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Speciation Analysis for Organotin Compounds in Biomaterials after Integrated Dissolution, Extraction, and Derivatization in a Focused Microwave Field

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Hydrolysis with acetic acid carried out in a low-power focused microwave field in the presence of sodium tetraethylborate and nonane is shown to shorten the sample preparation time for gas chromatographic determination of organotin compounds in biological materials. After a 3-min reaction time, ethyl derivatives of mono-, di-, and tributyltin, and triphenyltin are quantitatively (>95%) found in the supernatant organic phase that is injected onto a capillary GC column. Two rapid one-step analytical procedures, using flame photometric and atomic emission detection, respectively, were developed on this basis and validated by analyzing the NIES11 certified reference fish tissue.

Tributyltin (TBT), which has been used widely as a biocide in antifouling paints, turned out to be toxic also to nontarget biota.[1] It was found to be responsible for the degradation of edible aquatic resources, thus raising economic and ecotoxicological concerns. The interest in rapid analytical methods for biomaterials is reinforced, apart from ecotoxicology, by concerns about the triphenyltin (TPhT) pesticide residues in foodstuffs.[2] The difference in toxicity between the target compounds—TBT and TPhT and products of their degradation—di- and monosubstituted organotins, and inorganic tin makes speciation analysis necessary.

The analytical protocols available are based mostly on the coupling of chromatography and atomic spectrometry (AS).[3] Despite the increasing popularity of high-performance liquid chromatography−inductively coupled plasma mass spectrometry (HPLC-ICP MS),[4] the coupling of gas chromatography (GC) with AS remains the preferred choice due to good resolution and availability of sensitive detectors. The usual choice for detection is atomic absorption spectrometry (AAS),[5,6] flame photometric detection (FPD),[7−9] microwave-induced plasma atomic emission spectrometry (MIP-AES),[10−12] or ICPMS.[13] They generally show good performance, and it is the sample preparation step that determines the duration, efficiency, precision, and accuracy of the overall analytical procedure.

The time-consuming and tedious sample preparation protocols are a natural consequence of their being composed of three independent steps: sample decomposition/leaching, extraction of the analytes into a nonpolar solvent, and derivatization. In a typical case, organotin compounds are leached with an acid−methanol mixture for 10−60 min,[2,14−16] which is usually followed by centrifugation. The ionic organotins in the supernatant are volatilized directly via hydride generation (or ethylation) prior to cryofocusing and thermal desorption,[15−17] or they are extracted with tropolone (2 × 5 min).[2,14] The extracts are subjected to Grignard derivatization (∼10 min), followed by the decomposition of the reagent. In slightly improved procedures, the tissue dispersed in acid is subject to direct repeated (2−3 times) solid−liquid extraction with 0.1−0.5% solution of tropolone in toluene (10−20 min each time), and the combined extracts are usually subjected to Grignard reaction.[9,18−20] Although the individual operations are not time consuming, their number (in an extreme case over 20 operations[21]) makes the whole procedure error prone, costly, and unsuitable for automation.

In addition, the above protocols do not take into account that organotin compounds can be incorporated in the cellular tissue structure. In view of that, complete solubilization of the tissue

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(hydrolysis) was reported to be necessary to obtain accurate results.[22] The hydrolysis procedures are usually carried out with methanolic solutions of alkalis,[22,23] tetramethylammonium hydroxide (TMAH),[24] or enzymes.[25] They take 0.7−24 h at elevated and precisely controlled temperatures and are followed by an extraction−derivatization step, which increases the complexity of the whole sample preparation step.

Reactions in a microwave field have recently gained popularity in analytical, inorganic, and organic chemistry. They have been used to accelerate sample mineralization,[26,27] solid−liquid extraction (leaching),[17,28−30] and organic synthesis.[31] This paper aims at showing that, under carefully optimized conditions, sample decomposition, extraction, and derivatization of organotin compounds can be carried out simultaneously in a single reactor, which replaces hours-long multistep sample preparation procedures by a one-step, 3-min-long integrated approach.

EXPERIMENTAL SECTION

Apparatus. A Microdigest Model A301 (2.45 GHz, maximum power 200 W) microwave digester (Prolabo, Fontenay-sous-bois, France) equipped with a TX32 programmer was used. In contrast to the pressurized systems, in which the microwave energy is dispersed throughout the oven, the system used works under atmospheric pressure, and the microwave energy is focused into the vessel. Higher power density at the sample and thus higher heating efficiency can be obtained. The system allows the applied energy to be changed from 20 to 200 W by a step of 10 W. The time of exposure up to 99 min can be set by steps of 1 min.

Microwave-assisted reactions were carried out in 25-mL reaction tubes (22 mm i.d. × 80 mm) made of Pyrex glass. The volume in which microwaves were focused was ∼10 mL. This diameter is smaller than the cavity size. A holder was made of Teflon to keep the tube centrally in the cavity. The tube was fitted with an 18-mm-i.d. × 60-mm removable condenser with three condensation shelves. The temperature of the solution exposed to microwaves was measured in situ using a Megal 500 thermometer (Prolabo) connected to a microcomputer. This is an air thermometer suitable to work within hyperfrequency radiation fields in two modes: temperature measurement and temperature process control. The accompanying PC-compatible software allows the temperature to be plotted as a function of time. *Note: Safety guidelines regarding work with microwave fields should be observed.*[32] For atomic emission detection, the ethylated species were separated on a DB-210 (J&W) column (30 m × 0.32 mm × 0.25 μm) using an HP Model 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a split/splitless injection port. Detection was achieved with an HP Model 5921A atomic emission detector. Injections were made by means of an HP Model 7673A automatic sampler. Data were handled using an HP Model 5895A ChemStation. A piece of deactivated silica column (Hewlett-Packard, 0.32 mm × 0.17 μm) served as transfer line.

For FPD detection, the ethylated species were separated on an HP-1 column (25 m × 0.32 mm × 0.17 μm) using an AutoSystem model gas chromatograph (Perkin Elmer, St. Quentin-Yvelines, France) equipped with a split/splitless injection port. Injection was done manually. Data were handled using a PE Model 1020 Chemstation.

Reagents. Analytical grade chemicals (Merck, Nogent sur Marne, France) and water deionized and further purified in a Milli-Q system (Millipore, Milford, MA) were used throughout unless otherwise stated. The glassware used was cleaned with a common detergent, thoroughly rinsed with tap water, soaked for 12 h in a 10% nitric acid solution, and finally rinsed with deionized water just before use.

The plasma gas and carrier gas used for GC was helium, 99.9999%. The reagent gases for the AED were hydrogen, 99.9996%, and oxygen, 99.9996%. All the gases were supplied by l'Air Liquide (Floirac, France). Nitrogen (99.997%) was used as the carrier gas in GC-FPD. Hydrogen (99.995%) and air (99.995%) were used for the burner.

The derivatization reagent was a 2% (w/v) solution of sodium tetraethylborate ($NaBEt_4$, Strem, Bischheim, France); it was prepared daily by dissolving the reagent in water. The acetate buffer was prepared by dissolving 1 M sodium acetate in water, followed by adjusting pH to 5 with concentrated acetic acid.

Standards and Samples. Individual stock solutions (0.5 mg/mL as Sn) of $BuSnCl_3$ (MBT), Bu_2SnCl_2 (DBT), Bu_3SnCl (TBT), and Pr_3SnCl (TPrT) (Aldrich, St. Quentin Fallavier, France) were prepared in methanol. Mixed working solutions were prepared daily by diluting the stock solutions with methanol. Tetrabutyltin, used as internal standard to correct for injection volume, was prepared by dilution with octane or nonane as required. Multicompound working solution was prepared at 0.25 μg/mL and diluted with water as required. The internal standard solution was prepared by diluting the Pr_3SnCl stock solution with methanol to give a concentration of 1 μg/mL. A fish tissue NIES11 sample from the National Institute of Environmental Studies of Japan (NIES, Ibaraki, Japan) with the certified content for TBT and an indicative value for TPhT, was analyzed to validate the method.

Procedures. *Microwave-Assisted Ethylation of Organotin Compounds.* A sample of 5 mL of concentrated acetic acid was spiked with a known amount of organotin compound. Then, 1 mL of an organic solvent (isooctane or nonane) containing TeBT as internal standard and 1 mL of the $NaBEt_4$ solution (2% w/v unless stated otherwise in the Discussion section) were added. The mixture was made up to 8 mL with water and exposed to microwaves at the power of 40 W for 2 min. After cooling down, 1 μL of the organic supernatant was injected into the gas chromatograph. *Note: The volume of aqueous phase should not be lower than 8 mL; otherwise, there is a risk of violent boiling and considerable spray losses.*

Analysis of Biological Materials. An accurately weighed sample of 0.1−0.2 g of lyophilized tissue (or 1−2 g of wet tissue) was placed in an extraction tube and spiked with 100 μL of the TPrT solution. Then, 5 mL of acetic acid, 1 mL of nonane (possibly containing TeBT), and 3 mL of 2% (w/v) $NaBEt_4$ solution were added. The mixture was exposed to microwaves at the power of

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Table 1. Optimum GC and Detector Operating Conditions

conditions	GC-AED	GC-FPD
	GC	
injection port	splitless	splitless
splitless time	45 s	60 s
injection port temperature :	200 °C	225 °C
injection volume	1 μL	1 μL
column head pressure	130 kPa helium	6.7 psi nitrogen
	Oven Program	
initial temperature	100 (1 min)	120 (0.5 min)
1st ramp rate	20 °C min^{-1}	10 °C min^{-1}
intermediate temperature:		170 °C
2nd ramp rate		40 °C min^{-1}
final temperature	200 °C (5 min)	280 °C (5 min)
	Interface	
transfer line	HP-1	
transfer line temperature	250 °C	
	Detector	
wavelength	303.419 nm	600−620 nm
helium make-up flow	240 mL/min[a]	
H_2 pressure/flow	50 psi	90 mL/min
O_2 pressure/flow	20 psi	
air pressure/flow		90 mL/min
spectrometer purge flow	2 L/min	
solvent vent-off time	2.5 min	
cavity/detector temperature	250 °C	350 °C

[a] Measured at the cavity vent.

40 W for 3 min to reach a temperature of ~130 °C. After cooling (~2 min), the organic supernatant was injected directly (GC-AED) or after a cleanup (GC-FPD).

For cleanup, a Pasteur pipet was filled with alumina to form a plug of ~5 cm. Some silanized glass wool was inserted in the tip of the pipet and on the top of the alumina plug. The column was conditioned by passing 1 mL of diethyl ether. The sample extract was introduced onto the column and eluted with 1 mL of diethyl ether. The extract and the diethyl ether were forced through the column by applying a slight overpressure of air. The ether was evaporated from the combined eluate using a gentle stream of nitrogen. Then, the residue liquid was analyzed by capillary GC-FPD.

Gas Chromatographic Analysis. Optimum GC and detectors operation conditions are summarized in Table 1.

RESULTS AND DISCUSSION

Solubilization of Biomaterials in a Microwave Field. A possibility of mild (without destruction of carbon−metal bonds) solubilization of biomaterials in a low-power focused microwave field was recently evoked.[33] The strongly alkaline medium proposed needed to be neutralized to render it chemically compatible with the subsequent extraction−derivatization step. In addition, the shaking necessary for extraction caused foaming and difficulties in phase separation, thus increasing the number of individual operations. Attempts to use enzymic hydrolysis, which can be readily carried out in neutral media (suitable for the subsequent derivatization reaction and extraction), were made. They resulted in a conclusion that enzymic hydrolysis cannot be accelerated by a microwave field (under controlled temperature) and were thus abandoned.

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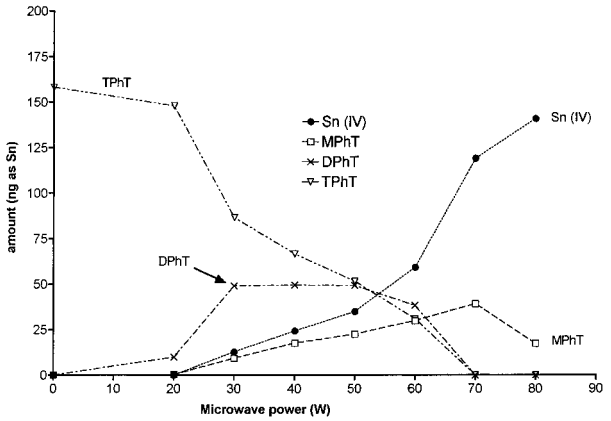

Figure 1. Effect of microwave power on the recovery and degradation of TPhT in concentrated acetic acid. Exposure time, 3 min.

Acid hydrolysis was then investigated. Acetic acid was preferred to hydrochloric acid because of the higher boiling point, lower acidity (and thus better compatibility with extraction−derivatization), and neutral behavior vs the C−Sn bond. In contrast to HCl, it was found to be impossible to dissolve the tissue in acetic acid completely within a few minutes. It was, however, noticed that the presence of $NaBEt_4$ and of organic solvent enhanced considerably the dissolution process, allowing a two-phase system to be obtained with transparent phases and a sharp phase boundary. The mechanism of this effect is not quite clear yet, but it is likely that the presence of an organic solvent shifts the hydrolysis equilibrium to the right by removing some of the reaction products into the organic phase. The best results (complete dissolution) were obtained for fatty fish tissues, whereas for some mussels, highly dispersed fine particulates were present after the hydrolysis. The particulates did not affect the recoveries in any case. In a few cases, formation of emulsion was observed, which was readily broken by addition of some anhydrous sodium sulfate.

Stability of Organotin Compounds. Earlier work[30] showed relatively good stability of organotin compounds in water, methanol, and isooctane at low microwave powers with a tendency of degradation in concentrated acids (especially HCl) at powers >60 W. It was thus necessary to test their stability in concentrated acetic acid used for hydrolysis of biomaterials. This was carried out by exposing a solution of an organotin compound (one at a time) to a microwave field at powers ranging between 20 and 80 W for a time of 3 min. Organotins in the solution were determined using a previously described procedure.[34] It was observed that each of the butyltin compounds (MBT, DBT, and TBT) and TPrT remained intact, except at powers exceeding 80 W, for which only 87−90% of the initial signal was recovered.

Under the same experimental conditions, poor stability of triphenyltin was observed. Figure 1 shows relative intensities of signals from TPhT and its degradation products as a function of the microwave power applied. Degradation products (DPhT) appear already at very low powers (20 W). At higher powers, further degradation products (MPhT and tin) are omnipresent. Note that the intensity of inorganic tin signal is only an indicative value, since the determination method has not been optimized for Sn(IV). It should be emphasized that, although the presence

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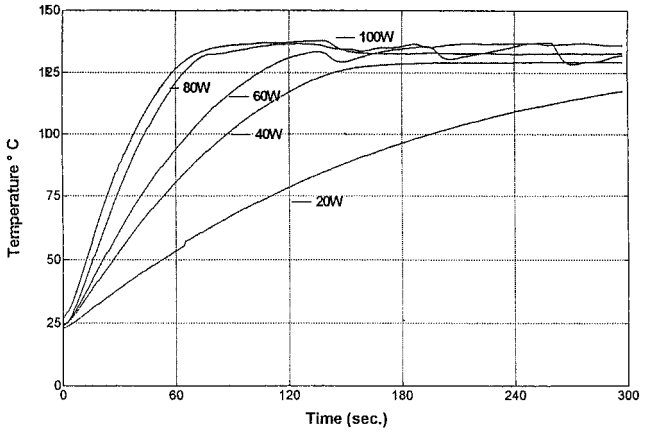

Figure 2. Effect of microwave power and time of exposure on the temperature of the extracted solution (5 mL of concentrated acetic acid and 1 mL of nonane).

of the matrix may modify the degradation reactions, usually resulting in a higher stability of the analytes, it is unlikely that phenyltin compounds can survive the acid hydrolysis in a microwave field. This agrees with the observation of Ceulemans *et al.*,[34] who found that, in acidic media at elevated temperatures, TPhT was degraded to DPhT (the only degradation product).

It was observed, however, that the TPhT moiety can be preserved, provided that a stabilizer converting the compound to a more stable species is present in the system. The ideal stabilizer turned out to be the derivatizing reagent, $NaBEt_4$. It was also possible to prevent the degradation of DPhT and MPhT, but drawing definitive conclusions regarding these compounds was hampered by the lack of sufficiently pure commercial standards.

Optimization of Microwave-Assisted Ethylation of Organotin Compounds. *Choice of Extraction Solvent.* The choice of extraction solvent is governed by the extraction thermodynamics, kinetics, and volatility of the solvent in view of the elevated temperature generated in the microwave field. Butyl- and phenyltin compounds can be extracted with hexane (bp 68 °C) upon 5 min of shaking.[34] Extraction of butyltins with isooctane (bp 99 °C) by 30 min of stirring was also reported.[35] In this work, nonane was examined as well, because this is the highest boiling solvent (151 °C) that can still be separated by capillary GC from the first-eluting compound, $BuEt_3Sn$, to a degree that it does not interfere on the level of detection.

The increase in temperature of acetic acid as a function of microwave power and time is shown in Figure 2. The maximum temperature (~130 °C), which is by ~10 °C higher than the boiling point of acetic acid, is achieved within 2 min, except for the lowest power investigated (20 W). Use of reflux condenser is necessary to prevent acid losses. Extraction solvent (nonpolar) is not heated dielectrically, but convection heating is responsible for complete loss of hexane after 30 s, even at relatively mild conditions (40 W). Isooctane could be kept up to 1.5 min without apparent evaporation loss, but an extended time will cause a complete loss of this solvent. Nonane was not lost at 40 W for 2 min, which favored its choice.

Nonane also shows faster extraction kinetics than isooctane or hexane. Table 2 shows that only nonane allows for quantitative (>95%) recovery of all the butyltin compounds after 5 min of shaking in acetic acid with $NaBEt_4$. The poor extraction efficiency with hexane and isooctane for MBT is in agreement with literature data.[34,35]

Table 2. Effect of the Choice of Solvent on the Efficiency of Ethylation−Extraction of Butyltin Compounds Spiked on 5 mL of Acetic Acid (1 mL of 2% $NaBEt_4$ Solution, 5 min Shaking)[a]

	recovery[b] ± standard deviation,[c] %		
compound	hexane	isooctane	nonane
MBT	42 ± 4	74 ± 8	96 ± 6
DBT	55 ± 4	86 ± 7	100 ± 8
TBT	61 ± 6	99 ± 11	98 ± 7

[a] Compound concentrations, ~0.4 μg/mL (as Sn). [b] Calculated against ethylated standards prepared according Ceulemans et al.[34] [c] Based on a triplicate measurement.

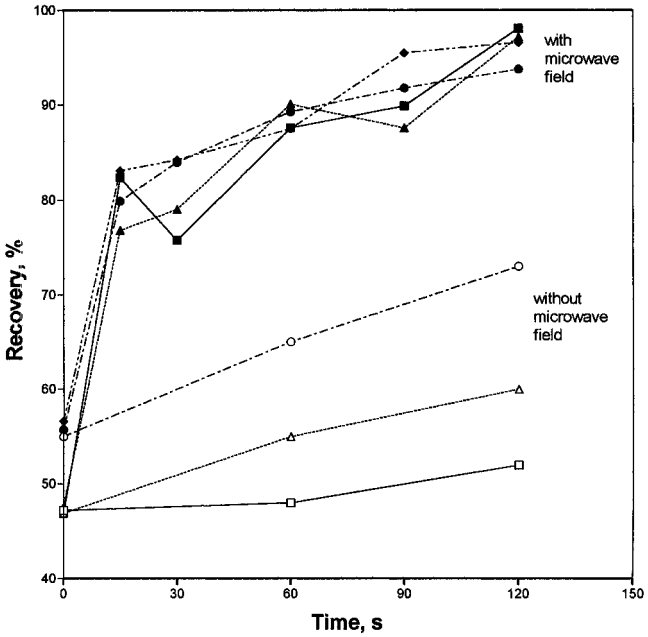

Figure 3. Kinetics of microwave-assisted derivatization−extraction of butyltin compounds from 0.33% $NaBEt_4$ in concentrated acetic acid into nonane (1 mL). Results corrected for TEBT as internal standard but not for TPrT (without microwave field, □ MBT, △ DBT, ○ TBT; with microwave field, ■ MBT, ▲ DBT, ● TBT, ◆ TPrT). Mean of three experiments.

Microwave-Assisted Extraction Conditions. Kinetics of simultaneous ethylation−extraction into nonane was studied at different times at 40 W. The recoveries shown in Figure 3 prove that ethylation is fast. Even at time 0 (just after mixing the reagents and extractant), about 50% of compounds are extracted, and extraction is quantitative after 2 min of heating. Figure 3 also shows that the extraction is much slower in the absence of microwave field. The fast kinetics of the microwave-assisted extraction can also account for the stabilizing effect of $NaBEt_4$ for TPhT. The quantitative extraction of TPhT, however, requires an addition of 3 mL of 2% borate solution (i.e., 3 times more than in the case of butyltin compounds). It is strongly discouraged to expose the mixture of acetic acid and 1 mL of nonane (in the absence of tissue) for more than 2 min, due to violent boiling causing potential spray losses.

Matrix Effects. The effect of fish tissue matrix on the ethylation−extraction efficiency was examined by spiking a sample of

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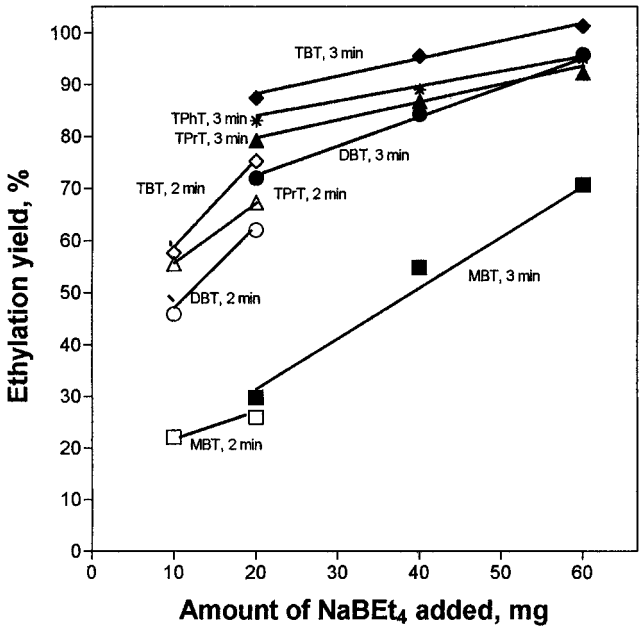

Figure 4. Effect of the $NaBEt_4$ concentration and time of microwave exposure on the recovery of butyltin compounds in the presence of 0.1 g of fish tissue.

0.1 g of dry tissue (free of organotins) with a mixture of butyl- and tripropyltin standards in methanol, equilibrating for 1 h to evaporate methanol, and submitting the tissue to the simultaneous derivatization−extraction procedure discussed above. Figure 4 shows that at the conditions optimum for water samples only 20% of MBT and about 50% of DBT and TBT are extracted. Furthermore, at low borate concentrations, standard deviations indicating irreproducible ethylation in the presence of biological matrix are serious (up to 40%). The precision is improved by the use of tripropyltin as internal standard.

The suppression of the recoveries by the biological matrix can be circumvented by increasing the concentration of $NaBEt_4$ and exposure time. It was observed that the latter can be prolonged in the presence of 0.1 g of tissue to 3 min at 40 W without loss of nonane. Figure 4 shows that, at a borate concentration of 0.75% in the reaction mixture (3 mL of 2% solution added), DBT, TBT, and TPrT are extracted quantitatively, whereas the recovery of MBT increases to ∼70%. At these conditions also, the recovery of TPhT exceeds 90%.

Speciation Analysis for Organotin in Biomaterials. Figure 5 shows chromatograms for a fish tissue obtained by the developed method using different detectors.

Figures of Merit. Values for detection limits and linearity are given in Table 3. Detection limits were estimated as three times the standard deviation of the noise (mV) in the vicinity of the relevant peak, divided by the response factor (mV/ng), and normalized to 0.2 g of dry tissue. Detection limits with FPD are sufficient for biomaterials for contaminated areas, whereas for cleaner samples the use of AED is strongly recommended. At the optimum conditions, the slopes of the calibration curve for spikes on the tissue and on acetic acid are equal for all the species. The different slopes for the particular butyltin species are a result of different FPD response and of not quantitative (70%) recovery of MBT. Common precision for butyltin compounds is between around 5% for butyltin compounds and 10% for triphenyltin.

Internal Standards. Two kinds of internal standards were used. Tetrabutyltin was added to the extracting solvent to control the precision of the manual injection. An ionic internal standard, TPrT, was used to control the ethylation efficiency and to correct for volume changes and spray losses during the microwave heating and the separation of the supernatant. The poor recovery of the internal standard can be considered as an early warning of the malfunction of the procedure.

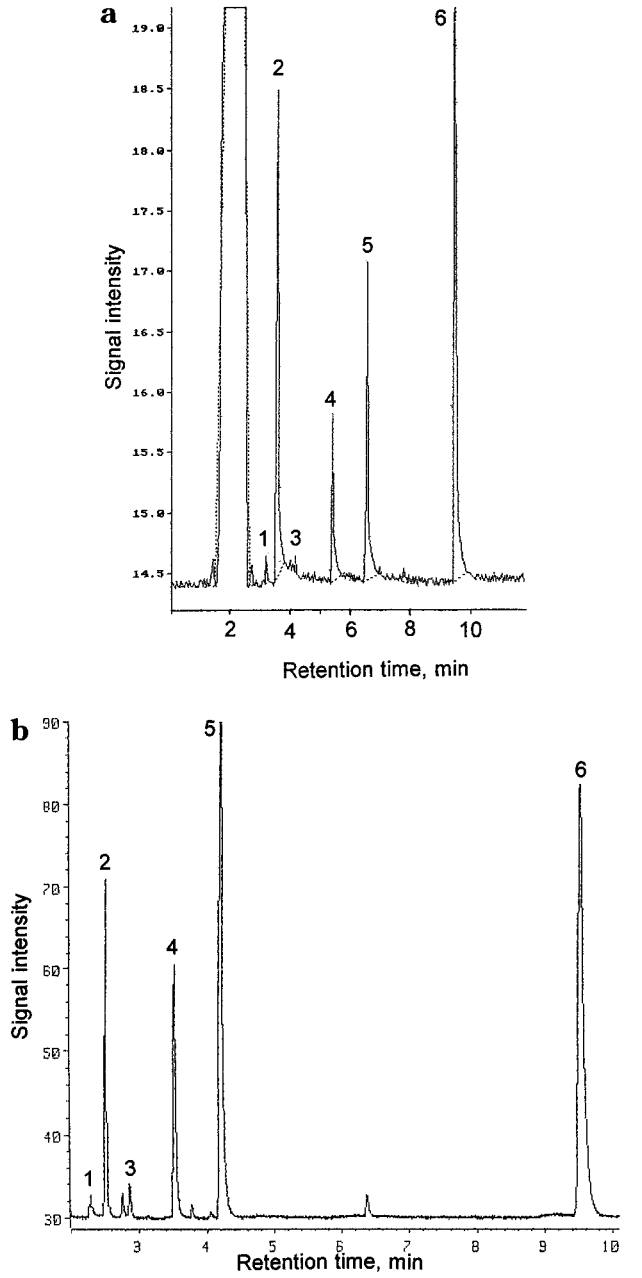

Figure 5. Chromatograms of fish tissue (NIES11) extracts obtained using the procedure developed (a) by GC-FPD and (b) by GC-AED. 1, MBT; 2, TPrT; 3, DBT; 4, TBT; 5, TeBuT; 6, TPhT.

Table 3. Figures of Merit of the Analytical Procedure

	calibration curve (GC-FPD)		detection limit,[a] ng/g	
compound	slope	R^2 coeff	GC-FPD	GC-AED
monobutyltin	0.00547	0.9986	23	5
dibutyltin	0.00597	0.9998	20	3
tributyltin	0.00376	0.9997	30	3
triphenyltin	0.00695	0.9984	40	10

[a] calculated as Sn on the basis of 0.2 g sample intake (dry tissue), 3 times standard deviation.

Accuracy. The method was validated by the analysis of the NIES11 reference fish tissue. For TBT, the values (as Bu_3SnCl) obtained by FPD, 1.18 ± 0.24 and 1.32 ± 0.05 μg/g (means of three replicates on two different days, calibration using a two-point standard graph), are close to the value found by GC-AED 1.37 ± 0.16 (mean of five experiments on the same day, calibration by one-point standard addition), and match well the certified value, 1.3 ± 0.1 μg/g.

The values for TPhT concentration (as Ph_3SnCl) obtained by FPD, 5.41 ± 0.85 and 6.18 ± 0.74 μg/g (means of three replicates on two different days, calibration using a two-point standard graph), match quite well the value reported by the NIES, 6.3 μg/g.[36] The latter is only indicative, as during the certification campaign analytical problems related to extraction, measurement, and cleanup occurred.[36] A similar result (5.80 ± 0.81 μg/g, mean of five experiments on the same day, calibration by one-point standard addition) was obtained from an independent series of measurements by GC-AED. Despite not being shown by the spike experiments, loss through degradation cannot be excluded, especially in view of the results reported by Ceulemans et al.,[24] who observed about 30% discrepancy between the results from a mild enzymic hydrolysis and harsher TMAH hydrolysis.

(36) Morita, M. Instructions for use report on NIES certified reference material No. 11 fish tissue; NIES: Japan, 1990.

CONCLUSIONS

The paper has demonstrated the possibility of radically accelerating each of the crucial steps of the sample preparation procedure for GC speciation analysis of biomaterials (sample decomposition, extraction, and derivatization) by carrying them out in a low-power focused microwave field. Conditions were found for integrating these steps into one, which is the prerequisite for automation of the procedure. The 3-min-long, single-step sample preparation developed is equivalent in terms of recovery and accuracy to hours-long multistep sample procedures commonly reported in the literature.

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