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Quality Control in the Speciation Analysis of Butyltin Compounds in Marine Biological Samples by Hydride Generation–Cold Trapping and On-line Quartz Furnace Atomic Absorption Spectrometry

F. Pannier, A. Astruc and M. Astruc
Laboratoire de Chimie Analytique, CURS, Université de Pau et des Pays de l'Adour, Avenue de l'Université, 64000 Pau, France

The determination of tributyltin (TBT) in sea food (mussel, oyster, fish) by a hydride generation–cold trapping–quartz furnace atomic absorption spectrometry procedure has been studied with emphasis on quality control. Large amounts of spiked materials have been prepared and stored either frozen, or frozen after freeze-drying. Extraction of TBT by 0.1 M hydrochloric acid in methanolic solution is efficient (88–96%) for wet samples or freeze-dried samples. It is also efficient with freshly spiked materials. Consumption of sodium borohydride by the matrix makes it necessary to use quite large amounts of reagent to obtain optimal sensitivity. Analysis of a fish-tissue Certified Reference Material seems to indicate a partial debutylation of TBT during transport and/or storage of this material.

Keywords: Speciation, buyltin compounds, marine biota, hydride generation, atomic absorption

INTRODUCTION

The presence of butyltin compounds in aquatic biota has been widely documented.[1] The lipophilicity of the very toxic tributyltin salts is such that very high bioconcentration factors have been evaluated (up to 60 000).[2–4] Some concentrations of TBT determined in sea food [*ca* 0.2 μg (Sn) g^{-1} (wet weight) in marine mussel, 0.17 in scallop muscle, 0.15 or even up to 0.8 μg (Sn) g^{-1} in oyster flesh[1,5,6]] are such that public health could be at risk. It is therefore becoming necessary to establish reliable analytical procedures for the speciation analysis of organotins in marine biological samples, to verify their overall validity and to develop Certified Reference Materials.

In this work the validity of the hydride generation–cold trapping–quartz furnace atomic absorption spectrometry speciation method applied to the determination of butyltins in sea food has been studied in detail with emphasis on sample pretreatment procedures. All concentrations of individual organotin compounds will be reported as tin, i.e. as ng (Sn) g^{-1}. Tributyltin will be denoted as TBT, dibutyltin as DBT and monobutyltin as MBT.

EXPERIMENTAL

Reagents

Reagents used in this study were as previously described[1] for methanol, hydrochloric acid, nitric acid, pure acetic acid and sodium borohydride ($NaBH_4$). Butyltin chlorides were obtained from BRC (Community Bureau of Reference, Commission of European Communities). The purity evaluated by comparison with Aldrich or Merck products is estimated at 91 ± 5% for MBT, 107 ± 8% for DBT and 86 ± 12% for TBT.

Sample preparation and spiking procedures

There is no shellfish reference material certified for its butyltin compounds content. The only available biological material is a fish sample (NIES No. 11: Certified Reference Material No. 11, 'Fish Tissue', National Institute of Environment Agency of Japan, Tsukuba, 1990) certified for its TBT content. It has therefore

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Table 1 Composition of spiked samples

Sample	Water content (%)	Calculated spike concentration (ng g^{-1})	
		Wet wt	Dry wt
Oyster	83	150	882
Mussel	88	150	1250
Salmon	85	100	667
		400	2670

been necessary to prepare large amounts of homogeneous spiked samples, stored wet or after lyophilization. Base material (oyster, mussel, salmon) were commercial products chosen to have undetectable butyltin content. Spiking operations took place from a few days to several months (up to 18 months) before analysis. No significant differences in extractability with frozen samples were noted during this period.

Mollusc samples (oyster and mussel)
The whole flesh of molluscs was removed from shells, pooled and frozen for two days. After thawing it was crushed and homogenized in a commercial blender; 120 g of this slurry were then spiked by slow addition of 3 ml of a solution of tributyltin chloride in methanol (6 mg l^{-1}) under vigorous stirring. Homogenization was then completed by a further 3 h of stirring.

Fish (salmon)
Fresh salmon fillets, carefully cleaned from skin, bones and scales, were homogenized and frozen. After thawing they were mixed with 50% (w/w) water; 50 g of this mixture was spiked by slow addition of 5 ml of a methanolic solution (1 or 4 mg l^{-1} respectively of tributyltin chloride under vigorous stirring. Finally this slurry was further homogenized in a commercial blender.

Sample storage

Each spiked sample was then divided in two parts, one being directly frozen and stored at −20 °C (in open-ended glass tubes closed with parafilm for molluscs, as chips in closed glass bottles for salmon); the other part was freeze-dried, crushed, homogenized in a blender and stored at −20 °C. Table 1 indicates the water contents of the wet spiked samples and TBT concentrations of the eight spiked samples.

Analytical procedure

A small subsample (50–200 µl) of shellfish extract was introduced to the reaction flask with 100 ml deionized water and 1 ml of pure CH_3COOH. Under stirring and helium flushing, organotin compounds were converted into their hydrides by addition of $NaBH_4$ (10%, m/v) via an Ismatec peristaltic pump (2.5 ml min^{-1}). Evolved hydrides were then carried by the helium flow (300 ml min^{-1}) to a glass GC column (650 mm × 4 mm, packed with Chromosorb W-HP, 10% OV 101) cooled in liquid nitrogen, where they were trapped. After removing the cooling bath, the column was left to warm at ambient temperature for 3 min and finally electrically heated by a Gilphy 80 wire for 4 min up to 180 °C. Separated hydrides, flushed by helium from the GC column, were introduced to an electrically heated (950 °C) quartz furnace placed in the light beam of an IL 151 atomic absorption spectrometer ($\lambda = 286.3$ nm). Hydrogen and oxygen, with respective flow rates of 200 ml min^{-1} and 45 ml min^{-1}, were introduced into the quartz cell, where their combustion increased sensitivity.[8] Automatization of $NaBH_4$ addition, cooling time in liquid nitrogen, column heating and absorption measurement was made by a TRS 80 microcomputer, signal integration being performed by a Varian 4270 integrator.

RESULTS AND DISCUSSION

Extract of butyltin compounds

In a preceding study[1] we examined two extraction procedures described in the literature as applied to wet mussel samples: extraction with pure acetic acid, which led to foam production, and extraction with an HCl–methanol solution using different HCl concentrations, sonication or shaking conditions. Methanolic HCl extraction was proved convenient provided that low HCl concentrations were used, as higher HCl concentrations led to a significant degradation of TBT and appearance of DBT and MBT.

Wet mussel samples in this study were therefore submitted to the following extraction procedure: 1 g wet sample was mixed wth 1 ml methanol and 5 ml 0.1 M HCl, sonicated for 1 h then made up to 10 ml with water. In these conditions a quantitative recovery of organic tin was

obtained and total debutylation of tin was always lower than 9% (Table 2).

This procedure has also been proved very efficient in the extraction of DBT and MBT from spiked wet mussel samples (98 and 98% recoveries) and in the extraction of TBT, DBT, MBT from spiked oyster samples (97, 97 and 99% recoveries respectively.[1]

Small modifications were necessary to apply the procedure to freeze-dried samples: 0.5 g of dry tissue was mixed with 2.5 ml methanol and 15.5 ml 0.1 M HCl before sonication. The volume was then made up to 25 ml with water. However, total ($BuSn_t$) recovery was significantly lower than 100% and partial degradation of TBT was noted. These difficulties were overcome with a prehumidification step as follows: 0.5 g of dry tissue was previously mixed with 5 ml of 1/1 methanol–water mixture before the addition of the 12.5 ml of 0.1 M HCl and sonication for 1 h. The results obtained are presented in Table 3 and appear satisfactory, TBT recovery being good and debutylation low.

Table 2 Fate and recovery of TBT with various methanolic HCl extraction procedures differing only in HCl concentrations applied to the same wet mussel sample

HCl (mol l^{-1})	TBT recovery (%)	$BuSn_t$ recovery[a] (%)
0.1	96	105
2.0	81	98
8.4	67	88

[a] Recovery is the ratio of measured concentration to calculated spiked concentration. $BuSn_t$ = TBT + DBT + MBT.

Table 3 Fate and recovery of TBT with the described extraction procedure applied to freeze-dried samples

Sample	TBT recovery (%)	$BuSn_t$ recovery (%)	Debutylation (%)
Oyster	95	102	9
Mussel	88	102	14
Salmon	94	104	10

Influence of the amount of $NaBH_4$ reagent

We demonstrated previously[8] that the analysis of sediment extracts needed some care as regard hydride generation, a large amount of sodium borohydride being necessary to obtain a maximum recovery of butyltin hydrides.

The analytical procedure used in this study was therefore optimized for each kind of tissue matrix by plotting the peak area of tributyltin hydride versus the amount of $NaBH_4$ added (in fact the time during which a constant 2.5 ml min^{-1} flow of a 10% $NaBH_4$ solution is added) (Fig. 1).

Optimal sensitivity, i.e. optimal production of tributyltin hydride in the reactor, necessitates a variable addition of $NaBH_4$ solution depending on the matrix, the more so as oyster and salmon extracts are analysed. From Fig. 1 reaction conditions were therefore chosen as 240 s at 2.5 ml min^{-1} of a 10% $NaBH_4$ solution in 1% aqueous NaOH.

Calibration curves, linear in the range 0–1000 ng (Sn) g^{-1}, were established by plotting peak areas obtained during the analysis of extracts of clean wet or freeze-dried tissue spiked

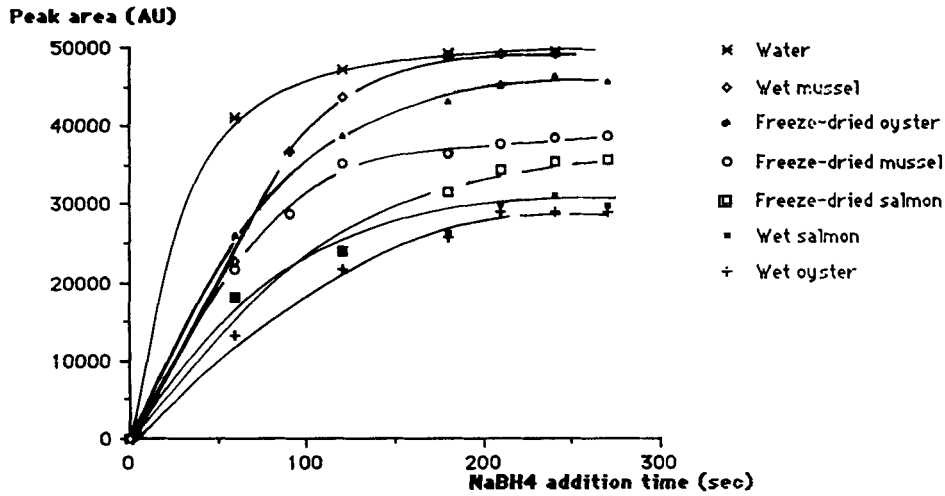

Figure 1 Effect of the amount of added $NaBH_4$ on a 5 ng TBT signal in various matrices.

Table 4 Sensitivity and detection limits of the determination of butyltin compounds in various matrices

Matrix	MBT			DBT			TBT		
	m^a	r^b	$C_L{}^c$	m^a	r^b	$C_L{}^c$	m^a	r^b	$C_L{}^c$
Water	14000	1.000	0.36	11500	0.999	0.44	11215	0.998	0.46
Wet mussel	13945	0.997	1.8	11340	0.999	2.2	8355	0.994	3
Wet oyster	11560	0.994	2.2	9470	0.992	2.7	4970	0.996	5
Wet salmon	13145	0.999	3.9	9270	0.998	5.5	5830	0.995	8.8
Freeze-dried mussel	13250	0.996	16	11510	0.999	1.5	6940	0.994	31
Freeze-dried oyster	12135	0.999	12.5	10655	0.994	14	7715	0.996	20
Freeze-dried salmon	11925	0.996	13	10035	0.998	16	6575	0.998	24

[a] Slope of the calibration curve, in area units · ng (Sn)$^{-1}$. [b] Correlation coefficient. [c] Method detection limit in ng (Sn) g^{-1} for solid samples or ng (Sn) l^{-1} for water.

just before analysis with standard solutions of butyltin chlorides and using previously determined $NaBH_4$ quantities. Quite wide variations of sensitivity and detection limits have been noted (Table 4) with different matrices studied and the butyltin species concerned.

Analysis of shellfish tissues

Mussel tissue

The analysis of wet mussel spiked samples has already been described[1] and a very good recovery of added TBT (96%) was obtained with a very slight degradation of TBT in DBT and MBT (Table 5). As it is more convenient to store and handle dry material, the analysis of freeze-dried samples was conducted. It is clear from Table 5 that a prehumidification step is necessary to promote a satisfactory recovery of organotins from these samples; when it is used, TBT recoveries (R_{TBT}) ranging from 87.5 to 95% are observed. TBT spikes added during the extraction procedure or TBT spikes added long ago, before several months of sample storage, have the same behaviour. It may also be noted in Table 5 that total butyltin recovery (R_t) is often higher than 100%; this fact must draw attention to precision problems that not only are linked to the analytical determination but also rely heavily on the precision of the determination of the water content (dryness) of these water-rich samples: a 1% error in humidity results in a *ca* 8% error on analyte content (dry mass).

Oyster tissue

Wet oyster tissue samples may be analysed directly with a very good recovery of TBT (95%) and only a very slight degradation to DBT and MBT (Table 6). Analysis of freeze-dried samples also necessitates a prehumidification step; then the recovery of TBT is close to 100%. This very good efficiency of the extraction procedure holds as well as for fresh spikes, made just before the extraction step, as for very old spikes realized months previously during the preparation of large amounts of samples.

Fish tissues

Salmon

As for shellfish tissues, the analysis of wet sam-

Table 5 Recovery of TBT from mussel tissue spiked with TBT

Sample	Prehumidification step	Concentration [ng (Sn) g^{-1}]				R_{TBT} (%)[b]	R_t (%)[c]
		MBT	DBT	TBT	$BuSn_t{}^a$		
Wet mussel + spike (250 ng g^{-1})		10	13	240	263	96	105
Dry mussel + spike (1250 ng g^{-1})	No	24	38.5	873	936	70	75
Dry mussel + spike (1250 ng g^{-1})	Yes	77	105	1090	1270	87.5	102
Dry mussel + spike (1250 ng g^{-1}) + addition (500 ng g^{-1})	Yes	137	133	1550	1820	88.5	104
Dry mussel + addition (500 ng g^{-1})	Yes	38	50	475	563	95	112.5

[a] $BuSn_t$ = TBT + DBT + MBT. [b] Percentage recovery of the TBT spike. [c] Total recovery of butyltin compounds. R_t = 100 $BuSn_t$/TBT spike.

Table 6 Recovery of TBT from oyster tissue spiked with TBT

Sample	Prehumidification step	Concentration [ng (Sn) g^{-1}] MBT	DBT	TBT	$BuSn_t$[a]	R_{TBT} (%)[b]	R_t (%)[c]
Wet oyster + spike (200 ng g^{-1})		6.8	6.5	189	202	95	101
Dry oyster + spike (882 ng g^{-1})	No	23.1	40.6	584	648	66	73
	Yes	23.4	48.8	827	899	94	102
Dry oyster + spike (882 ng g^{-1}) + addition (500 ng g^{-1})	Yes	32.5	79.8	1290	1405	93	102
Dry oyster + addition (500 ng g^{-1})	Yes	26.5	57.5	463	547	93	109.5

[a, b, c] As in Table 5.

ples is quite satisfactory, wiht a TBT recovery of 93% and little degradation in DBT or MBT (Table 7). Analysis of freeze-dried samples is just as efficient if a prehumidification procedure is used (R_{TBT} 94–96%). Once more, the recovery of total butyltin often exceeds 100%; the likely reason is again the precision of the determination of the humidity content.

Sea bass–NIES No. 11 CRM "Fish tissue"

Two different samples of the Certified Reference Material, NIES No. 11, were obtained directly from NIES (Japan) and submitted to analysis using a 1 g subsample (Table 8). It must be noted that tributyltin [certified value 1.3 ± 0.1 μg g^{-1} as TBTCl, i.e. 474 ± 36 ng (Sn) g^{-1}] and triphenyltin [reference value 6.3 μg g^{-1} as TPTCl, i.e. 1940 ng (Sn) g^{-1}] should be the only tin-containing species in this CRM as total tin content is certified at 2400 ± 100 ng g^{-1}. It may therefore be supposed that degradation products such as DBT, MBT, diphenyltin (DPT) or monophenyltin (MPT) were not present in this material at the time of certification.

Direct analysis of the dry material was not successful as only 52% TBT was detected and BuSn was only 60% of the certified TBT value. Prehumidification, even for a long time, slightly increased TBT recovery to 63–67% and $BuSn_t$ reached only 75–79% of the certified TBT value. This sample was then spiked with 400 ng (Sn) g^{-1} TBT and mixed for 2 or 4 h before analysis: the TBT spike was recovered with 96–101% yields. The presence of scales and pieces of bone in the material may have contributed to the scatter of these results.

A second sample of NIES No. 11 CRM was then obtained and analysed. TBT values determined wih or without prehumidification were 75 or 72% of the certified value; it must be noted that $BuSn_t$ values were 99 or 91% respectively of the certified TBT value, because both MBT and DBT were measured in significant amounts.

Several hypotheses may explain these results. Sample 1 of NIES No. 11 contained scattered large particulates of scales and bones which may have significantly reduced the actual amount of fish tissue present in the 1 g sample. It is therefore difficult to propose elaborate explanations.

Sample 2 did not contain so many large particulates; however, measured TBT concentrations were 25–28% lower than the certified value, the difference being very close to the sum of measured DBT and MBT. As TBT spikes in this matrix are quantitatively recovered without giving rise to DBT or MBT increases, it must be

Table 7 Recovery of TBT from salmon tissue spiked with TBT

Sample	Prehumidification step	Concentration [ng (Sn) g^{-1}] MBT	DBT	TBT	$BuSn_t$[a]	R_{TBT} (%)[b]	R_t (%)[c]
Wet salmon + spike (400 ng g^{-1})		28.3	18.6	371	418	93	104
Dry salmon + spike (667 ng g^{-1})	No	28	26.7	465	520	70	78
Dry salmon + spike (667 ng g^{-1})	Yes	25.8	45.8	627	699	94	105
Dry salmon + spike (667 ng g^{-1}) + addition (500 ng g^{-1})	Yes	58	90.0	1110	1260	96	108

[a, b, c] As in Table 5.

Table 8 Analysis of NIES fish tissue No. 11 Certified Reference Material

NIES No. 11 fish tissue	h[a]	Concentration [ng (Sn) g^{-1}] (dry wt) MBT	DBT	TBT	$BuSn_t$[b]	R_{TBT} (%)[c]	R_t (%)[d]
Sample 1	No	20	22.4	245	287	52	60
	+	30.1	29.8	297	357	63	75
	++	26.9	27.2	318 A	373	67	79
Sample 1 spiked with TBT (400 ng g^{-1}) (2 h mixing)		24.3	33.2	698 B (380)[e]	755	(95)	—
Sample 1 spiked with TBT (400 ng g^{-1}) (4 h mixing)		26.1	31	724 C (406)[e]	781	(101)	—
Sample 2	No	41.9	50.6	339	432	72	91
	+	57.6	53.6	357	468	75	99

Certified values: tributyltin: 474 ± 36; total tin: 2400 ± 100 ng (Sn) g^{-1}
Reference value: triphenyltin: 1900 ng (Sn) g^{-1}
[a] h, Prehumidification step (+, 4 hurs; ++, overnight). [b] $BuSn_t$ = TBT + DBT + MBT. [c] Ratio between TBT measured and the certified value (%). [d] Ratio between $BuSn_t$ measured and the certified TBT value (%). [e] Values in parentheses were obtained by subtracting TBT measured in line A from TBT measured in lines B or C.

supposed that DBT and MBT were present in the analysed material. As they were not present at the time of certification, DBT and MBT must have been produced by TBT degradation during transportation or storage of these sames.

CONCLUSION

A detailed investigation of the whole analytical procedure for determination of tributyltin in seafood by the hydride generation procedure has been made, using carefully spiked samples. Extraction of TBT from wet oyster, mussel or salmon tissues by 0.1 M HCl–methanol allows nearly quantitative recoveries with very limited degradation to dibutyltin and monobutyltin. This procedure is also convenient for the analysis of freeze-dried samples, provided that they are submitted to a rehumidification step prior to extraction. Biological matrices induce a serious increase of sodium borohydride consumption to produce butyltin hydrides, and have an influence on sensitivity. Quality control of the method by analysis of a Certified Reference Material was not completely satisfactory and the stability of this material may be questioned.

REFERENCES

1. F. Pannier, A. Astruc and M. Astruc, *Anal. Chim. Acta* **287** 17 (1994).
2. C. Stewart and J. De Mora, *J. Environ. Technol.* **11**, 565 (1990).
3. C. Zuolian and A. Jensen, *Marine Poll. Bull.* **20**, 281 (1989).
4. R. B. Laughling, in *Oceans Proceedings 1986*, Organotin Symposium vol. 4, p. 1206 (1986).
5. T. L. Wade, B. Garcia-Romero and J. M. Brooks, *Environ. Sci. Technol.* **22**, 1488 (1988).
6. C. D. Rice, F. A. Espourteille and R. J. Huggett, *Appl. Organomet. Chem.* **1**, 541 (1987).
7. V. Desauziers, F. Leguille, R. Lavigne, M. Astruc and R. Pinel, *Applied Organomet. Chem.* **3**, 469 (1989).
8. P. M. Sarradin, Thesis, University of Pau (1993).