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Determination of butyl- and phenyltin compounds in biological material by gas chromatography–flame photometric detection after ethylation with sodium tetraethylborate

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Abstract

A reliable and rapid speciation method for the simultaneous determination of butyl- and phenyltin species in biological samples has been developed. Three extraction procedures are compared: enzymatic hydrolysis and solubilization by ethanoic and hydrochloric acids. Derivatization is performed by the one-step ethylation/extraction procedure using the sodium tetraethylborate reagent directly in the aqueous phase in the presence of an isooctane layer. Analysis is performed using capillary gas chromatography coupled to flame photometric detection. The detection limits are in the range of a few ng(Sn)/g. Analysis of the environmental samples and the certified reference material demonstrates the accuracy of the analytical method.

Keywords: Ethylation; Derivatization, GC; Extraction methods; Butyltin compounds; Phenyltin compounds; Organotin compounds; Sodium tetraethylborate

1. Introduction

Organotin compounds have been extensively used as biocides. Contamination of marine and freshwater environments by the highly toxic tributyltin (TBT) is primarily due to its use in antifouling paints on vessels [1]. The ecotoxicological impact of TBT has been demonstrated [1,2]. Triphenyltin (TPhT) compounds are used as agricultural fungicides in crop protection. They are employed to control potato, celery, sugar beet, coffee and rice against fungal diseases [3]. However, TPhT was found to be a non-selective pesticide, detrimental to freshwater fauna [4]. Moreover, TPhT has been shown to have a high bioaccumulation potential [3]. Considerable concentrations of these compounds and their metabolites were detected in Swiss Lakes [5]. The en-

vironmental risks of organotins include their bioaccumulation, persistence and high toxicity to some animals [6,7]. The recognition of organotin toxicity at low concentration levels has stimulated the development of accurate and sensitive analytical methods for organotin determinations. Many efficient analytical procedures combine chromatographic separation and various detection techniques such as atomic absorption spectrometry (AAS) [8,9], mass spectrometry (MS) [10], atomic emission spectrometry (AES) [11] or flame photometric detection (FPD) [12–14].

Despite recent improvements in instrumentation, several limitations remain present in the field of sample preparation. Prior to gas chromatography (GC) organotin compounds need to be liberated from the biological matrix, before being derivatized into forms suitable for chromatography. Extraction of organotin compounds after acid leaching from the

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biological matrix followed by hydridization using NaBH_4 or alkylation with Grignard reagent are the most common approaches. The alternative to acid leaching is the use of tissue solubilizers such as TMAH [11,15], KOH [16,17] or enzymes [11,18,19] to decompose the sample matrix.

A new organotin derivatization procedure directly applied in the aqueous phase using sodium tetraethylborate (NaBEt_4) has been recently proposed in which the derivatization and extraction of compounds are realized in a single operating flask. It allows the simultaneous speciation of butyl- and phenyltin and is much more rapid than procedures using the Grignard reaction [9–11,15,20]. In this paper, the optimal conditions for the simultaneous extraction of butyl- and phenyltin compounds have been evaluated on freshwater fish samples. Different approaches for breaking down the biological matrix have been tested. They are followed by a rapid *in situ* derivatization with NaBEt_4 reagent and a direct analysis performed using capillary GC–FPD. The optimized method was applied to the analysis of a reference fish tissue (NIES) and a freshwater mussel sample.

2. Experimental

All organotin concentrations reported in this paper are expressed as the mass of tin per sample mass or solution volume unit.

2.1. Apparatus

A Varian 3300 gas chromatograph (Les Ulis, France) was used for this study. It was fitted with a split/splitless injector, a J&W Scientific capillary column (Folsom, CA, USA) coated with polydimethylsiloxane (30 m×250 μm I.D., film thickness 0.25 μm) and a FPD system. The detector was operated with a 610 nm optical filter (MTO Optique Instrumentale, Massy, France) and an air–hydrogen flame. Nitrogen was used as the carrier gas.

2.2. Reagents

Tripropyltinchloride (TPrT, 98%), monobutyltin-trichloride (MBT, 95%), dibutyltin-dichloride (DBT,

97%), tributyltinchloride (TBT, 96%), monophenyltin-trichloride (MPhT, 98%), diphenyltin-dichloride (DPhT, 96%) and triphenyltinchloride (TPhT, 95%) were purchased from Aldrich (Saint-Quentin-Fallavier, France). Tetrabutyltin (TeBT, 98%) was obtained from Fluka (Saint-Quentin-Fallavier, France). Stock organotin solutions (1000 mg/l as Sn) were prepared in methanol. Stored at +4°C in the dark, they are stable for several months [21]. Working standards were obtained by dilution in methanol weekly for 10 mg/l and daily for 100 $\mu\text{g/l}$ with storage in the dark at +4°C.

Methanol and sodium ethanoate were purchased from Prolabo (Gradignan, France), nitric acid and ethanoic acid were obtained from Merck (Nogent-sur-Marne, France), and isooctane from Fluka (Saint-Quentin-Fallavier, France). The deionized water used was 18 M Ω (Millipore system).

Protease Type XIV and Lipase Type VII enzymes were obtained from Sigma (Saint-Quentin-Fallavier, France). The phosphate buffer (0.1 mol/l) was prepared by dissolving ammonium dihydrogenphosphate (Prolabo RP from Prolabo) in deionized water and the pH was adjusted to 7.5 with concentrated ammonia (Merck). Ethanoate buffer (pH 4.8, 0.2 mol/l) was prepared by dissolving 16.4 g of sodium ethanoate in 1 l of deionized water followed by pH adjustment with ethanoic acid.

Sodium tetraethylborate (NaBEt_4) was obtained from Strem Chemicals (Bischheim, France). The working solution was made up daily by dissolving 0.02 g in 1 ml of deionized water and storing in the dark at +4°C.

Glassware was rinsed in deionized water, decontaminated overnight in 10% (v/v) nitric acid solution and then rinsed again.

2.3. Samples

Freshwater fishes (Chevesnes or Chubs) were caught in the Bidouze River in the Pyrenees Mountains. Fillets were peeled and the bones were carefully removed. After freezing for 48 h at –20°C, the sample was thawed and ground in a blender, before being spiked. Freshwater fish flesh was found to be free of organotin before spiking. Freeze-dried *Dreissena Polymorpha* mussel samples from the Zurich Lake were given to us by Dr. Becker Van Slooten

from the Polytechnic Federal School of Lausanne. They were mixed to obtain a homogenized powder prior to analysis.

2.4. Spiking process and storage

300 g of sample slurry (precisely weighed) were spiked by slow addition of a minimal volume (5 ml) of a methanolic solution of TBT and TPHT chlorides under stirring. Wet samples (spiked and unspiked) were then stored at -20°C in closed polyethylene flasks.

Performing extraction on wet tissue after mixing and homogenization is a rapid and sure way to analyze real samples restricting degradation processes. But it is more convenient to store, handle and transport dry material [22]. Therefore, parts of both spiked and unspiked samples were freeze-dried before storage at -20°C in polyethylene flasks. Siccity was estimated on eight aliquots and found equal to 87%.

2.5. Extraction and analytical procedure

A scheme of the different procedures is shown in Fig. 1.

2.5.1. Extraction

Three extraction methods were tested on spiked wet and freeze-dried samples.

Enzymic hydrolysis

0.1–0.3 g of freeze-dried or 0.5–2 g of wet samples were introduced in a capped 50 ml polycarbonate tube with 0.05 ml of a $10\ \mu\text{g}(\text{Sn})/\text{ml}$ TPPrT methanolic solution as the internal standard, and 5 ml of phosphate buffer (pH=7.5), 0.010 g of Lipase Type VII and 0.010 g of protease Type XIV. The tubes were incubated for 4 h in a thermostatic bath at 37°C under magnetic stirring.

Ethanoic acid digestion

0.1–0.3 g of freeze-dried or 0.5–2 g of wet samples were introduced in a capped 50 ml polycarbonate tube with 0.05 ml of a $10\ \mu\text{g}(\text{Sn})/\text{ml}$ TPPrT methanolic solution as the internal standard, and 20 ml of glacial ethanoic acid. The tubes were shaken at 420 rpm for 4 h.

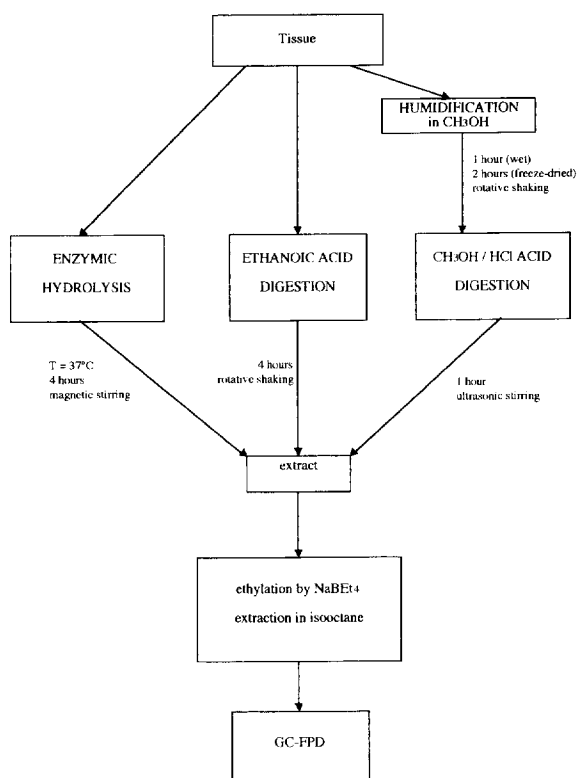


Fig. 1. Schematic representation of different sample preparation procedures used for isolation of organotin compounds from biological matrices.

Methanolic–hydrochloric acid digestion

0.1–0.3 g of freeze-dried or 0.5–2 g of wet samples were introduced in a capped 50 ml polycarbonate tube with 0.05 ml of a $10\ \mu\text{g}(\text{Sn})/\text{ml}$ TPPrT methanolic solution as the internal standard, and 2.5 ml of methanol. The tubes were shaken at 420 rpm for 1 h to humidify the material. This rehumidification step was carried out for 2 h for freeze-dried samples. 12.5 ml of 0.12 M HCl in methanol was then added and the mixture was treated ultrasonically for 1 h.

2.5.2. Derivatization and analysis

Subsamples (0.1–0.5 ml) of raw extracts were directly introduced in the derivatization reactor without any further treatment. Organotin compounds were ethylated in 100 ml of ethanoate buffer (pH=4.8) with 0.1 ml of NaBEt_4 solution in the presence of 0.3 ml of isooctane. The mixture was shaken at

Table 1
Analytical conditions for determination of organotin compounds by GC–FPD

Injector parameters			
	Sample amount		2–4 μl
	Injection temperature		290°C
	Relay	Initial relay	+1 (splitless)
		Relay time	1 min
		Final relay	–1(split)
GC parameters			
	Carrier gas	Nitrogen	
		Flow-rate	0.7 $\text{cm}^3 \text{min}^{-1}$
	Oven program	Initial temperature	70°C for 1 min
		1st heat-up rate	30°C min^{-1}
		Intermediate temperature	190°C for 0 min
		2nd heat-up rate	15°C min^{-1}
		Final temperature	270°C for 5 min
FPD parameters			
	Detection temperature		290°C
	Flame	Air/hydrogen	
		Hydrogen flow-rate	185 $\text{cm}^3 \text{min}^{-1}$
		Air flow-rate	250 $\text{cm}^3 \text{min}^{-1}$
	Make up	Nitrogen	
		Flow-rate	30 $\text{cm}^3 \text{min}^{-1}$

420 rpm for 30 min. 2–5 μl of isooctane extracts were directly injected into the GC–FPD system. The analytical parameters were optimized and they are precisely described elsewhere [20]. The chromatographic conditions are summarized in Table 1.

The determination of organotin concentrations in samples were made on three extracts using TPrT as the internal standard, and were checked on one other extract by standard addition in triplicate to compare the analytical performances.

3. Results and discussion

3.1. Extraction

3.1.1. Procedures

To our knowledge, enzymic hydrolysis or acid leaching of biological matrix directly followed by NaBEt_4 derivatization for the determination of phenyltin compounds as described in this paper had been previously reported only by Ceulemans et al. [11]. In the present study, the number of sample handling steps was reduced because the clean-up step was found to be unnecessary. Although extracts of

samples rich in organic matter, such as biological tissues, contain large amounts of coextractives (fats, high boiling hydrocarbons), we did not note any problem of column contamination or background interferences as indicated by Ceulemans et al. [11], probably because only small aliquots (0.1–0.5 ml) of extracts were used in the derivatization step. Moreover, in the present study enzymic hydrolysis was carried out in a pure phosphate buffer without any of the additives used by Ceulemans et al. [11] or Forsyth and Cleroux [18]. These authors added ethanol and citric acid to the phosphate buffer which could help the solubilization of organotin compounds, apart from the pure enzymic attack.

Ethanoic acid digestion was developed by Desauziers et al. [23] to analyze butyltin compounds in sediments. It has not been commonly applied to break down biological matrices or extract simultaneously butyl- and phenyltin compounds although it is a simple method. When analysis is carried out by hydride generation–gas chromatography–quartz furnace atomic absorption spectrometry (HG–GC–QFAAS), some interferences with the matrix may occur [24].

Methanolic–HCl treatment is one of the most

common ways used to solubilize matrices. Organotin compounds are further complexed by complexing agents like tropolone [25–27] to improve their extraction into an organic solvent before a Grignard derivatization. Such procedures are more time consuming and manpower consuming; losses and degradation may occur.

3.1.2. Recovery

Recoveries of butyl- and phenyltin species presented in Table 2 are calculated by comparison with the spike concentrations [250 ng(Sn)/g(wet mass) TBT and TPhT in wet tissue and 1925 ng(Sn)/g(dry mass) in freeze-dried tissue].

Spiked fish flesh extracts of both freeze-dried and wet tissue were homogeneous and suitable for analysis, regardless of the extraction process. In all cases, the same partial decomposition in mono- and disubstituted degradation products appears. This may indicate that DBT, MBT, DPhT and MPhT were in the material before extraction. Moreover, the possible decomposition of butyl- and phenyltin compounds during the extraction step was checked by incubation of TBT and TPhT in extraction con-

ditions. Analysis of the incubated standard solutions led to a quantitative recovery of TBT and TPhT. These metabolites could have been formed during the quite long spiking process realised at daylight and room temperature.

Quantitative recovery of total butyltin compounds is obtained by all methods tested. Phenyltin recovery is satisfactory when acid digestion is applied, less for ethanoic than for methanolic–HCl acid digestion, especially in wet matrices. We noted the formation of lumps in this case that may disturb the sampling of the extract. When enzymic hydrolysis is performed, only 51–56% of the TPhT spiking value is found. These low yields are verified on environmental samples. Tests conducted on phenyltin standard additions in extracts before enzymic attack alone on unspiked fish samples lead to quantitative recoveries. Phenyltin compounds in fish flesh seem to be strongly linked to matrix sites not reached with this extraction procedure.

In this work, methanolic–HCl solubilization has been investigated with a 0.1 M HCl solution. Preliminary studies were carried out with higher HCl concentrations in methanol and significant degra-

Table 2
Analysis of organotin in spiked fish flesh

	Enzymic hydrolysis		Ethanoic acid digestion		Hydrochloric acid digestion	
	wet ng(Sn)/g(w.m.)	freeze-dried ng(Sn)/g(d.m.)	wet ng(Sn)/g(w.m.)	freeze-dried ng(Sn)/g(d.m.)	wet ng(Sn)/g(w.m.)	freeze-dried ng(Sn)/g(d.m.)
MBT ^a	18 (10%)	86 (9%)	26 (10%)	104 (5%)	23 (9%)	108 (6%)
DBT ^a	8 (9%)	93 (7%)	16 (10%)	114 (2%)	14 (7%)	107 (7%)
TBT ^a	212 (4%)	1660 (2%)	206 (6%)	1726 (2%)	205 (5%)	1636 (1%)
\sum Bu ^a	238 (5%)	1839 (1%)	248 (7%)	1944 (2%)	242 (3%)	1851 (1%)
R\sumBu	95±5%	95±1%	99±7%	101±2%	97±3%	96±1%
MPhT ^a	13 (12%)	86 (10%)	26 (8%)	217 (5%)	21 (12%)	237 (3%)
DPhT ^a	6 (13%)	80 (12%)	24 (8%)	224 (8%)	17 (6%)	216 (10%)
TPhT ^a	121 (16%)	816 (7%)	170 (5%)	1309 (4%)	195 (8%)	1388 (6%)
\sum Ph ^a	140 (12%)	982 (6%)	220 (6%)	1750 (4%)	233 (5%)	1841 (1%)
R\sumPh	56±1%	51±3%	88±5%	91±4%	93±5%	96±1%

^a Mean concentration (R.S.D. %) ($n=4$).

\sum Bu = MBT + DBT + TBT.

R \sum Bu: butyltin recovery (calculated by comparison with the TBT spiking concentration).

\sum Ph = MPhT + DPhT + TPhT.

R \sum Ph: phenyltin recovery (calculated by comparison with the TPhT spiking concentration).

R.S.D.: Relative Standard Deviation.

d.m.: dry mass.

w.m.: wet mass.

dation was observed. This fact agrees with results reported in the literature. For example, Mediterranean mussel samples were analyzed by Tolosa et al. using methanolic 4 M HCl followed by tropolone/diethylether/hexane extraction and Grignard methylation [28]. Low extraction yields were found for MBT and DPhT (19%) and yields ranging from 60% to 91% were obtained for DBT, TBT and TPhT.

Compared to enzymic hydrolysis and ethanoic acid digestion, methanolic 0.1 M HCl solubilization is the most rapid procedure: 2 h for wet and 3 h for freeze-dried samples. Derivatization by NaBEt₄ can then be carried out directly on subsamples of the extracts without any further treatment and butyl- and phenyltin compounds can be simultaneously and quantitatively determined. Moreover, when ethanoic digestion is applied to large volumes of subsample, lumps appear. Therefore, for low-concentration samples methanolic-HCl digestion may be preferred.

3.2. Analytical procedure performances

The reproducibilities obtained for different extraction procedures and each compound are given by means of relative standard deviation and are reported in brackets in Table 2.

The detection limits reported in Table 3 in both wet and freeze-dried samples were determined according to IUPAC recommendations with $k=3$. They were calculated from the calibration curves of all compounds.

The detection limits are higher for freeze-dried samples than for wet ones. They range between 1 and 3 ng(Sn)/g for wet tissue analysis and between 7 and 23 ng(Sn)/g for freeze-dried tissue analysis. They are generally lower than most of those reported

in the literature when organotin determinations are performed using GC-FPD after Grignard derivatization [Becker et al. [5]: about 33 ng(Sn)/g(wet mass); Garcia-Romero et al. [27]: 5–10 ng(Sn)/g(wet mass); Tolosa et al. [28]: 1–4 ng(Sn)/g(wet mass); Stegmüller et al. [29]: 4–14 ng(Sn)/g(wet mass)].

3.3. Applications

Enzymic hydrolysis and hydrochloric acid digestion were validated by analysis of two different environmental samples.

3.3.1. NIES fish tissue

The only available biological certified reference material is a freeze-dried fish sample prepared by the National Institute of Environmental Studies (Japan). This material is certified for its total tin and TBT contents:

$2.4 \pm 0.1 \mu\text{g/g}$ (dry mass) total tin

$1.3 \pm 0.1 \mu\text{g/g}$ (dry mass) TBTCl,

i.e. $474 \pm 36.5 \text{ ng(Sn)/g}$.

This material contains also $6.3 \mu\text{g/g}$ (dry mass) TPhTCl, i.e. 1940 ng(Sn)/g . This value is given as the indicative one.

The mean results obtained from four determinations are given in Table 4. The recoveries are calculated by comparison with certified or indicative concentrations. The results obtained by HG-GC-QFAAS and both extraction procedures [19] are also reported for comparison.

Degradation products MBT, DBT, MPhT and DPhT appear at significant levels, as represented by

Table 3
Relative detection limits

	Enzymic hydrolysis		Ethanoic acid digestion		Hydrochloric acid digestion	
	wet	freeze-dried	wet	freeze-dried	wet	freeze-dried
MBT ^a	1	9	2	13	1	10
DBT ^a	1	7	2	14	1	11
TBT ^a	1	7	2	11	1	10
MPhT ^a	2	13	3	16	2	19
DPhT ^a	1	11	3	16	2	20
TPhT ^a	1	11	3	20	3	23

^a Concentrations are expressed in ng(Sn)/g(wet mass) for the wet sample and in ng(Sn)/g(dry mass) for the freeze-dried one.

Table 4
Concentrations of butyl- and phenyltin in NIES reference material

	Eth.-GC-FPD		HG-GC-QFAAS [19]	
	enzymic ng(Sn)/g(d.m.)	acidic ng(Sn)/g(d.m.)	enzymic ng(Sn)/g(d.m.)	acidic ng(Sn)/g(d.m.)
MBT ^a	68±3	93±1	66±2	58±7
DBT ^a	47±4	57±4	66±1	54±6
TBT ^a	355±10	319±24	383±4	357±8
∑Bu ^a	470±16	469±28	515±6	468±6
R∑Bu	99±3%	99±6%	109±1%	99±1%
MPhT ^a	353±7	383±30	n.a.	n.a.
DPhT ^a	107±7	163±8	n.a.	n.a.
TPhT ^a	416±39	1251±44	n.a.	n.a.
∑Ph ^a	869±50	1797±80	n.a.	n.a.
R∑Ph	45±3%	93±4%	n.a.	n.a.

^a Mean concentration±SD (R.S.D. %) ($n=5$).

∑Bu=MBT+DBT+TBT.

R∑Bu: butyltin recovery (calculated by comparison with the TBT certified concentration).

∑Ph=MPhT+DPhT+TPhT.

R∑Ph: phenyltin recovery (calculated by comparison with the TPhT indicative concentration).

n.a.: non-analyzable.

d.m.: dry mass.

w.m.: wet mass.

Fig. 2. However, the total concentrations ∑Bu and ∑Ph are very close to the respective certified or indicative values. This may indicate that metabolites were in the material before analysis. This degradation happened probably during transport or storage. The results obtained by the completely different technique HG-GC-QFAAS confirms this analysis.

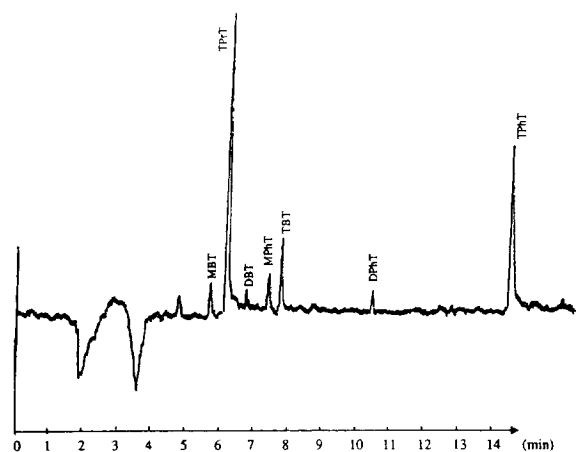


Fig. 2. Typical chromatogram of NIES analysis after HCl-methanol digestion.

On the other hand, the low phenyltin recovery obtained by enzymic hydrolysis in comparison with the one obtained by acidic digestion confirms the previous spiked-fish analysis results. In contrast, Ceulemans et al. [11] determined TPhT in NIES material at a higher concentration than the indicative one (TPhT recovery of 115±6%). Due to the presence of citric acid (21 g/l) and ethanol in the phosphate buffer, the conditions of extraction they used seem close to those of the acidic digestion of this study which gives quantitative TPhT recoveries.

3.3.2. *Dreissena Polymorpha* mussels from Zurich Lake

The results obtained after both enzymic hydrolysis and hydrochloric acid digestion are reported in Table 5. They are compared with those obtained in our laboratory using HG-GC-QFAAS and those determined by Dr. Becker Van Slooten using Grignard derivatization-GC-FPD after tropolone-diethyl ether-hexane extraction [30].

TPhT concentration reported in Table 5 is lower when enzymic hydrolysis is performed. This result confirms previous remarks. The results obtained by

Table 5
Concentrations of butyl- and phenyltin in *Dreissena Polymorpha* mussels

	Eth.-GC-FPD		HG-GC-QFAAS	Results from Ref. [30]
	enzymic	acidic	enzymic	
MBT ^a	1440±40	1140±40	1350±220	n.c.
DBT ^a	960±40	1210±70	1780±130	1403±510
TBT ^a	9760±470	9540±1040	9000±200	10630±1230
TPhT ^a	1070±70	1930±50	n.a.	n.c.

^a mean concentration + SD ($n = 3$) in ng(Sn)/g(dry mass).

n.a.: non-analyzable.

n.c.: non-communicated.

the three different analytical techniques are relatively similar. Organotin concentrations in *Dreissena Polymorpha* mussels from the Zurich Lake are quite high. Becker Van Slooten and Taradellas showed that *Dreissena polymorpha* mussels are subject to important TBT bioaccumulation [31].

4. Conclusion

After optimization of the operating conditions, butyl- and phenyltin species were determined in biological matrices. Results show that methanolic 0.1 M HCl digestion is a convenient method for the simultaneous and quantitative release of butyl- and phenyltin compounds. With enzymic hydrolysis, quantitative recoveries can be achieved only for butyltin species. Although both procedures are very simple and rapid with a minimal number of sample handling steps, acidic digestion is preferred because of its performance to butyl- and phenyltin species. Moreover, it is directly compatible with the one-step aqueous ethylation/extraction-capillary GC-FPD method. It allows accurate determination of butyl- and phenyltin compounds in wet and freeze-dried tissue from freshwater and seawater fish with detection limits of a few ng(Sn)/g. Therefore, this method is a convenient analytical technique for the analysis of real-world samples.

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