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Analytical Determination of Stannanes in Different Biological Samples

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Abstract: *Three species of stannanes were determined in different biological samples obtained from the sea water of the Arabian Gulf. Following digestion with acetonitrile, and extraction with n-hexane, the analytes were derivatized and subsequently analyzed in gas chromatography mass spectrometric system. Five-point calibrations using the concentration range of 0.05-10 µg/g were linear with coefficients of determination between 0.9892 and 0.9992. LODs of 7.7-14.1 µg/g with their corresponding LOQ were determined at S/N of 3 and 10 respectively with precision of ≤14.3%. All the three species of the phenylated tin determined were quantified in all the samples with the exceptions of Trivially fish, Crab, Indian Mackerel, Stripped red mullet and Emperor fish. Only monophenyltin was detected in Trivially fish and Crab. Triphenyltin tin was absent in Indian mackerel, and diphenyl tin was not detected in both Stripped red mullet and Emperor fish. The range of 0.097-5.870 µg/g wet weight was obtained for the individual species of stannanes in the samples, an indication for the contamination of the biota samples. Results of calculation for the ratios of triphenyltin to its two main degradation products may suggest long residence of triphenyltin in the environment due to its slow rate of degradation.*

Keywords:*Biological samples, derivatization, GC-MS, liquid-liquid extraction, stannanes.*

I. Introduction

Stannanes are various organometallic compounds of tin and hydrocarbons. Following the discovery of diethyltin, in 1849 by Edward Frankland, stannanes have been put to various uses: as wood preservatives, heat stabilizers, catalysts and glass coatings. Probably, the most popular usage is on ship hulls as biocidal paints. Different forms of organotin are frequently encountered in marine environments, and a strong correlation has been reported between organic matter in sediments and water, and butyltins [1]. High concentrations of tributyltin have been found in the marine bivalve, *Mytilus edulis* [2].

In vitro studies of the effects of dibutyltin (DBT), tributyltin (TBT) and triphenyltin (TPT) on the enzyme, 5α-reductase provides an evidence of antiandrogenic activity of organotins, and this can hamper normal masculinity in humans. The IC_{50} ranges between 2.7 and 11.2µM [3]. In mollusks, the effect can manifest in the condition known as imposex, where male characteristics may appear in females. Research has found that location with high concentration of TBT correlated with high frequency of imposex in the Arctic marine whelk, *Buccinum finmarkianum* [4]. These effects and other toxicological potentials of organotin have led the international maritime organization to put a global ban on the use of organotin as active biocide in antifouling paints. In a one-year study conducted between March 2007 and March 2008 on the surface seawaters from Dona Paula Bay, India, investigators have found significant variations in the seasonal levels of butyltins (BT). Increasing concentrations were observed from March to May which correlated with increased shipping and tourism activities in these months of the year [5]. Since geographically closely related areas of the Skagerrak and German Bight, for instance, were found to contain different contamination levels of TBT, a pointer that factors other than shipping density might also be working, environmental factors that could contribute to sipping of organotin into marine environments should also be confronted in order to tackle this issue more effectively [6].

Before any sensitive determination of organotin is made, a variety of sample preparation procedures may often be employed. These include sieving, digestion and extraction. Sludge samples are first dried at 50^{o}C for 3 days, homogenized and sieved using 0.2 mm mesh and the butyl, phenyl and octyltin contents can be extracted quantitatively with glacial acetic acid [7]. Percent recoveries between 88 and 101% were obtained when microwave-assisted extraction of organotin compounds with acetic acid-hexane mixture (20:80) was performed for flour samples [8]. A solid-phase microextraction can also be employed as applied by Pawslizyn and Millan [9] for the determination of butyl species. Here, a 100µm thick poly (dimethylsiloxane), PDMS, was used in a headspace approach to achieve the extraction of the volatile and non-polar tetraethyl- and tetrabutyltin compounds in water and sediment; equilibrium conditions were achieved for tetraethyltin at 40^{o}C, 1200rpm for 30 min and at pH 4.3. For sorptive extraction, a 1-cm stir bar coated with 55µL PDMS was used to pre-concentrate organotin compounds from mussels, after digestion with tetramethylammonium hydroxide [10]. A

method of liquid-phase microextraction, LPME, was later explained for the extraction of tributyltin and triphenyltin in seawater using 2µL drop of trifluorotoluene as the extractant solvent [11]. Interference from sulphur in sulphur-rich sediments can be minimized by pressurized liquid extraction (PLE) [12], while degradation of trialkyl- and triaryltin during sonication is minimized using toluene-acetic acid in 10:4 mixtures [13].

Following these sample prep steps, different methods have been employed for the separation and quantitative determination of species of organotin in various matrices. Using gas chromatography atomic emission spectrometry, GC-AES, a fast and accurate method was developed for the determination of butyltins in several sea foods. The limit of detection was reported as 3-6ng/g [14]. As previous studies have failed to obtain baseline resolution between dibutyltin (DBT) and triphenyltin (TPT), Ace C-18 stationary phase with decreased particle size was used to achieve resolution in mussel and oyster matrices. The concentration of the analytes could be determined down to 40pg/g with HPLC-ICP-MS set up [15].

For the determination of eight organotin compounds in water and sediments, gas chromatography with pulsed flame photometric detector, GC-PFPD, was used. In this method, tripropyltin and diheptyltin were applied as internal standards for volatile and semi volatile compounds respectively [16]. Based on commercially available spike solution containing mixture of mono-, di- and tributyltin, MBT, DBT and TBT, enriched with ^{119}Sn, isotope dilution method was used in conjunction with gas chromatography electron impact ionization mass spectrometry, GC-EII-MS, for the identification of MBT, DBT and TBT in water. This method limit of detection was calculated as 0.18-0.25ng/L [17]. Good resolution was obtained with methanol: water: acetic acid (80:19:1) mixture as mobile phase for ion-pair reversed phase chromatography with hydride generation quartz furnace atomic absorption spectrometry detection, IP-RPC/HG-QFASS. Ion pairs for the organotin compounds were generated by reaction with decane solfonate [18]. High butyltin concentrations, 0.05-5.48mg Sn/Kg, in Gipuzkoa sediments of North Spain, were found with gas chromatography flame ionization detector (GC-FID) determination, reflecting pollution related to the area's historical industrial as well as fishing activities [19]. In the same vein, GC-FID analysis of butyltins and phenyltins in sediments, plankton and mussels at Port of Osaka, Japan, has revealed higher concentrations of tributyltin (TBT) than triphenyltin (TPT) in all matrices. The levels of TBT were also high in marinas and mooring areas of small and medium-hull vessels [20]. During determination of TBT and TPT in sea brass, *Dicentrachus labrax*, with gas chromatography tandem mass spectrometry, GC-MS-MS, under controlled laboratory conditions, D. labrax accumulated the analytes from first week, with higher concentrations present in liver than in muscles [21]. Organotin determination using GC-MS under retention time locked conditions has availed easy peak location based on mass spectra and retention time of target analytes-concentrations ranging between 15µg/kg and 43mg/kg were recorded at port of Antwerp, Belgium and near ship repair station respectively, in water and sediments samples [22].

In many instances, derivatizations of the analytes have been performed in order to improve recoveries and detectability. Ethylation using sodium tetraethyl borate, STEB, is commonly employed in the gas chromatographic determination of organotin compounds [23, 24]. When liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry, LC-APCI-MS, was harnessed for the separation and quantitation of TBT and its hydroxylated intermediate in seawater, tropolone was used as complexing agent and recoveries of 72-96% were obtained [25].

In this study, we aimed to determine the concentrations of triphenyltin, diphenyltin and monophenyltin in 10 different biota matrixes from local environment of the Eastern Province of Saudi Arabia using liquid-liquid extraction and GC-MS analysis of derivatized analytes.

II. Materials And Methods

2.1 Chemicals and Reagents

Analyte standards of high purity were used in this study; monophenyltin (MPhT) and diphenyltin (DPhT) were supplied in their chloride forms by Sigma-Aldrich (St. Louis, MO), while triphenyltin (TPhT)was supplied as triphenyltinchloride by Fluka (Buchs, Swizerland). Stock solution of the mixture of these standards was prepared in acetone at a concentration of 1000 mg/ml from which necessary dilutions were made as needed. N-hexane was supplied by J.T. Baker Chemical Co, USA. Acetonitrile was supplied by Romil (Cambridge, UK). Sodium sulfate anhydrous was supplied by Riedel-de-Haen, AG, Switzerland, and dichloromethane (DCM) by Sigma-Aldrich (St. Louis, MO). Nanopure water purification system (Barnstead, Dubuque, IA, USA) was used for preparing the ultrapure water for analysis.

2.2 Sample Collection

Ten different biota samples comprising of Trivially fish (TF), Barracuda fish (BF), Stripped red mullet (SM), Emperor fish (EF), Solea (SL), Indian Mackerel (IM), oyster (OT), crab (CR), squid (SQ) and shrimp (SP)were purchased from local fishermen within the Eastern Province of Saudi Arabia.

2.3 Extraction and Derivatization of Stannanes

All the glassware for use in this study for the extraction and derivatization of the three phenylated tins were first washed with hot detergent water and then rinsed with ultrapure water. They were then immersed in a pool of 12 M hydrochloric acid and left for 24 hrs. Methanol and ultrapure water were then used in succession to rinse these wares followed by oven drying at 50^{o}C. Liquid-liquid extraction was then used for extracting the biota samples after digestion. Before digestion, 5 g wet of each sample was weighted and minced. The minced sample was digested with 20 ml of acetonitrile for 15 min and decanted. Digestion was repeated with 20 ml of the solvent and also decanted. The fractions of the digestate were pooled and n-hexane (30 ml) was used for the liquid-liquid extraction. The hexane layer was removed and the liquid-liquid extraction repeated for one more time. The extracts were transferred to 100 ml beaker and the residual water was removed with 20 g of anhydrous sodium sulphate. 60 ml of the hexane extract was evaporated to 2ml using a rotary evaporator (Buchi Rotavapor R-200 equipped with heating bath B-490). This 2 ml of the organic extracts was concentrated to 1 ml under a gentle stream of nitrogen, and this was derivatized with 500 µl of 2M n-propylmagnesium bromide for 20 min. The derivatized extract was then filtered using a syringe filter and analyzed in GC-MS.

2.4 Determination of Stannanes

GC-MS 6890N system (Agilent) equipped with autosampler 7683B series and a 6890B injector was employed in the separation and detection of the three species of organotin. The system was operated through a Chemstation with installed wiley7n.l and NIST 98.L libraries. Agilent 19091Z-213 column of 30m x 320 µm (i.d) x 1µm film thickness of HP-1 methyl siloxane stationary phase was used. High purity helium gas that was flowing at a rate of 2.0 ml min^{-1} was used as the carrier gas. Injection volume was 2 µL in splitless mode. Injection port temperature, MS detector temperature and interface temperature were set at 250^{o}C each. Column temperature was initially set at 40^{o}C and held for 5 min. This was ramped to 300^{o}C at the rate of 12^{o}C/min. It was held at this final temperature for 4 min. Total ion current (TIC) in SCAN mode for ions of masses 50-550 was used for acquisition, and selected ion monitoring (SIM) mode was employed for quantitation using m/z of 283, 361 and 351 for MPhT, DPhT and TPhT respectively.

III. Results And Discussion

Table 1 shows the calibration parameters for the concentration range of 0.05-10 µg g^{-1}, and analytical figures of merit. Linearity for the determination in biota samples was demonstrated by the coefficients of determination between 0.9892 and 0.9992. Limits of detection (LOD) (7.7-14.1 ng/g) and their corresponding limits of quantitation (LOQ) were estimated at signal-to-noise ratio of 3 and 10 respectively. Precision (repeatability) was determined as percent relative standard deviation (%RSD, n=3) and found to be ≤14.3%.The average percent recovery of 81-90%, obtained by spiking the respective samples at 0.1 µg g^{-1} concentration, showed that the method did not suffer considerably from matrix effect in the biota matrixes.

Table 2 shows the concentrations of the three different species of stannanes in the biota samples under study. All species of stannanes were detected in the samples with the exceptions of TF, CR, IM, SM and EF. In TF and CR, however, only MPhT was detected and quantified. TPhT was not detected in IM, and DPhT was absent in both SM and EF. In Fig. 1, the mean organotin detected in the different samples ranged between 0.403 and 2.341 µg/g wet weights; the highest total stannanes, 7.112 µg/g wet weight, was recorded in OT while the lowest value, 0.875 µg/g wet weight, was obtained in TF samples due, partly, to the absence of TPhT and DPhT in this sample. For the individual species, however, OT recorded the highest value of 5.870µg/g wet weight (MPhT) while SM had the lowest value of 0.097µg/g wet weight (TPhT). Previously, de Mora et al. [26] have found 7.6 ngSn g^{-1} dry weight of DPhT and 8.3 ngSn g^{-1} dry weight of TPhT in grouper from Khawr, Qatar [26]. In addition to boat traffic, some agricultural and industrial activities can also contribute appreciable amounts of OTs into the marine environment [27].The biota samples used in this study were caught inthe portions of the sea within the Eastern Province of Saudi Arabia which is frequented by small to large size vessels and is home to many commercial as well as industrial/agricultural activities.

In Fig. 2, ratios of TPhT to its degradation products of DPhT and MPhT were calculated. The generally >1 values for TPhT/DPhT may suggest either long residence time where degradation occurs at slow rate or an evidence of new inputs of TPhT from anthropogenic sources. The low values of TPhT/MPhT appear to support the former. Some bacterial and cyanobacterial mats can facilitate biodegradation of organic pollutants [28, 29].

IV. Figures And Tables

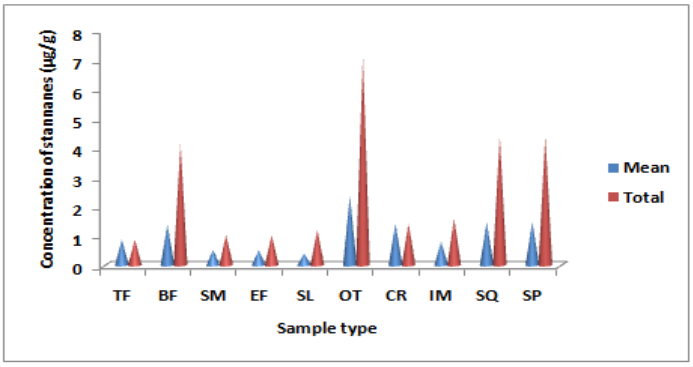

Fig. 1: Mean and total concentrations of TPhT, DPhT and MPhT in ten different biota samples: TF-trivially fish, BF-barracuda fish, SM-stripped red mullet, EF-emperor fish, SL-solea, OT-oyster, CR-crab, IM-Indian mackerel, SQ-squid, and SP-shrimp.

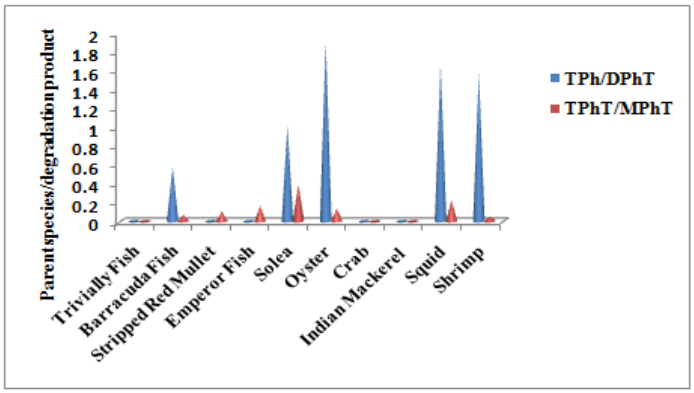

Fig. 2: Ratio of TPhT to its two main degradation products in each biota sample

Table 1: Calibration parameters and analytical figures of merit

Analytes	Slope (x 10^{-3}) Mean±SD	Intercept (x 10^{-5}) Mean±SD	R^2*	LOD (ng g^{-1})	LOQ (ng g^{-1})	% R	% RSD (n=3)
MPhT	0.19±0.01	0.09±0.01	0.9892	7.7	23.1	88	9.3
DPhT	0.53±0.6	1.02±0.17	0.9992	9.0	27.0	90	11.0
TPhT	1.6±0.5	0.36±0.03	0.9986	14.1	42.3	81	14.3

*For concentration range of 0.05-10 µg/g
%R: Average percent recovery for 0.1 µg/g spiked biota samples

Table 2: Concentration of phenylated tin species in ten different biota samples

Biota Species	Concentration (µg/g), Mean±SD		
	TPhT	DPhT	MPhT
Trivially Fish	ND	ND	0.875±0.081
Barracuda Fish	0.243±0.035	0.419±0.046	3.472±0.323
Stripped Red Mullet	0.097±0.014	ND	0.928±0.086
Emperor Fish	0.150±0.021	ND	0.887±0.082
Solea	0.267±0.038	0.261±0.029	0.680±0.063
Oyster	0.815±0.117	0.427±0.047	5.870±0.546
Crab	ND	ND	1.420±0.132
Indian Mackerel	ND	0.297±0.028	1.307±0.122
Squid	0.735±0.105	0.439±0.048	3.234±0.300
Shrimp	0.221±0.032	0.140±0.015	4.054±0.377

ND: Not detected

V. Conclusion

Three species of stannanes were determined in different biological samples. Digestion, extraction and derivatization were employed to prepare the polar analytes for GC-MS analysis. All the three species of the phenylated tin determined were quantified in all the samples with the exceptions of TF, CR, IM, SM and EF. Only MPhT was detected in TF and CR. TPhT was visibly absent in IM, and DPhT was not detected in both SM and EF. The range of0.097-5.870µg/g wet weight was obtained for the individual species of organotin in the samples, an indication of the contamination of the biota samples. The ratios of TPhT to its two main degradation products may suggest long residence of the TPhT in the environment due to slow rate of degradation.

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