeated.

Reagents

Solid, 98% pure $NaBEt_4$, from Strem Chemicals, (1 g) was dissolved in 100 mL water (at 0°C) with 2 g KOH. Further dilutions were performed with Milli-Q water and the solutions were stored in the freezer. Acetate buffer, pH 5, was prepared by diluting 59 mL acetic acid (CH_3COOH 96%, Merck; pro analyse) and 136 g sodium acetate (CH_3COONa, Merck; pro analyse) to 500 mL with Milli-Q water. Methanol (Merck LiChrosorb) and *n*-hexane (Merck Suprasolv) were purchased from Vel. Tetramethylammonium hydroxide (TMAH) was obtained from Acros Organics as a 25 % (*w*/*v*) solution. Monobutyltin (MBuT) was directly acquired as a 10 ng μL^{-1} solution in methanol (reference material) from Dr Ehrenstorfer. DBuT, TBuT, and TPrT were available as their chloride salts (Merck, purity >99%). A triphenyltin (TPhT) stock solution was kindly provided by the Department of Organic Chemistry of the Vrije Universiteit van Brussel. Ultra pure water was produced by a Milli-Q water system (Millipore).

Standard solutions

Dibutyltin (DBuT), TBuT, and tripropyltin (TPrT) stock solutions of approximately 1 g (as Sn) per liter were gravimetrically prepared in methanol. These stocks were stored under refrigeration and could be used for several months. The stock solutions were further diluted with methanol to 10 ng μL^{-1}. These standard solutions and the MBuT standard were stored under refrigeration and used for about 1 week. Solutions with lower concentration were stored for 1 day only.

The TPhT solution was used for GC optimization only and not for quantification.

Digestion of mussel samples

Dry, ground mussel samples (10–100 mg) were placed in 25-mL flasks. A known volume of TPrT standard solution was added as internal standard. A solution of TMAH in methanol (25% *w*/*v*, 2 mL) was used for digestion. The flasks were then shaken for approximately 1 h in a water bath at 60°C. The solution was then transferred to a 50-mL bottle for ethylation of the organotin compounds.

Derivatization

Standards

Derivatization was performed with $NaBEt_4$. Ethylation was preferred to a Grignard reaction because it could be applied in aqueous media and no special safety measures were required. Also, reduction of the number of analytical steps by in-situ derivatization is beneficial.

Hydride generation was also tried out during this work but seemed less suitable than ethylation, maybe because no special measures were taken to prevent volatilization of the derivatized organotin compounds [6–9] .

Ethylation of the standard solutions was performed in 50-mL bottles. To the standard solution 1 mL acetate buffer, 1 mL $NaBEt_4$ solution (0.1 or 1%, *w*/*v*), and Milli-Q water were added. To extract the derivatized organotin compounds 1 mL *n*-hexane was added and the bottle was then filled (total volume 50 mL) with Milli-Q water. After stirring for 40 min the hexane phase containing the organotins was isolated with a Pasteur pipette.

Samples

After digestion, samples were transferred to 50-mL bottles and 20 mL acetate buffer, $NaBEt_4$ solution, and 1 mL *n*-hexane were added. The rest of the bottle was filled with Milli-Q water. After stirring for 40 min the hexane phase was isolated with a Pasteur pipette. No further clean-up was performed. To reduce foaming, 50 μL of 2-propanol (Merck, pro analyse) was added to "break the emulsion" and thus enable clean separation of the hexane phase. The sample digestion and derivatization/extraction procedures are largely based on the method described by Gallina et al. [10].

Specifications of the GC

GC was performed with a Varian CP-3800 equipped with a split/splitless injector and a PFPD

Column

A CP-Sil 5 CB column coated with 100% PDMS stationary phase was used. The dimensions of this FSOT column were: length 15 m, inside diameter 0.32 mm, outside diameter 0.35 mm, film thickness 0.25 µm. PDMS, a non-polar stationary phase with a high crosslinking, is often used for organotin speciation analysis. The organotins form a rather uniform series when considering polarity, so separation will be largely because of their different boiling points (and thus their molecular mass after derivatization). Helium was used as carrier gas. A constant pressure of 10 psig was applied, so gas flow rates changed during a chromatographic run because of the temperature gradient.

Detector

General

The PFPD is a new type of GC-detector. The difference from the classic FPD is that a pulsed rather than continuous flame is used. This has several advantages, for example greater selectivity, higher sensitivity, and lower fuel gas consumption [11]. For Sn the sensitivity, and thus the detection limit, of the PFPD is approximately 100 times better than that of the FPD detector. The PFPD can be used for selective detection of many compounds for example S, P, N, As, Se, Ge, Te, Sb, Br, Ga, In, Cu, and Sn (this list is not complete).

Two different fuel gas flows (O_2 + H_2) are used. The first mixes with the eluent from the GC column and fills the middle part of the combustor tube (or combustion chamber). The second, with lower hydrogen-gas content, fills the outside of the combustor tube and the ignitor chamber. When the fuel gas mixture ignites the sample molecules (from the GC eluent) are broken down into simpler molecules or atoms but also form electronically excited species.

The flame-fuel is consumed completely after only 3–4 ms but the excited species (from the GC eluent), depending on the species, emit for over 30 ms. Because of this property it is possible to separate background and analyte emission from each other. The frequency of pulses is very important because ignition of the fuel must occur when the organotin compounds are also in the ignition chamber. The fuel gas flows should therefore be well optimized.

Background emission arises mostly from flame-fuel combustion products, for example excited CH, C_2, and OH species, formation of which is favored in hydrogen-rich flames. It is possible, however, that sulfur compounds present in the (environmental) sample matrix interfere during Sn analysis. These sulfur compounds, mostly excited S_2, reach a maximum emission after 5–6 ms. Interference with Sn emission can be avoided by using optical filters (to filter out the sulfur signal) or appropriate choice of gate settings (delay time and gate width, see below).

Settings for the organotin analysis

The settings that are most specific for the PFPD are the different fuel gas flow rates and the gate settings. During preliminary experiments with the apparatus the fuel flow rates were not at all optimized. As a result the sensitivity of the detector for organotin speciation analysis was orders of magnitude below that reported in the literature ([12–16], see also Table 1).

For organotin compounds, emission at two different wavelengths is important. Sn–C bonds emit at 390 nm (blue) and Sn–H bonds at 610 nm (red). There can be a problem with the Sn–C emission, because sulfur species also emit at 390 nm. With the classic FPD, therefore, an optical filter is usually used and only Sn–H emission at 610 nm is measured. Sn–C emission is, however, 100–1,000 times more intense than that of Sn–H, hence using the signal at 390 nm is necessary for sensitive analysis. With the PFPD this is possible—an optical filter was used to select the 390 nm wavelength and appropriate gate settings excluded sulfur interference.

Table 1 Reported limits of detection for PFPD organotin analysis using one of two possible tin emissions

Compound	Absolute limits of detection (pg)	
	Sn–H	Sn–C
MBuT	0.48	0.10
DBuT	0.35	0.07
TBuT	0.30	0.07
TPhT	0.38	0.11

In this way the sensitivity is significantly higher than with a classic FPD.

Another problem with the classic FPD is contamination of the detector by non-volatile (tin) oxides. The PFPD is self-cleaning, because of the pulsed flame propagation mechanism.

Results and discussion

Optimization of the analysis

Oven program

The goal was to optimise the separation of MBuT, DBuT, TBuT, TPrT, and TPhT. The initial temperature program was similar to settings recommended by the instrument supplier (Table 2). Obviously, good resolution between the first four eluting compounds was not obtained (Fig. 1). At the start of the program the column temperature is apparently increased too fast (30° min^{-1}), so the very volatile ethylated organotin compounds are almost unretained by the stationary phase of the column (shortly after the start of the chromatographic run the boiling points of all the early-eluting compounds have already been exceeded).

Reducing the rate of temperature increase at the start of the program to 8° min^{-1} yielded much better separation of the first four eluting compounds (Fig. 2). We calculated the resolution for the pair of peaks which were closest to each other, those from MBuT and TPrT, the first two compounds to elute. R_S was 2.5, which is much better than the value required for baseline separation ($R_S = 1.5$). However, because TPhT elutes much later than the four other compounds, it is important that after their elution the temperature is increased much more quickly (30° min^{-1}). At the end of each run, the oven was kept at 270°C for 15 min to recondition the column for the next run (Table 2).

Injection

For injection the most important settings are injector temperature, initial column temperature, split delay, split ratio, and injection volume. In this work the injector was used in splitless mode to maximize sensitivity. This means that during and shortly after injection the split outlet is closed and all the flow entering the liner goes to the column. After a delay of 1 min, however, the split outlet is opened to vent vapors that remain in the injector. This is very useful when analyzing "dirty" environmental samples, because less volatile compounds are predominantly retained in the injector and thus, after

Table 2 Optimization of column and injector system

Ramp	End	Stay
Initial, adapted and final oven temperature program		
Initial		
Start	80°C	1 min
30° min^{-1}	180°C	
10°C	270°C	15 min
Total time: 28.3 min		
Adapted		
Start	80°C	1 min
8° min^{-1}	130°C	
30° min^{-1}	270°C	15 min
Total time: 26.9 min		
Final		
Start	50°C	1 min
50° min^{-1}	100°C	
7° min^{-1}	130°C	
20° min^{-1}	270°C	15 min
Total time: 28.3 min		

	MBuT	TPrT	DBuT	TBuT
Optimization of column oven start temperature (peak heights in μV)				
80°C	17,000 ± 1,000	33,000 ± 3,000	39,000 ± 5,000	56,000 ± 9,000
69°C	37,000 ± 2,000	79,000 ± 2,000	77,000 ± 3,000	89,000 ± 5,000
60°C	63,000 ± 2,000	97,000 ± 2,000	89,000 ± 1,000	102,000 ± 3,000
50°C	91,000 ± 2,000	126,000 ± 4,000	116,000 ± 11,000	118,000 ± 6,000
Optimization of the split delay (split delay in minutes, peak heights in μV)				
0.75	139,000 ± 3,000	201,400 ± 400	180,000 ± 6,000	153,000 ± 1,000
1.00	160,000 ± 10,000	240,000 ± 13000	218,000 ± 9,000	189,000 ± 8,000

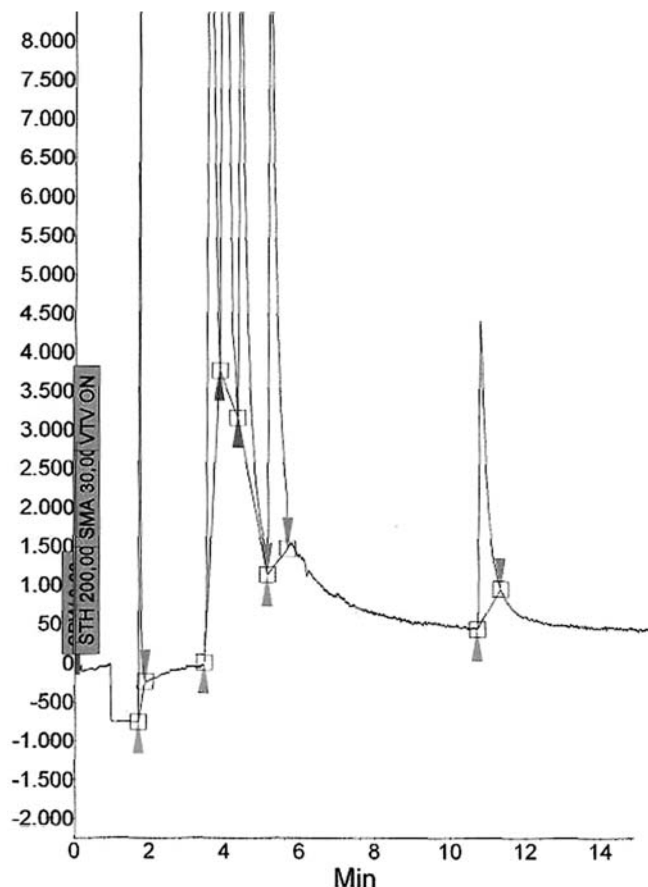

Fig. 1 Chromatogram of organotin compounds obtained with the initial oven program shown in Table 2. Peaks from left to right: MBuT, TPrT, DBuT, TBuT, TPhT

a short delay, removed through the split vent (and not through the column). Another advantage of this purging of the injector is a sharpening of the tail of the solvent peak, so that early-eluting compounds do not disappear in the solvent peak.

A split ratio of 1/20 (after injection) and an injection volume of 1 μL were always used. The injector was kept

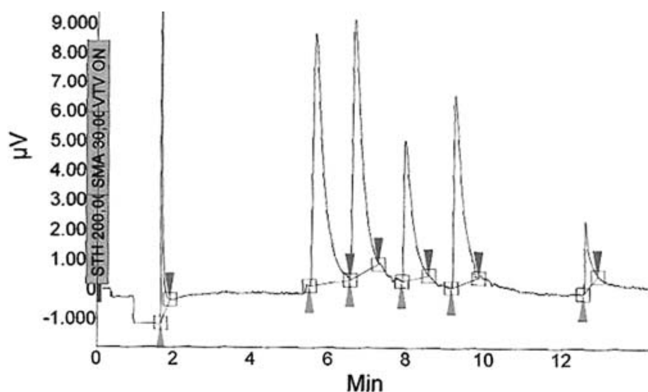

Fig. 2 Chromatogram of organotin compounds obtained with the adapted oven program shown in Table 2. Peaks from left to right: MBuT, TPrT, DBuT, TBuT, TPhT

at 220°C, because increasing it to 250°C had no significant effect. The temperature of the GC column at the start of the program was optimized using 250 ppb standards of MBuT, DBuT, TBuT and TPrT. Injections were repeated six times at each temperature. Average signal heights and standard deviations (SD, expressed in μV) are shown in Table 2. The highest peaks are obtained at the lowest GC start temperature (50°C), which is well below the boiling point (69°C) of the solvent, *n*-hexane.

In the literature no oven programs starting below 70°C could be found, even for analysis where the same solvent (*n*-hexane) and injector mode were used. It was decided to use starting temperatures equal to or above 50°C. A few tests were performed at 40°C, which resulted in a substantial increase in signal for the early eluting compounds (MBuT and DBuT). This lower temperature was not further tested, however, because the TPhT peak came out very badly and was 50% too small.

Two different split delays were tried. For each delay, a 250 ppb standard was analyzed three times. The results are shown in Table 2. Peak heights increase as the split delay is increased, because more of the sample enters the column. A 1 min delay was therefore chosen. Longer split delays are not recommended, in part because of the increased band broadening but also because of the risks to the column when analyzing "dirty" environmental samples (e.g. fish) and because SD increase with increasing split delay. The results (average peak heights and SD) are not corrected with an internal standard (TPrT).

The final settings used for gas chromatography are summarized in Table 2.

PFPD detector

Gate settings

The gate settings of the PFPD were optimized using 100 ppb standards. For each setting, the same standard was injected three times. Results are shown in Table 3.

First a 3 ms delay and 5 ms width setting was tested. When the gate width was increased to 6 ms or 7 ms no signal increase was observed. Although this contradicts the Varian PFPD manual [17], which suggests greater signals at longer measurement times, it is possible when maximum Sn emission occurs within 8 ms of measurement (for example 3 ms delay + 5 ms width) and when the detector baseline is shifted. In the literature no gate settings [11, 13] of more than 8 ms are reported. In addition, to avoid interferences, the gate width should be as small as possible. Increasing the gate delay from 3 ms to 4 ms and reducing the gate width from 5 ms to 4 ms had very little effect on peak heights for the organotin compounds (Table 3). Hence, 4 ms delay and 4 ms width are the preferred gate settings.

Table 3 Optimization of PFPD gate settings (gate settings in ms, peak heights in μV)

Gate delay, width	MBuT	TPrT	DBuT	TBuT	TPhT
3 ms, 5 ms	3,220 ± 90	2,900 ± 100	1,680 ± 40	2,490 ± 30	7,600 ± 500
3 ms, 6 ms	2,900 ± 200	2,500 ± 200	1,490 ± 90	2,200 ± 100	6,500 ± 600
3 ms, 7 ms	2,590 ± 40	2,280 ± 20	1,367 ± 7	2,000 ± 20	5,900 ± 100
4 ms, 4 ms	3,240 ± 40	2,880 ± 40	1,770 ± 30	2,360 ± 60	7,000 ± 1,000

Detector temperature

To reduce peak tailing as a consequence of the formation of non-volatile tin oxides inside the detector, high detector temperatures are recommended. In the literature temperatures of 300–350°C are mentioned. When chromatograms obtained with detector temperatures of 250°C and 300°C were compared it seemed there was less tailing at 300°C. A detector temperature of 350°C was not considered, because it would seriously reduce the lifetime of the column. During this work only peak height quantification was used, because even at 300°C there is still too much tailing to enable correct and reproducible peak area integration.

Other instrument settings

Gas flow rates to the detector were left unchanged (Air1 = 17.0 mL min^{-1}, H_2 = 13.0 mL min^{-1}, Air2 = 10.0 mL min^{-1}). The photomultiplier voltage was set at 510 V. Increasing the voltage from 510 V to 560 V increased the signal heights but also the noise. There was no real benefit.

Instrument properties

Linearity of the PFPD

To evaluate the linearity of the detector, a series of standard solutions from 1 ppb to 100 ppb was prepared. They were ethylated as described before. Peak heights were corrected for the signal of TPrT, the internal standard (IS). For each compound the mean value of the ratio (compound signal/IS, $n = 6$) was calculated and this was multiplied by the mean value of the TPrT signal height. The best linear equation through the data points for each of the compounds was calculated using Microsoft Excel.

Linearity of all calibration plots was satisfactory: MBuT ($R^2 = 0.95$), DBuT ($R^2 = 0.99$), TBuT ($R^2 = 0.99$) and TPhT ($R^2 = 0.98$). However, the signals for the highest standards were, relative to the others, always somewhat too low. To check derivatization was not the cause, a second test was performed. A standard of 250 ppb was ethylated, extracted with hexane, and diluted to appropriate concentrations. A calibration plot from 1 ppb to 250 ppb was established. A very good linearity was obtained up to 100–150 ppb for all organotin compounds, but above that concentration deviations from linearity occurred.

Instrument detection limits

To determine the detection limits of the instrument it was assumed that the yield from ethylation of the standard solutions was 100%. Limit of detection is often defined as the concentration corresponding to a signal of three times the noise from a blank. A blank was measured eight times per day during 3 days, yielding an average noise of 308 μV. By using the calibration curves described in the previous subsection, the following detection limits were obtained: MBuT 0.74 ppb, DBuT 0.75 ppb, TBuT 0.63 ppb.

Reproducibility and repeatability

Repeatability for the butyltin compounds (determined by analysis of the same standard six times consecutively) was mostly approximately 5%, with a few exceptions exceeding 10%. Repeatability was also tested over a much longer time period. A 5-ppb standard was analyzed 38 times consecutively (each run took approximately 30 min). The relative standard deviations (RSD) obtained were: MBuT (RSD = 6%), DBuT (RSD = 7%) and TBuT (RSD = 13%).

The reproducibility of the instrument was checked by analysis of the same 5 ppb standard on three different days (six times each day). Between measurements the standard was stored under refrigeration. The inter-day reproducibility is comparable with the repeatability if the instrument is in routine operation, this means that it is on for at least 24 h. Otherwise, RSD of 20% can be observed.

Instrument blank

Pure *n*-hexane was used to measure the instrument blank, to assess possible contamination during the instrumental analysis. Injections of pure solvent, before starting a series of standards/samples or between samples and standards, never generated a peak. This means that all parts of the instrument—column, detector, injector, needle, vials etc.—are free from contamination.

Biological samples

Organotin concentrations

To evaluate the digestion and derivatization/extraction procedures a mussel certified reference material, CRM

477 [18], was used. This material is certified for its content of MBuT (1.50 ± 0.28 mg kg^{-1}), DBuT (1.54 ± 0.12 mg kg^{-1}) and TBuT (2.20 ± 0.19 mg kg^{-1}).

The sample-preparation step, including digestion, derivatization, and extraction, was performed on three different 100-mg portions of CRM 477. All three were prepared in parallel so that they were submitted to the same treatment at the same time. Acetate buffer (20 mL), 0.1 % (*w*/*v*), $NaBEt_4$ solution (1 mL), and *n*-hexane (1 mL) were added to the mineralized sample. An external calibration curve was used. Each standard and the CRM were analyzed three times to assess repeatability.

For DBuT and TBuT the average recovery was $50 \pm 10\%$ and $63 \pm 7\%$, respectively. A check of the pH indicated a value of 4. To verify whether these relatively low recoveries were because of the age of the CRM (from 1997), the CRM was spiked. Similar recoveries were found for the spike. Therefore the effects on the derivatization of the amount of sample, the concentration of $NaBEt_4$, and pH were evaluated.

Increasing the pH to 5 or 5.5 did not result in greater recovery of the organotin compounds from the CRM. Better results were obtained by adding larger amounts of ethylating agent to the mineralized sample but similar improvements could be achieved by using smaller amounts of sample. TPrT (0.1 ng μL^{-1}) was added to 10-mg portions of the CRM, equivalent to approximately 1 mg kg^{-1} TPrT. The recovery results are shown in Table 4. Under these conditions the organotin compounds can be assessed with accuracy between 90% and 110%.

Table 4 Checking recovery with CRM 477 at pH 4

Mass	Compound	Recovery (%)	Recovery/I.S. (%)
10 mg	DBuT	90 ± 20	110
	TBuT	70 ± 15	90
	TPrT (I.S.)	80 ± 15	

Standard and procedural blanks

Two types of blank were prepared. One type (the standard blank) was treated in the same way as the standards, i.e. Milli-Q water plus acetate buffer, ethylating reagent and hexane, stirred for 40 min. The second type (the procedural blank) was subjected to the same analysis procedure as the samples (digestion at high temperature with TMAH, derivatization, and extraction) and includes all reagents and solvents added to a sample. Together with the instrumental blank (pure *n*-hexane), these blanks were analyzed regularly within each standard or sample series to detect any possible contamination.

As an illustration, Figs. 3 and 4 show the average signal of six replicate analyses of the same blank (standard or procedural blank, respectively) over a period of time. A signal of 0 μV means the signal is equal to the noise level.

For the standard blanks, only the MBuT signal could be seen. The average noise is higher than during the evaluation of the instrument (mentioned above, see *Instrument detection limits*), because a change was made from a 0.1% (*w*/*v*) to a 1% (*w*/*v*) $NaBEt_4$ solution during optimization of the method for biological samples.

Levels were significantly higher for procedural blanks than for standard blanks. This is, of course, because of the increased amounts of reagents and number of treatments. Especially, contact with materials that are not entirely decontaminated remain a critical point in the procedure—in our experiments magnetic stirring bars coated with Teflon (PTFE). In an experiment in which parallel ethylations were performed on four subsamples of the same extract only one furnished organotin peaks discernible from the noise—the TBuT and MBuT signals were three and ten times the noise, respectively. The bottles that were used were new whereas all other conditions (reagents, solvents, and internal standard) were kept as usual. Magnetic stirring bars may thus be the cause of accidental contamination. A more encouraging observation was that reagents, solvents, and internal standard solutions were largely free from organotin contaminants—no detectable contamination was observed for 3 out of 4 extracts.

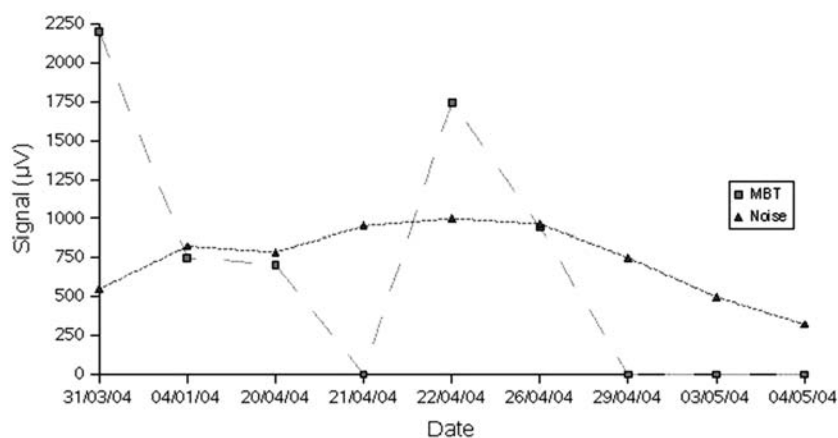

Fig. 3 The average signal above the noise for six replicate analyses of the standard blank over a period of time. The noise signal is also shown

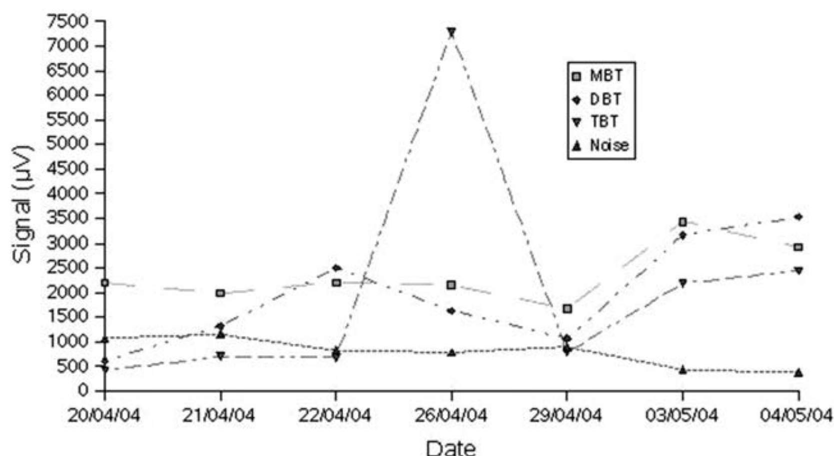

Fig. 4 The average signal above the noise for six replicate analyses of the procedural blank over a period of time. The noise signal is also shown

To examine whether the higher procedural blank values were because of the use of extra glassware, namely the digestion flasks, a procedural blank was prepared using a new flask. Derivatization/extraction was carried out in the same 50 mL bottle as usual. No organotin signals (except for the internal standard) could be seen. This means that the digestion flasks are largely responsible for the higher procedural blank values and that the TMAH reagent used in the work is free from organotin. There is large between-bottle and even between-magnet variation of organotin contamination. Material that came into contact with increased organotin concentrations needs thorough cleaning and contamination-free storage before use in a new analyses of standards or samples.

Reproducibility of analysis of biological samples

To gain an idea of reproducibility for biological samples, three CRM 477 sub-samples were analyzed. The digestion and derivatization/extraction procedures were performed on al three subsamples at the same time. Each sub-sample solution was analyzed three times to assess the repeatability and during 3 days to assess the reproducibility. External calibration was performed each day. The results are shown in Table 5. It seems that, with daily calibration:

1. all RSD values are smaller than 10% except for DBuT; DBuT RSD were much larger than for the two other compounds, with a maximum RSD of 18%; and
2. for a given sub-sample for a given compound, the range of daily averages is smaller (for example MBuT subsample 1: 164–181) than the averages over 3 days for the three subsamples (for example MBuT subsamples 1, 2 and 3: 174–195).

Conclusions

An analytical procedure for separation and quantification of organotin compounds (MBuT, DBuT and TBuT) by GC–PFPD was optimized on standard samples. Derivatization of the organotin compounds was performed by adding $NaBEt_4$ and buffer to standard solutions. The derivatized compounds were then extracted with 1 mL *n*-hexane and separated and quantified by GC–PFPD. The instrument settings also enable quantification of TPhT, but better settings for this compound might be more suitable because this work focused mainly on the butyltins.

The gas-chromatographic conditions were as follows:

- Splitless injection of 1μL is performed, with a split delay of 1 min and split ratio of 20. The injector is continuously kept at a temperature of 220°C. The column temperature at the time of injection is, for butyltin analysis, best kept well below the boiling point of the sample solvent (hexane) which is 69°C. For less volatile (late-eluting) compounds, e.g. TPhT, a higher column start temperature might be recommended.
- At the beginning of the analysis the column temperature should increase slowly to enable good separation of early-eluting compounds. It is very important at the end of each run to keep the column at a temperature higher than 250°C for approximately 15 min to recondition the column and to increase reproducibility.
- For the gate settings of the PFPD it was found that a gate delay of 4 ms and a gate width of 4 ms were well suited to selective and sensitive organotin detection. During this work no problems with (sulfur) interferences were encountered. The PFPD was continuously kept at a temperature of 300°C, leading to reduced peak tailing in comparison with 250°C. Some authors state that at 350°C there is still significant improvement, but to do so the PFPD must be isolated with an aluminum cap. All other settings, e.g. fuel gas flow rates, were left on the defaults recommended by the supplier (Varian).

With the settings mentioned here above, and running the instrument for several days continuously, instrument reproducibility well below 5% was observed. The PFPD has a good linearity from the detection limit, which was

Table 5 Repeatability and reproducibility of organotin analysis (three subsamples of CRM 477 measured during 3 days)

Compound	Mean signal ($n = 3$) ± SD			Average over 3 days
	16/03/04	17/03/04	18/03/04	
MBuT subsample 1	181 ± 9	176 ± 9	164 ± 7	174 ± 9
MBuT subsample 2	197 ± 6	197 ± 7	192 ± 7	195 ± 3
MBuT subsample 3	189 ± 5	172 ± 9	177 ± 4	179 ± 9
MBuT Average per day	189 ± 8	182 ± 13	178 ± 14	
DBuT subsample 1	180 ± 10	170 ± 30	190 ± 30	180 ± 10
DBuT subsample 2	230 ± 30	199 ± 8	210 ± 20	210 ± 20
DBuT subsample 3	260 ± 20	220 ± 30	230 ± 10	240 ± 20
DBuT Average per day	223 ± 40	196 ± 25	210 ± 20	
TBuT subsample 1	43 ± 4	41 ± 6	42 ± 6	42 ± 1
TBuT subsample 2	37 ± 1	39 ± 2	38 ± 5	38 ± 1
TBuT subsample 3	40 ± 2	43 ± 7	44 ± 7	42 ± 2
TBuT Average per day	40 ± 3	41 ± 2	41 ± 3	

found to be approximately 0.70 ppb for the butyltins, to concentrations higher than 100 ppb (as Sn) in hexane. Between 100 and 150 ppb the detector begins to deviate from linearity.

Blanks were, in general, very acceptable, although procedural blanks were sometimes very high during analysis of natural samples. It seemed that materials that were insufficiently decontaminated, for example magnetic stirring bars, were the origin of the problem.

Derivatization and extraction steps during analysis of natural samples are considered the most critical in the analysis of organotin compounds. The problem is that many factors can affect the derivatization yield. During this work it also became apparent that optimization of the procedure for real samples is a complex process which must be conducted in several steps. $NaBEt_4$ concentration proved to be important, and either an increase in its concentration or a reduction in sample mass improved recovery after spike additions. The ideal buffer pH was best below the originally set value of 5. At pH 4 a much higher recovery was obtained, organotin hydroxides formed during digestion with TMAH probably need a more acidic environment to return to their ionic form and to react with the $NaBEt_4$. Finally at pH 4, with a sample (CRM) mass of 10 mg, and using an internal standard (TPhT) the organotin compounds could be assessed with accuracy between 90% and 110%. However, validation on natural solid (biota and sediment) samples is still necessary. Attention should be paid to the effect of small changes in procedural conditions, for example the ratio of sample/ethylating reagent or a small deviation from the ideal buffer pH, and how the effect of these small changes can be minimized so that a robust analytical procedure is achieved.

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