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Speciation of organotin compounds in marine biomaterials after basic leaching in a non-focused microwave extractor equipped with pressurized vessels

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Abstract

A rapid method for the speciation of butyl- and triphenyltin compounds in marine biotissues is described. A non-focused microwave extractor, operating at a power of 950 W and equipped with 12 pressurized vessels, was used to achieve fast sample leaching with tetramethylammonium hydroxide. The pH of the liquid extract was adjusted to 5. Organotins were ethylated with sodium tetraethylborate, extracted in isooctane and determined by means of a microwave-induced plasma atomic emission detector coupled to a gas chromatograph. The stability of butyl and phenyl compounds, exposed to the microwave energy, was studied as a function of the vessel temperature. The possibility of simultaneous carried-out extractions and the use of microwave to perform the ethylation and extraction of organotin compounds was also studied. The full procedure was validated with certified material NIES-11 and with real samples, by comparison with a classic leaching method using tetramethylammonium hydroxide without microwave.

Keywords: Microwave induced plasma atomic emission detection; Microwave extraction; Extraction methods; Organotin compounds

1. Introduction

Organotin compounds, in particular tributyltin (TBT) and triphenyltin (TPhT) to a lesser degree, have been used as biocides in agriculture but mainly in antifouling paints to control the growth of marine organisms on the hulls of ships [1,2].

Both compounds and their degradation products, resulting from progressive loss of the organic chains bonded to the Sn atom [3], are accumulated by aquatic species (fish, molluscs, algae, etc.) until concentrations in the range of $\mu g\, g^{-1}$ of tissue are reached, for marine organisms that live in waters with organotin concentrations in the $ng\, g^{-1}$ range [4,5]. This accumulation of organotin compounds in the food chain leads to a potential risk for human health.

Although the use of organotin paints on ships smaller than 25 m has been banned and their use in larger vessels restricted [6], recent studies show that organotin compounds retained in sediments are continuously released into the aquatic environment [7].

Because the toxicity of organotin compounds is a function of the number and type of the organic radical bonded to the Sn atom [3], it is necessary to develop analytical techniques, with the capability to determine the concentration of the different or-

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ganometallic species in the original matrix. Coupling liquid chromatography–inductively coupled mass spectrometry has being used for the speciation of organotins [8]. Nevertheless, gas chromatography coupling with different detectors as atomic absorption spectrometry (AAS) [9,10], flame photometric detection (FPD) [11,12], mass spectrometry (MS) [13,14] and microwave induced plasma atomic emission spectrometry (MIP-AED) [15–17], is by far the most popular technique in the determination of organotins.

From the analytical point of view, it has to be considered that organotins are present in the environment as polar compounds, so as a previous step to their separation by gas chromatography it is necessary to transform them into less polar compounds. The most common derivatization techniques are: hydride generation with $NaBH_4$ [18], alkylation with Grignard reagents [11] and direct ethylation with $NaBEt_4$ [19,20]. Hydrides have high volatility so it is difficult to obtain quantitative results without using a cryogenic trap; Grignard reagents can be used only with non-protic apolar solvents, so to extract organotins in such solvents it is necessary to complex them previously with, for instance, tropolone. Sodium tetraethylborate is the only reagent which is able to produce quantitative ethylation in aqueous solutions or in polar solvents.

On the other hand, organotin compounds in biological materials are incorporated into the cellular tissue structure [21], so regardless of the extraction–derivatization step it is necessary to achieve a complete tissue solubilization in order to obtain accurate results. Tissue hydrolysis can be carried out with acetic acid, tetramethylammonium hydroxide (TMAOH) [22], or by using enzymes [23]. All these procedures require several hours to achieve complete tissue solubilization.

The use of an open focused, low-power microwave system has been successfully used to accelerate this step, making complete solubilization possible in a few minutes while keeping the integrity of the different organotin compounds [22,24–26]. Nevertheless, these systems show an important disadvantage: as the leaching procedure takes place in open glass containers, exposition time is limited by the boiling point of the mixture.

Non-focusing microwaves, operating over closed vessels (used successfully for the extraction of polycyclic aromatic hydrocarbons [27,28], organochlorine pesticides and polychlorinated biphenyls from soils and foods, [29,30]), could solve this problem. This type of microwave device works at high powers (950 W) and, as far as we know, have never been used in the extraction of labile compounds such as organotins.

The object of this study is, in the first place to check the stability of organotin compounds in TMAOH solutions exposed to microwave action and secondly to use the microwave extractor to accelerate the leaching of the biological matrix in TMAOH. The biological extracts thus obtained, are neutralized and organotin compounds ethylated using $NaBEt_4$ according to a published procedure [23]. The possibility of performing simultaneous leaching, extraction and ethylation steps in the microwave extractor was also studied. The full procedure is validated using certified material NIES-11 and by comparison with a conventional hydrolysis with TMAOH at 60 °C for 4 h [23].

2. Experimental

2.1. Reagents

HPLC grade methanol, isooctane, diethyl ether and acetic acid were supplied by Merck (Darmstadt, Germany). Sodium acetate, activated acidic alumina, tetramethylammonium hydroxide (TMAOH 25% water) and standards of monobutyltin trichloride 95% (MBT), dibutyltin dichloride 96% (DBT), tributyltin chloride 96% (TBT), tripropyltin chloride 98% (TPT), phenyltin trichloride 98% (MPhT), diphenyltin dichloride 96% (DPhT), triphenyltin chloride 95% (TPhT), tetrabutyltin 93% (TeBuT) and tetraethyltin 97% (TeEtT) were obtained from Aldrich (Milwaukee, WI, USA) and used without further purification. Standards with a concentration of 3 mg ml^{-1} as tin, were prepared by dissolving the ionic compounds in methanol, for polar compounds and in isooctane for TeEtT and TeBuT. These solutions are stable at least for one month. Diluted standards of each compound and mixtures of them were prepared weekly dissolving the concentrated standards in methanol or isooctane. Standard solutions were stored refrigerated at 4 °C in the dark.

Sodium tetraethylborate ($NaBEt_4$), was supplied

by Strem Chemicals (Strasbourg, France), in containers of 1 g. The reagent was kept in a desiccator and manipulated in a dry box to prevent its degradation. Fresh solutions 1% (w/v) were prepared every 8 h in Milli-Q water.

Buffer solutions at pH 5 were prepared by dissolving 1 *M* of sodium acetate in Milli-Q water and adjusting the pH to 5.00±0.05 with acetic acid.

2.2. Apparatus

An HP 5890 Series II gas chromatograph from Hewlett-Packard (Avondale, PA, USA) equipped with a split/splitless injection port and coupled to an HP 5921A microwave-induced plasma atomic emission detector was used. Injections were made by means of an HP model 7673A automatic sampler. The whole system was controlled by an HP 35920A Chemstation. Separations were performed in a 30 m×0.25 mm I.D. methylphenylsilicone capillary column, of 0.25 μm film thickness, (DB-5; J&W Folsom, CA, USA). He 99.999% was used as plasma and carrier gas, H_2 99.999% and O_2 99.99% were also used as auxiliary gases in the plasma. Optimum settings for the determination of the analytes are given in Table 1.

Table 1
GC–AED operating conditions used for the separation of organotin compounds

GC parameters	Settings
Injection port	Split/splitless
Purge time	60 s
Injection port temperature	260 °C
Injected volume	1 μl
Column head pressure	20 p.s.i (138 kPa)
Split flow	9 ml/min
Oven programme	
Initial temperature	80 °C
Initial time	1 min
Rate	20 °C/min
Final temperature	260 °C
Final time	10 min
AES parameters	
Transfer line temperature	270 °C
Cavity block temperature	270 °C
Wavelength	303.419 nm
Helium make-up flow	270 ml/min
Ferrule purge	28 ml/min
Spectrometer purge flow	2 l/min N_2
Solvent vent	3.85 min
H_2 pressure	50 p.s.i (345 kPa)
O_2 pressure	22 p.s.i (152 kPa)

Microwave extractions were performed in a MES-1000 microwave extraction system (CEM, Matthews, NC, USA). This extractor can perform 12 simultaneous extractions, (under identical conditions), into closed vessels with a volume of 100 ml. The system controls temperature and pressure in one vessel and assumes the same values for the others. To assure identical conditions in all the vessels, they are rotating continuously in the interior of the microwave oven. The system is programmed to reach a selected temperature or pressure and to keep them for a preprogrammed time. The power is always 950 W, but it is possible to achieve slow temperature rates controlling the real time that the system applies energy, (e.g. working at 50% the system is switched on and off at equal times).

2.3. Samples

Three different samples were used in this study:

- Reference material NIES-11. This was a gift from the National Institute for Environmental Studies, Japan. This material has a certified level of TBT (1.3 $\mu g\,g^{-1}\pm0.1$) and a reference level of TPhT (6.3 $\mu g\,g^{-1}$), both as ionic compounds. Nowadays this is the only certified material for organotins in marine biological matrix.
- Tuna tissue. This material was prepared from tuna muscle, cut in small pieces, homogenized and freeze-dried. Then it was analysed using conventional leaching with TMAOH for 4 h [23] and no organotins were detected, nevertheless a high amount of inorganic tin is present in the material, (this compound is ethylated non quantitatively to TeEtT). An amount (25.00 g) of this material was mixed with 100 ml of methanol containing known concentrations of MBT, DBT, TBT and TPhT. The mixture was stirred periodically, kept in the dark, allowed to air-dry until it again reached the initial weight and then stored at −20 °C. If no losses occurred during the drying or storage, the theoretical concentrations, as tin, in this sample should be: MBT (0.864 $\mu g\,g^{-1}$), DBT (0.682 $\mu g\,g^{-1}$), TBT (0.625 $\mu g\,g^{-1}$) and TPhT (0.754 $\mu g\,g^{-1}$).
- Mussel. Sample was prepared from a pool of

mussels collected in the local area, freeze–dried and homogenized.

2.4. Sample extraction

Two different leaching procedures were carried out:

- Conventional leaching. A sample amount, (NIES-11, mussel or tuna), between 0.2–1 g, was placed in a 50 ml tube, TPT was added as internal standard, (this compound has never been reported in environmental samples) and TMAOH. The mixture was left at 60 °C for 4 h and then neutralized and derivatized [23].
- Microwave leaching. Between 0.2–1 g of freeze-dried biological tissue were placed in an extraction vessel, TPT was added as internal standard and also 15 ml of TMAOH. This mixture was exposed to microwave action until 120 °C, this temperature was kept for 2–3 min. After cooling down, the mixture was transferred to a 50-ml glass tube, neutralized (3.8 ml of acetic acid and 5 ml of buffer solution) and derivatized (2 ml isooctane and 2 ml borate 1%), shaking manually for 5 min.

In both cases (conventional and microwave leaching) the mixture was centrifuged to allow phase separation and the isooctane phase cleaned by passing through a Pasteur pipette filled with alumina [23]. Operating in this way, polar compounds are retained over the alumina, preserving the chromatographic column.

An alternative ethylation and extraction protocol was performed. After microwave leaching, mixtures were neutralized in the microwave vessels; 2 ml of isooctane and 2 ml of borate (1% in water) were added. Vessels were closed and exposed again to microwave action (90 °C for 2 min); working in this way the centrifugation step is not necessary. Microwaves break the foam that appears with manual shaking and achieve a very clear interface between aqueous and organic phase, so it is possible to speed up sample preparation, to minimize sample handling and to reduce the volume of isooctane needed to extract the ethylated compounds to only 1 ml.

2.5. Derivatization of standards

A known amount of ionic organotins was spiked over 5 ml of buffer solution, 2 ml of isooctane and 2 ml of $NaBEt_4$ solution (1% in water), were added. The mixture was shaken for 5 min and the organic phase injected in the chromatographic system [31].

3. Results and discussion

3.1. Optimization of AED conditions

Optimal AED conditions for the determination of organotin compounds have been recently studied by different authors. All of them point to the necessity of using H_2 and O_2 as auxiliary gases in the detector and also high make-up flows, stressing the influence of these parameters in the system sensitivity. Nevertheless, there are important disagreements in the values of helium make-up flow, (from 150 ml/min [13], to 220–240 ml/min [16,17]) and so in the reported detection limits (ratio $S/N=3$): about 4 pg as tin, [13,17] and less than 1 pg as tin, [16].

Helium make-up flow, H_2 and O_2 pressures, influence the sensitivity of the system, were tested with a solution of TeBuT and TeEtT in isooctane, at the two emission lines of tin (270.651 nm and 303.419 nm), recommended in the AED software. For both compounds, at both wavelengths, maximum sensitivities were achieved at helium flow of 270 ml/min and H_2 and O_2 pressures of 345 and 152 kPa.

The linearity of the AED response for ethylated organotin compounds was checked from 10 to 1000 ng ml^{-1}, as tin. In this range of concentration, calibration curves for all the compounds studied have practically the same slopes, so detector response is dependent only as the amount of tin and not on column temperature or the molecular structure of the different compounds. Table 2 summarize detection limits, as tin, for ionic compounds at 271 and 303 nm.

Because the emission line at 303 nm has a slightly better sensitivity than the 271 nm line, it was used to register the emission signal in further experiments. TPT was chosen as internal standard to build calibration curves. Organotin concentrations in NIES, tuna and mussel samples were determined using these calibration curves and a constant amount of TPT as internal standard.

Table 2
Limits of quantification ($S/N=10$) obtained under conditions described in Table 1

Compound	LOQ (ng ml^{-1} as tin)	
	303.419 nm	270.651 nm
MBT	4.9	8.9
DBT	6.9	8.4
TBT	3.8	5.3
TPT	6.2	8.1
MPhT	6.2	7.6
DPhT	5.4	7.4
TPhT	5.1	6.7

3.2. Stability of organotin compounds.

Previously to any attempt of sample leaching in the CEM microwave system, it was necessary to check the stability of organotin compounds under these conditions. With this aim, individual standards of MBT, DBT, TBT, TPT, MPhT, DPhT and TPhT where spiked over TMAOH into the microwave vessels and exposed to microwave energy until different temperatures were reached (90 °C, 115 °C and 130 °C). The final temperature was kept for 3 min.

The mixtures were neutralized with acetic acid, the pH adjusted to 5 with buffer and derivatized according to the procedure described in Section 2.5 for standards. Concentrations for each compound were compared with the value obtained for the same amounts of ionic compounds spiked over buffer solutions and derivatized without being exposed to the microwave field.

The results obtained (Table 3) show that all the compounds are quite stable at 90 °C. At higher temperatures alkyl compounds stay unaltered, while phenyl compounds, especially MPhT and DPhT, are much less stable. TPhT, was stable to 115 °C but at 130 °C presented similar behaviour to MPhT.

Table 3
Study of the stability of organotin compounds in a TMAOH solution, as a function of the microwave temperature

Compound	Recovery (%)[a]		
	90 °C, 3 min	115 °C, 3 min	130 °C, 3 min
MBT	98.6	101.6	101.3
DBT	96.9	91.0	88.4
TBT	98.4	99.2	106.4
TPT	98.0	84.5	88.7
TPhT	95.9	83.0	47.9
MPhT	89.0	60.5	48.5
DPhT	90.8	50.1	13.8

[a] Percentages of recovery estimated over the same concentration of ionic compounds not exposed to microwave action.

The pattern of decomposition for phenyl compounds consisted in progressive loss of phenyl groups. For example, MPhT, was the main product of degradation from DPhT at 115 °C; at 130 °C inorganic tin is the major product of degradation.

Although the presence of a biological matrix in the interior of the microwave reservoirs, could increase little the stability of these compounds, it was practically impossible to obtain quantitative extractions of MPhT and DPhT, at temperatures over 90 °C. For TPhT stability, it is recommended not to work over 115 °C.

3.3. Analysis of biological samples

NIES-11

First leaching experiments in the microwave system were preformed using a mixture of 5 ml Milli-Q water +5 ml TMAOH (25% water), [23]. Vessels were placed one at a time in the microwave oven, so its power was fixed at 25% in order to avoid an excessive rate of heating. 200 mg of NIES were taken and different temperatures (90, 100 and 115 °C) were tested, although 90 °C was not enough to achieve complete hydrolysis. The final temperature was kept for 2 min. The results obtained (Table 4) were compared with those produced by conventional hydrolysis for 4 h at 60 °C, using the same amounts of tissue and TMAOH–water mixture. Each experiment was triplicated. For TBT concordance between conventional hydrolysis, microwave leaching and certified values was excellent; for TPhT results show bigger discordance because of poor repeatability. Nevertheless, microwave leaching at 115 °C produced a concentration of TPhT very close to the reference value.

Tuna samples

To confirm the good results obtained for TBT and TPhT in NIES-11, for MBT and DBT, (MPhT and DPhT are not expected to be stable at 115 °C for their quantitative determination) a tuna sample spiked with MBT, DBT, TBT and TPhT, according to the instructions given in Section 2.5 was subjected to the same procedure as NIES-11.

Table 4
Analysis of NIES-11 material[a]

	TBT ($\mu g\ g^{-1}$ as chloride)	TPhT ($\mu g\ g^{-1}$ as chloride)
Certified values	1.3±0.1	6.3[b]
MW leaching, 100 °C, 2 min.	1.24±0.05	4.1±0.8
MW leaching, 115 °C, 2 min.	1.28±0.08	6.0±0.9
Classic leaching, 4 h	1.24±0.08	4.9±0.3

[a] 200 mg of sample with 5 mL TMAOH (25% water) plus 5 mL Milli-Q water.
[b] Reference value.

Using microwave leaching at 115 °C, it was impossible to recover even the TPT spiked as internal standard immediately before starting the leaching. Conventional hydrolysis shows the same problem. After some proof it was found that a modified conventional procedure using 15 ml of TMAOH (25% water) for 4 h was enough to get complete sample leaching; microwave leaching working with the same amount of TMAOH and using some slight stronger conditions, (120 °C, 3 min) produces similar results (Table 5). NIES-11 was leached into the microwave under these modified conditions, and results were also satisfactory, (TBT 1.27±0.10 $\mu g\ g^{-1}$ and TPhT 5.96±0.22 $\mu g\ g^{-1}$ as chlorides).

3.3.2. Simultaneous extractions

Until now all the experiences were performed placing one vessel at a time in the microwave oven. Under these conditions, with the system operating at 25% of power, the time necessary to raise the temperature of one single vessel (containing 200 mg of tissue plus 15 ml TMAOH), to 120 °C is about 2 min. This is in fact the time that the vessel receives the microwave energy, in the second step the system only supplies energy when the temperature decreases below 120 °C.

At this point measurements with 3 and 6 vessels were performed simultaneously. With the microwave system operating at 25% and 50% of power, respectively, 4.5 and 4 min of microwave exposure were necessary to reach the final temperature. Under these conditions rate of sample heating was slower, so results obtained could be different if complete sample leaching was not achieved or if the organotins were not stable under these conditions.

Table 6, shows results obtained for tuna spiked samples as a function of the number of vessels extracted simultaneously. Three samples of mussel were placed simultaneously in the microwave and results were tested with those obtained using classic leaching (Table 7). In both cases results obtained did not depend on the number of samples placed in the microwave oven.

As for the mussels, MBT concentration was much higher than DBT and TBT levels. Furthermore, the sample has a lot of inorganic tin (Fig. 1); this could suggest that the presence of organotins in the area was quite old and that the degradation process was in an advanced state. MPhT was also present in the mussels; this compound has not been quantified due to its low stability in the microwave system.

3.3.3. Influence of sample amount in the efficiency of leaching procedure

In all the experiments 200 mg of sample were

Table 5
Analysis of tuna spiked material[a]

	MBT (ng g^{-1} tin)	DBT (ng g^{-1} tin)	TBT (ng g^{-1} tin)	TPhT (ng g^{-1} tin)
MW 120 °C, 3 min	827.1 (5.8)	573.4 (8.1)	636.7 (3.8)	691.4 (11.6)
Classic leaching (4 h)	744.8 (4.2)	538.2 (1.5)	606.1 (4.2)	852.1 (6.2)
Spiking levels (ng g^{-1})	864.0	681.9	625.0	754.0

[a] 200 mg of sample with 15 mL TMAOH (25% water).
Mean R.S.D. (%) values in brackets.

Table 6
Analysis of spiked tuna samples (200 mg)

No. vessels at a time	MW power (%)	Compound concentration (ng g^{-1} as tin)[a]			
		MBT	DBT	TBT	TPhT
1	25	827.1 (5.8)	573.4 (8.1)	636.7 (3.8)	691.4 (11.6)
3	25	794.7 (5.6)	551.5 (3.1)	645.6 (6.6)	708.2 (7.6)
6	50	846.1 (5.6)	572.4 (6.0)	639.2 (7.0)	722.7 (7.0)
Spiked levels		864.0	682.0	625.0	754.0

[a] Mean R.S.D. (%) values in brackets.

used. In order to improve the sensitivity of the technique, higher amounts of sample were subjected to the leaching procedure with 15 ml of TMAOH. Table 8 shows results for NIES-11 and tuna spiked samples.

NIES-11 is a quite easy sample so it is possible to use at least 1 g of sample without differences in the results. Tuna is more difficult to destroy, so for a sample amount of 1 g the recoveries obtained, for DBT and MBT were poorer than for 200 or 500 mg. Probably using the same microwave conditions, but with a greater amount of TMAOH, better results may be obtained. In any case, it is clear that the amount of TMAOH has to be fixed not only as a function of sample size but also depending on the sample matrix nature.

3.4. Assisted microwave derivatization

With the results obtained we may conclude that the non-focused microwave extractor is useful to leach simultaneously several samples, keeping the molecular structures of MBT, DBT, TBT and TPhT unaltered. The full procedure needs, as well as leaching, three more steps: neutralization, extraction–derivatization and centrifugation to break the foam produced during the extraction.

Microwave systems have been reported to be useful in breaking the interface foam improving phase separation [23] and in performing liquid–liquid extractions. So the possibility of carrying out all the steps of the analysis in the microwave was examined.

Here samples where neutralized in the microwave vessels, then borate and 2 ml of isooctane were spiked. Vessels were closed and exposed to MW action (90 °C, 2 min). Under these soft conditions no degradation was expected for ionic compounds. Moreover, ethylated compounds were extracted in the organic phase, where they did not receive microwave energy.

Phase separations were much better than after centrifugation, so the organic phase is easily removed (it was possible to use only 1 ml of isooctane) and ready to be injected in the chromatographic system after the clean-up step. Table 9 shows that results obtained with this procedure are similar to those obtained after manual shaking for 5 min and centrifugation, so this allows reduction of the analysis time and sample preparation.

Quantification limits based in 1 g samples and using 1 ml of isooctane, expressed as tin, were MBT (4.9 ng g^{-1}), DBT (6.9 ng g^{-1}), TBT (3.8 ng g^{-1}) and TPhT (5.1 ng g^{-1}).

Table 7
Comparison between classic and microwave leaching for 3 mussel samples (200 mg) at a time

	Compound concentration (ng g^{-1} as tin)[a]			
	MBT	DBT	TBT	TPhT
Classic leaching, 60 °C, 4 h	1460.2 (9.9)	188.1 (5.7)	312.1 (8.8)	159.1 (8.6)
MW leaching, 25% power	1465.4 (6.8)	216.1 (10.9)	376.0 (5.2)	202.3 (6.9)

[a] Mean R.S.D. (%) values in brackets.

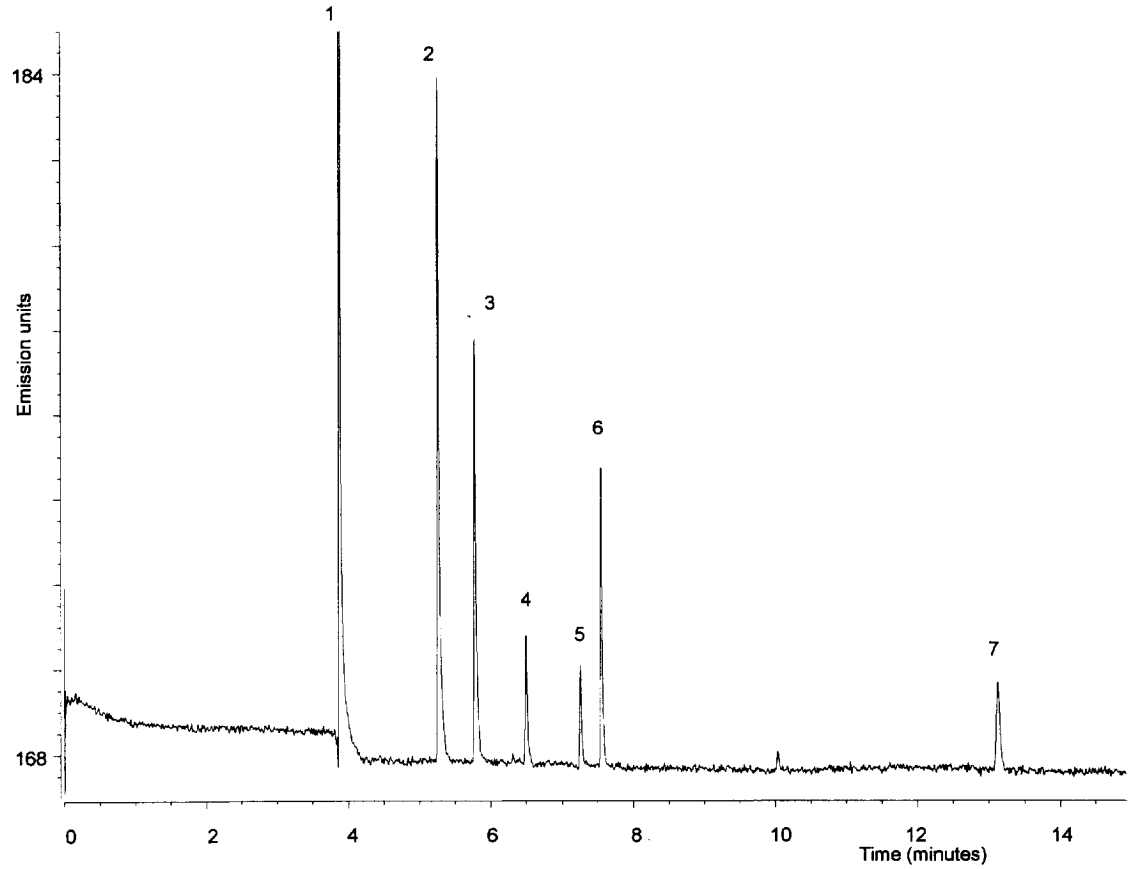

Fig. 1. Chromatogram for a sample of 200 mg of mussel. 1=Sn(IV), 2=MBT, 3=TPT (I.S.), 4=DBT, 5=MPhT, 6=TBT, 7=TPhT.

4. Conclusions

It has been shown that most organotin species of environmental concern can be extracted from biological materials by means of a non-focused microwave extractor device. Up to 12 samples can be simultaneously extracted, however, in this paper a maximum of six samples were processed simultaneously. Extraction temperatures have to be fixed as a function of the stability of the species to be extracted as well as the completeness of hydrolysis. Working at temperatures not higher than 120 °C all the studied species but MPhT and DPhT can be quantitatively extracted. Most steps in sample prepa-

Table 8
Estimated recovery, as a function of sample amount, for NIES-11 and tuna spiked material.

Sample	Amount (mg)	Recovery (%)			
		MBT	DBT	TBT	TPhT
Spiked tuna	200	95.7±6	84.1±8	101.9±4	91.7±12
	500	87.4±9	80.6±6	99.5±4	89.7±5
	1000	81.6±11	64.8±10	101.3±8	108.8±8
NIES-11	200			98.5±6	94.9±15
	1000			96.9±3	98.4±6

Table 9
Results obtained for samples of NIES-11 and spiked tuna, leached, extracted and derivatized under the microwave field[a]

Sample	Amount (mg)	Compound concentration[b]			
		MBT	DBT	TBT	TPhT
Spiked tuna[c]	200	820.8 (3)	531.6 (4)	629.1 (9)	635.2 (6)
NIES-11[d]	200			1.24 (7)	6.4 (6)

[a] The full procedure was run with 3 samples at a time.
[b] Mean R.S.D. (%) values in brackets.
[c] Concentrations in ng g^{-1} as tin.
[d] Concentrations in μg g^{-1} as chloride.

ration (leaching, extraction and ethylation) can be carried out inside the extraction vessels thus shortening sample preparation as well as minimizing sample handling and enhancing detection limits. The sample size that can be accurately processed depends on the matrix nature but, even for difficult samples (e.g. tuna) the sample size is high enough to allow detection limits as needed in environmental studies.

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References

[1] R. Maguire, Appl. Organomet. Chem., 1 (1987) 475–498.
[2] M.J. Waldock, J.E. Thain and M.E. Waite, Appl. Organomet. Chem., 1 (1987) 287–301.
[3] S.J. Blunden, L.A. Hobbs and P.J. Smith, in H.J.M. Bowen (Editor), Environmental Chemistry, Royal Society of Chemistry, London, 1989, pp. 51–77.
[4] R.B. Laughlin, W. French and H.E. Guard, Water, Air, Soil Pollut., 20 (1983) 69–79.
[5] R.B. Laughlin, W. French and H.E. Guard, Environ. Sci. Technol., 20 (1986) 884–890.
[6] H. Vrijhof, Sci. Total Environ., 43 (1985) 221–231.
[7] S. Rapsomanikis and R. Harrison, Appl. Organometal. Chem., 2 (1988) 151–157.
[8] Y. Inoue, K. Kawabata and Y. Suzuki, J. Anal. At. Spectrom., 10 (1995) 363–366.
[9] S.S. Forsyth, Sci. Total Environ., 89 (1989) 299–304.
[10] W.M.R. Dirkx, R. Lobinski and F.C. Adams, Anal. Sci., 9 (1993) 273–278.
[11] M.D. Muller, Anal. Chem., 59 (1987) 617–623.
[12] Y. Cai, R. Alzaga and J.M. Bayona, Anal. Chem., 66 (1994) 1161–1167.
[13] J.A. Stäb, W.P. Cofino, B. Vam Hattum and U.A. Brinkman, Fresenius J. Anal. Chem., 347 (1993) 247–255.
[14] J.A. Stäb, U.A. Brinkman and W.P. Cofino, Appl. Organometal. Chem., 8 (1994) 577–585.
[15] R. Lobinski and F.C. Adams, Trends Anal. Chem., 2 (1993) 41–49.
[16] R. Lobinski, W.M.R. Dirks, M. Ceulemans and F.C. Adams, Anal. Chem., 64 (1992) 159–165.
[17] Y. Liu, V. López-Ávila and M. Alcaraz, Anal. Chem., 66 (1994) 3788–3796.
[18] T.M. Dowling and P.C. Uden, J. Chromatogr., 644 (1993) 153–160.
[19] P. Michel and B. Averty, Appl. Organometal. Chem., 5 (1991) 393–397.
[20] M. Ceulemans, J. Spzunar-Lobinska, W.M.R. Dirkx, R. Lobinski and F.C. Adams, Int. J. Environ. Anal. Chem., 52 (1993) 113–125.
[21] F. Pannier, A. Astruc and M. Astruc, Anal. Chim. Acta, 287 (1994) 17–24.
[22] J. Szpunar, V.O. Schmitt, R. Lobinski and H.L. Monod, J. Anal. At. Spectrom., 11 (1996) 193–199.
[23] M. Ceulemans,C. Witte, R. Lobinski and F.C. Adams, Appl. Organometal. Chem., 8 (1994) 451–461.
[24] S. Shawky, H. Emons and H.W. Durbeck, Anal. Comun., 33 (1996) 107–110.
[25] J. Szpunar, V.O. Schmitt, O.F.X. Donard and R. Lobinski, Trends Anal. Chem., 15 (1996) 181–187.
[26] O.F.X. Donard, B. Lalere, F. Martin and R. Lobinski, Anal. Chem., 67 (1995) 4250–4254.
[27] J.R. Dean, I.J. Barnabas, I.A. Fowlis and P. Owen, Analyst, 120 (1995) 1897–1904.
[28] V. López-Ávila, R. Joung and W.F. Becker, Anal. Chem., 66 (1994) 1907–1106.
[29] K.K. Chee, M.K. Wong and H.K. Lee, J. Chromatogr. A, 723 (1996) 259–271.
[30] F.I. Onuska and K.A. Terry, J. High Resolut. Chromatogr., 18 (1995) 417–421.
[31] M. Ceulemans, R. Lobinski, W.M.R. Dirkx and F.C. Adams, Fresenius J. Anal. Chem., 347 (1993) 256–262.