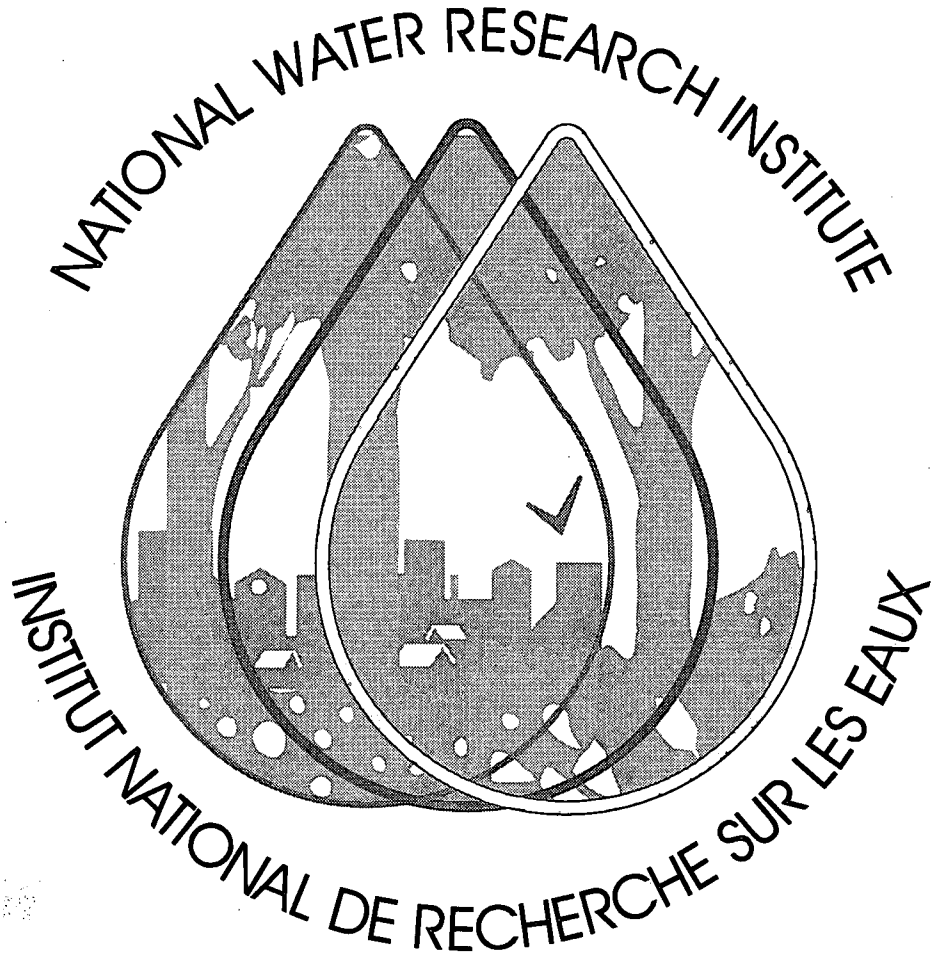

EVALUATION OF DERIVATIZATION TECHNIQUES FOR THE ANALYSIS OF ORGANOTIN COMPOUNDS IN BIOLOGICAL TISSUE

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NWRI CONTRIBUTION NO. 96-159

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MANAGEMENT PERSPECTIVE

The two commonly used derivatization techniques, namely, (1) direct *in situ* derivatization with sodium tetraethylborate and (2) tropolone extraction followed by ethyl Grignard derivatization for the determination organotin compounds in mussels were compared and evaluated with respect to their recoveries from spiked samples and a Reference fish tissue. These methods are used in our study of concentrations of butyltin compounds in mussels in Canadian harbours and rivers.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Les deux méthodes de dérivation couramment utilisées pour le dosage des composés organiques de l'étain dans les moules, soit 1) la dérivation directe *in situ* à l'aide de tétraéthylborate de sodium, et 2) l'extraction de tropolone suivie d'une dérivation par réaction éthylique de Grignard, ont été comparées et évaluées par rapport aux taux de récupération de ces composés à partir d'échantillons dopés et d'un tissu de poisson de référence. Ces méthodes sont employées dans notre étude sur les concentrations des composés de type butylétain dans les moules provenant de ports et de rivières du Canada.

ABSTRACT

The two commonly used derivatization techniques for the determination of organotin compounds in biological tissue, namely, (1) direct *in situ* derivatization with sodium tetraethylborate, and (2) tropolone extraction followed by derivatization with ethyl Grignard reagent, were compared and evaluated. It was found that Method 1 recovered well the three butyltin species and the triphenyltin species from spiked mussel and from a certified reference fish tissue, but recoveries became highly variable when larger mussels were used as indicated by the recovery of the internal standard.

Method 2 did not recover the triphenyltin species because of its decomposition in the acidic medium required for the extraction, but it gave more consistent results than Method 1 with larger mussels, e.g *Elliptio*. Method 2 is also simpler in operation. When only the butyltin compounds are of interest in the study, Method 2 is preferable.

RÉSUMÉ

Les deux méthodes de dérivation couramment utilisées pour le dosage des composés organiques de l'étain dans les moules, soit 1) la dérivation directe *in situ* à l'aide de tétraéthylborate de sodium, et 2) l'extraction de tropolone suivie d'une dérivation par réaction éthylique de Grignard, ont été comparées et évaluées. On a constaté que la méthode 1 permettait de récupérer efficacement les trois espèces de butylétain et le triphénylétain à partir de moules dopées et d'un tissu de poisson de référence certifié, mais que le taux de récupération devenait très variable avec les moules de grande taille, comme en fait foi le taux de récupération de l'étalon interne.

La méthode 2 n'a pas permis de récupérer le triphénylétain, en raison de sa décomposition dans le milieu acide nécessaire pour l'extraction, mais les résultats étaient meilleurs qu'avec la méthode 1 et les moules de grande taille, p. ex. *Elliptio*. En outre, la méthode 2 est plus simple à appliquer. Lorsqu'on s'intéresse uniquement aux butylétains, la méthode 2 est préférable.

INTRODUCTION

Extraction of organometallic compounds from biological tissues for speciation analysis has long been a challenging problem for analytical chemists. Although complete dissolution of biological tissue without rupturing the chemical structure of the analytes can now be achieved by using enzyme hydrolysis with mixed enzymes, lipase and protease (Forsyth and Marshall 1983), or by a tissue solubilizer, tetramethylammonium hydroxide (TMAH) (Chau et al. 1984), problems still exist in the quantitative extraction of analytes from the digested samples due to interferences of fats, lipid and organic matter present in these samples. Recently, a study has been published on the evaluation of three digestion methods, namely, acid digestion, TMAH digestion and enzyme hydrolysis followed by the direct, *in situ* derivatization technique with sodium tetraethyl borate ($NaBEt_4$) for the determination of butyltin and phenyltin spiked in fish tissue (Ceulemans et al. 1994). These authors concluded that both the TMAH and enzyme digestion methods gave similar recoveries for all the butyltin species and the triphenyltin species (except for the mono- and di-phenyltin), but the acid digestion method caused decomposition of the triphenyltin.

The present study, however, evaluates the two most used derivatization techniques, namely, the tropolone extraction followed by derivatization with ethylmagnesium bromide, and the direct *in situ* derivatization with $NaBEt_4$, for the determination of several organotin species in biological samples after sample dissolution with TMAH. A Reference fish tissue (NIES No. 11) was used to evaluate the recovery and accuracy of the methods. Analysis of real mussel samples are given to illustrate their applications. It will serve as a complementary study to the earlier investigation (Ceulemans et al. 1994).

EXPERIMENTAL

Apparatus

The GC-AED system consists of a gas chromatograph (HP 5890, Series II, Hewlett-Packard, PA) equipped with a split/splitless injection port, a HP microwave plasma atomic emission detector (Model 5921A), and a HP automatic sampler (Model 7673A). The system was controlled by a computer using the HP35920A ChemStation software.

Operation of the GC-AED system

The ethyl-derivatized organotin compounds contained in hexane (1μl) were injected to the GC-AED system. The integrated peak areas were compared to that of tripentyltin (TPeT) as an internal standard. Operation parameters are listed in Table 1.

Reagents

The carrier gas for chromatography and make-up gas for the plasma was high purity helium, 99.999%. The spectrometer purge gas, nitrogen, was of ultra-high purity. The reagent gases for the AED operation were oxygen (99.999%), and hydrogen (99.999%). All gases were supplied by Canox (Canada). The organotin compounds and special reagents were obtained from Alfa Chemicals (Danvers, MA) or from Aldrich (Milwaukee, WI). Reference fish tissue (NIES No. 11) was obtained from National Institute for Environment Studies, Ibaraki, Japan. Other solvents, acids and common laboratory reagents were of analytical grade. Distilled water purified by a Milli-Q system (Millipore, USA) was used throughout the experiments. Individual stock solutions of organotin compounds (1000 μg ml^{-1} as Sn) were prepared by dissolving the equivalent amounts of organotin in methanol or in toluene.

PROCEDURES

Method 1 - direct derivatization with $NaBEt_4$

The derivatization procedure followed that given in a previous study (Kuballa et al 1995) with modifications. Standard solutions of phenyltins (MPT, DPT, TPT), butyltins (MBT, DBT, TBT) and TPeT as an internal standard (0.5 ml of 1μg Sn ml^{-1}) were spiked to 0.2 g of freeze-dried mussel sample in a 50 ml Erlenmeyer flask followed by addition of 5 ml 25% TMAH. The mixture was digested at 60°C for 60 min. After addition of 25 ml of sodium acetate-acetic acid buffer solution (1M $NaOOCCH_3$ adjusted to pH 4 $\pm$ 0.1 by CH_3COOH), 2 ml toluene and 0.6 ml 2% $NaBEt_4$, the mixture was magnetically stirred for 10 min. A further addition of 0.6 ml 2% $NaBEt_4$ was made and stirred for 20 min. Then 1 ml of the toluene was removed and cleaned up in a micro column containing approx. 1 g of silica gel packed in a Pasteur pipette (10 cm x 5 mm i.d.) and eluted with 5 ml of hexane. After reduction of the eluate volume to 1 ml by nitrogen, 1 μl of the eluate was injected to the GC-AED system for analysis.

Method 2 - tropolone extraction and ethyl magnesium bromide derivatization

After additions of 10 ml water, 5 ml acetic acid, 6 g NaCl and 4 ml 0.2% tropolone/toluene solution to the digested mussel sample solution containing the spikes as described above, the mixture was magnetically stirred for 60 min. Then 2 ml of toluene was removed and dried by nitrogen. The volume was brought back to 1 ml with hexane and allowed to react with 0.5 ml of ethylmagnesium bromide for 10 min. After destruction of the excess ethylmagnesium bromide by adding 2 ml of 1 N H_2SO_4, the organic layer was quantitatively removed and cleaned by silica gel in a micro column, and analyzed as described above. The recoveries of the phenyltin and butyltin species are listed in Table 2.

ANALYSIS OF FISH REFERENCE TISSUE

Both Methods 1 and 2 were applied to the analysis of a Reference fish tissue (NIES No. 11) following the procedures described above. Recovery results are given in Table 3.

RESULTS AND DISCUSSION

The TMAH method was used for sample digestion because of its effectiveness in breaking up tissue in a relatively shorter period of time (1-2 hr), without altering the chemical form of the analytes.

Contrasting the two methods in the analysis of butyltin in mussel tissue, it was observed that Method 1, the direct *in situ* derivatization method with $NaBEt_4$, has a reasonable recovery for the three butyltin species and the triphenyltin species from spiked mussel and from a Reference fish tissue, but failed to recover the DPT species satisfactorily from the spiked mussel (Tables 2 & 3). Table 4 summarizes the analysis of real mussel samples by both methods with the concentration of each organotin species calculated with reference to the internal standard without correction of recovery. With real mussel samples, less problems were experienced in the recovery of butyltin compounds with smaller mussels, e.g. zebra mussels. With larger-sized mussels, e.g. *Elliptio,* recoveries were inconsistent, as indicated by the recovery of the internal standard TPeT with values ranging from 22 to 59%. Because of the poor and highly variable recovery of Method 1 as indicated by the internal standard, low levels of analytes may be totally missed (eg. MBT, DBT at Sorel, St.Lawrence R.). The variation is likely due to interferences caused by the

large amounts of protein and fibre in larger mussels. It was observed that large amounts of fluffy, fibrous material were produced in the solution with larger-size mussels after the direct ethylation reaction with $NaBEt_4$. Such phenmenon was not observed with Method 2 where the tropolone/toluene extraction isolated the analytes in the organic solvent from the sample solution for ethylation by the Grignard reagent. It must be borne in mind that recovering analytes from spiked samples is not exactly the same as from real samples in which the analytes are bound in a more complex structure. If problems already exist in the recovery of a spiked internal standard, the recovery of native organotin compounds in the biological sample cannot be expected to be problem-free.

Method 2, which employs the conventional ethyl Grignard derivatization after sample digestion, also gave reasonable recovery for the butyltin species in spiked tissues (Table 2), in Reference tissue (Table 3) and consistent results for real mussel samples (Table 4). However, a major drawback of Method 2 was that it did not recover the triphenyltin species. It was possibly due to the acidic condition (pH 2) required for the tropolone extraction caused decomposition of the triphenyltin species. The decomposition of triphenyltin to diphenyl- and monophenyltin species in acidic medium has been reported by other workers (Ceulemans et al. 1994). Such decomposition was evidenced by the large amount of the degradation product, monophenyltin, being recovered by this method 2 (Table 2).

From our experience with mussel samples, both Methods 1 and 2 have their advantages and disadvantages. They both recover satisfactorily the butyltin species from spiked mussel samples. With larger-sized mussels, Method 2 gave more consistent results and higher recovery than Method 1, and also avoided the interferences caused by the fibrous material in the mussel. From an operation point of view, Method 2 is simpler and straightforward which does not need laborious pH adjustments and daily preparation of the fresh $NaBEt_4$ reagent. From the economical point of view, the reagent ethylmagnesium bromide is far less expensive than $NaBEt_4$. Method 2 is adopted for routine use in our laboratory.

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TABLE 1. GC-AED OPERATION PARAMETERS

GC Parameters	
Injection port	splitless
Injection port temp.	250°C
Injection volume	1 μl
Column	SPB-1, 30-m length x 0.53-mm,
Column Head Pressure	Helium, 100 KPa (14.5 psi)
Temperature program	60°C(2min)x20°C/min to 250°C (3.5min)
AED parameters	
Transfer line	SPB-1
Transfer line temp.	270°C
Cavity temperature	270°C
Solvent vent off time	1.2 min
Spectrometer purge gas	N_2 at 2 l min^{-1}
Helium carrier and makeup gas	240 ml min^{-1}
Sn wavelength	271 nm
H_2 pressure	414 KPa (60 psi)
O_2 pressure	138 KPa (20 psi)

TABLE 2. RECOVERIES OF PHENYLTIN AND BUTYLTIN SPECIES FROM SPIKED MUSSEL TISSUE.

	MBT	DBT	TBT	MPT	DPT	TPT	TPeT
Method 1	81±8	102±3	103±5	89±15	27±3	91±9	100±5
Method 2	92±2	88±5	85±4	198±8	109±7	-	93±7

MBT - monobutyltin; DBT - dibutyltin; TBT - tributyltin; MPT - monophenyltin; DPT - diphenyltin; TPT - triphenyltin; TPeT - tripentyltin Internal Standard.
Spikes: 0.5 μg as Sn of each species; recovery expressed as % with relative standard deviation (%); n = 3; - not detectable

TABLE 3. ANALYSIS OF REFERENCE FISH TISSUE (NIES NO. 11)

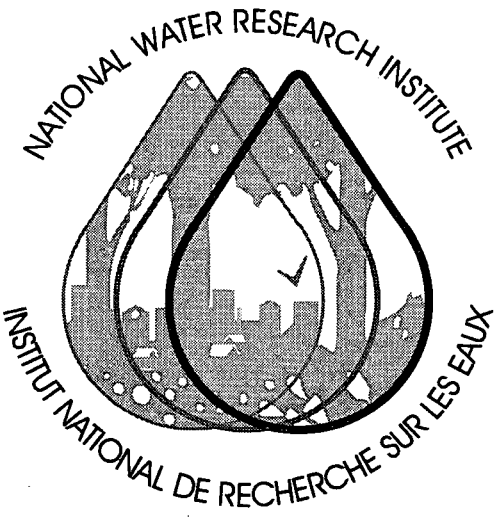
	TBT	TPT
Certified Value (μg/g as chloride)	1.3±0.1	6.3
Method 1	1.16± 0.07	5.06±0.30
Method 2	1.04±0.02	-

Results in μg/g as organotin chloride; n = 3; - not detectable

TABLE 4. DETERMINATION OF BUTYLTIN COMPOUNDS IN MUSSELS BY METHOD 1 AND 2.

Sampale Location	Method	MBT	DBT	TBT	Recovery of TPeT
Kingston Harbour dry dock	1	819±81	1322±170	9408±938	82.6±0.9
(zebra, 2.5-3 cm)	2	1221±107	1330±70	8799±303	95.3±1.0
Detroit R. at Lakeview Marina,	1	201±16	198±10	1750±43	86.9±11.5
Windsor (zebra, 2.5-3 cm)	2	326±84	239±23	1890±38	80.6±4.7
St.Clair R. at Bridgeview Marina,	1	53±9	58±5	502±22	89.9±3.9
Sarnia (zebra, 2-2.5 cm)	2	118±11	78±5	587±36	86:0±4.9
St.Lawrence R. at Sorel	1	-	-	88±22	22.3±16.6
(*Elliptio*, 9-10 cm)	2	41±4	47±4	117±15	90.7±9.3
Penetang Harbour	1	126±9	96±6	191±9	58.9±5.6
(*Elliptio*, 10-11 cm)	2	186±39	100±9	213±10	88.4±9.5
Midland Bay at Wye Heritage Marina	1	161±28	136±23	102±8	48.7±21.7
(*Elliptio*, 7-9 cm)	2	298±25	171±8	137±6	97.2±1.8

All locations are in Ontario; Internal Standard added, 100 ng TPeT as Sn;
The dried mussel was digested in TMAH, and split for analysis by the two methods;
Concentration of butyltin compounds was calculated with reference to the Internal Standard;
n = 3; - not detected



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