IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 5 Ver. I (Sep - Oct. 2015), PP 10-15
www.iosrjournals.org

Determination of butylated tin species in biota samples by derivatization and GC-MS analysis

Abdulmumin A. Nuhu*[1],Mohsen A. Al-shatri[2]
[1]*Department of Chemistry, Ahmadu Bello University, PMB 1069, Zaria, Kaduna 2222, Nigeria*
[2]*Department of Chemistry,King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia*

Abstract: *Tributyltin and its degradation products were determined in ten species of biota collected from the Eastern Province of Saudi Arabia. Following digestion of the samples with acetonitrile, liquid-liquid extraction was performed and the extract was analyzed on GC-MS system after derivatization of the polar analytes. The method has good linearity in the concentration range of 0.05-10 µg/g wet weight. Limits of detection at signal-to-noise ratio of three (S/N = 3) for this analysis ranged between 9.3 and 12.9 ng/g wet weight. Precision (%RSD) for three determinations was 10-13%. Results indicated that both TBT and DBT were found in all species while MBT was only present in Indian Mackerel, Solea and Barracuda fish. In each biota sample, the total of the three organotin species averaged 8.21 µg/g wet weight with trivially fish having the highest value of 11.73 µg/g wet weight, and Emperor fish recording the lowest value of 4.085µg/g wet weight. The highest individual species quantified was DBT at 8.986±1.090µg/g wet weightwhich was detected in the Barracuda fish; this shows that the principal species in the biota samples was DBT. These results indicate long residence of TBT in the biota samples and slow degradation of DBT to MBT as supported by the low ratios of TBT to DBT and high ratios of TBT to MBT. Contamination of the various biota samples with the organotin species was evident in this study.*

Keywords:*Biota samples, derivatization, digestion, GC-MS, liquid-liquid extractionorganotin species.*

I. Introduction

Organotins are organometallic compounds that can be formed from the butylation of tin to produce tributyltin (TBT). Its main degradation products include dibutyltin (DBT) and monobutyltin (MBT). These are found to contaminate different environmental and biological matrices including biota.Analysis of butyl tins has revealed the presence of TBT in plankton and mussels at Port of Osaka, Japan [1]. Ruedel*et al*. [2] determined the concentration levels of organotin compounds including TBT and its degradation products in marine biota samples that have been collected between 1985 and 1999 from North and Baltic Sea areas and stored at the German Environmental Specimen Bank. Through the years, the concentrations of TBT remained relatively constant at 17 - 3 ng/g for mussels from a site close to marine traffic and 8 - 2 ng/g for remote areas. This indicated that the banning of TBT in antifouling paints in 1991 for small boats within the European Community seems not to have resulted in a decrease of TBT levels in marine biota.

In a study by Harino*et al*. [3], organotin compounds were detected in mussels *Mytilusedulis* from two major estuaries of the UK, the Mersey and the Thames, approximately one decade after legislation banning the use of TBT on small boats. The study showed that TBT concentration can be correlated to shipping activity in the Manchester Ship Canal (MSC). Results also showedthat concentration of BTs in mussels can be correlated to the total extractable tin in sediment, though in contrast to sediments, 85% of the total tin in mussels was made up of BTs, the most predominant of which was TBT.In addition, during determination of TBT in sea brass, *Dicentrachuslabrax*, under controlled laboratory conditions, *D. labrax* accumulated the analyte from first week, with higher concentrations present in liver than in muscles [4].

Ikeda *et al.* [5], who studied bioaccumulation of organotin compounds through the food web in the deep water of Japan Sea, found that TBT was present in all samples but at a lower concentration compared to coastal areas. The range of TBT determined was 1.8-240 ng/g-dry weight.

A survey of endocrine disrupting chemicals in fish and shellfish conducted by Chatani*et al*. [6]in Japan found TBT in the range of 10 to 30 ppb in 3 of 24 samples analyzed. Michel and Averty [7] found that the organotin contamination of the French coast is still a problem fifteen years after regulatory measures were introduced to limit their use to protect the oyster-farming industry.Several studies have shown the effects of TBT compounds, such as shell malformations of oysters, imposex in marine snails, and reduced resistance to infection.For example, it has been demonstrated that imposex (the growth of male reproductive organs in females) can be initiated in some gastropod mollusks by TBT in the low ng/L range [8, 9]. TBT at these concentrations is also known to cause shell deformity and larval mortality [10].

The effects of TBT on marine organisms in Arcachon Bay led to the legislation for an eventual worldwide ban. In Arcachon Bay imposex was first observed in the predatory gastropod

Ocenebraerinacea(oyster drill) in 1970, which was attributed to TBT in early 1980s and led to its near extinction [11]. Eventually oyster stocks too were adversely affected through the late 1970s and into the 1980s, which decreased from 10,000 – 15,000 tons in the mid-seventies to 3000 tons in 1981, resulting in massive financial losses to the shellfish industry. In addition to reproductive failure, shell deformation leading, in severe cases, to 'ball-shaped' specimens in adult oysters made them worthless [12].

A widespread imposex was observed in female dogwhelks (*Nucella lapillus*) in coastal waters off southern United Kingdom with the highest incidence near ports and harbors [8, 13, and 14]. Subsequently imposex has been observed in over 140 species worldwide.Despite the severity of the phenomenon of imposex, the connection to shipping was not established until analytical capabilities improved towards late 70s to early 80s.

Different methods have been employed for the separation and quantitative determination of species of these compounds 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[15]. 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 this resolution in mussel and oyster matrices. The concentration of the analytes could be determined down to 40pg/g with HPLC-ICP-MS set up [16].

For the determination of eight organotin compounds in water and sediments, gas chromatography with pulsed flame photometric detector, GC-PFPD, was used [17]. In this method, tripropyltin and diheptyltin were applied as internal standards for volatile and semi volatile compounds respectively. 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 [18]. A 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 decanesulfonate[19].

In many instances, derivatization of the analytes has been performed in order to improve recoveries and detectability. Grignard reagents are commonly used for derivatization of the organotin compounds. Ethylation using sodium tetraethyl borate, STEB, is a type of derivatization employed in the gas chromatographic determination of organotin compounds [15, 20]. However, the reagent is highly flammable and toxic and not applicable for routine environmental applications. 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 [21].

While photochemical degradation of trialkyltin compounds in the water column converts them to less toxic di- and monoalkyltin compounds within days, once they are adsorbed in sediments or bioaccumulated in marine organisms they can be active for months if not years. Owing to the adverse effects of organotin compounds on marine life, revealed by the cases cited above and their potential presence in the global marine environment due to shipping, much attention has been focused on the determination of organotin compounds in the environment.

The aim of this study was to determine the concentration of three species of butylated tins in 10 different biota samples sourced from local environment using GC-MS analysis following derivatization of the polar analytes and liquid-liquid extraction.

II. Materials And Methods

Chemicals and Reagents

Analytical grade standards of monobutyltin (MBT) as monobutyltintrichloride anddibutyltin (DBT) as dibutyltin dichloridewere supplied by Sigma-Aldrich (St. Louis, MO), while standard of TBTas tributyltin chloride was purchased from Fluka (Buchs, Swizerland). 1000 mg/ml solutions were prepared in acetone and stored as stock from which necessary dilutions were made as needed. Sodium sulfate anhydrous was supplied by Riedel-de-Haen, AG, Switzerland, and dichloromethane (DCM) by Sigma-Aldrich (St. Louis, MO). N-hexane was purchased from J.T. Baker Chemical Co, USA. Ultra pure water was prepared using Nanopure water purification system (Barnstead, Dubuque, IA, USA).

Sampling

All biota samples were caught in the southern coast of the Arabian Gulf that is situated within the Eastern Province of Saudi Arabia. Ten different species of biota were used in this study: Trivially fish (TF), Barracuda fish (BF), Stripped red mullet (SM), Emperor fish (EF), Solea (SL) and Indian Mackerel (IM). Others were oyster (OT), crab (CR), squid (SQ) and shrimp (SP).

Extraction and derivatization of organotins

All glassware used for the extraction and derivatization were first washed with hot detergent water and rinsed with ultrapure water. These were then immersed in a pool of 12 M hydrochloric acid and left for about 24 hrs. They were then removed, rinsed with methanol and ultrapure water. They were subsequently dried in oven at 50^{o}C. Biota samples were extracted using liquid-liquid extraction method. Briefly, 5 g wet of each sample was weighted and minced. The minced sample was digested with 20 ml of acetonitrile (Romil, Cambridge, UK)for 15 min and decanted. Digestion was repeated with 20 ml of acetonitrileand also decanted. Organotins were then extracted with 30 ml of n-hexane and washed with 30 ml of deionized water by liquid-liquid extraction technique. The hexane layer was collected 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 anhydrous sodium sulphate (20 g). 60 ml of the hexane extract was evaporated to 2ml using a rotary evaporator (BuchiRotavapor R-200 equipped with heating bath B-490). 2 ml of organic extracts was concentrated to 1 ml under a gentle stream of nitrogen, and this wasderivatized 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.

Determination of organotins

Butylated species of organotin namely, MBT, DB and TBT were separated and detected using GC-MS 6890N system (Agilent) equipped with autosampler 7683B series and a 6890B injector. It was operated through a Chemstation with incorporated wiley7n.l and NIST 98.L libraries. Separation was carried out with the aid of an Agilent 19091Z-213 column of 30m x 320 µm (i.d) x 1µm film thickness of HP-1 methyl siloxane stationary phase. High purity helium flowing at a rate of 2.0 ml min^{-1} was the carrier gas for 2 µL injected sample volume. Injection port temperature, MS detector temperature and interface temperature were set at 250^{o}C each. The column temperature was initially set at 40^{o}C which was held for 5 min, and then 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 forquantitation using m/z of 246.8 (MBT), 277 (DBT), and 291.1 (TBT).

III. Results And Discussion

The concentration range of 0.05-10 µg g^{-1} was used to construct five-point calibration curves for each of the biota matrix and these werefound to be linear with coefficient of determinations ranging between R^2= 0.9891 and 0.9960) (Table 1). Limit of detection (LOD) was found to be 9.3-12.9 ng/g as calculated from signal-noise ratio of three (S/N = 3) and the corresponding limit of quantitation (LOQ) was estimated from S/N = 10. Repeatability of analysis was investigated using percent relative standard deviation (%RSD) of triplicate determinations and was found to be between 10 and 13%.In the biota matrix, recovery estimates for 0.1 µg g^{-1} spiked samples were between 81 and 94% which indicates that the method used did not suffer significantly from matrix effect. Table 2 shows the concentrations of different species of OTs in all the biota samples under study.The highest individual species quantified was DBT at 8.986±1.090µg/g wet weightwhich was detected in the Barracuda fish while the lowest was 0.113±0.010 µg/g wet weight MBT detected in the Indian Mackerel. While TBT and DBT were detected and quantified in all the ten biota species, MBT was only found in three samples, namely Solea, Indian Mackerel and Barracuda fish. The total of the three organotin species (Figure 1)averaged 8.21 µg/g wet weight with trivially fish having the highest value of 11.73 µg/g wet weight and Emperor fish recording the lowest value of 4.085µg/g wet weight.Previously, concentration of butyl tin in Mediterranean Sea ranged between 16 and 230 ng/g wet weight [22]. Apart from boat traffic, some agricultural and industrial activities are known to contribute appreciable amounts of OTs into the maritime systems [23]. The portion of the sea within the Eastern Province where the biota samples were caught is frequented by small to large size vessels and is home to many commercial as well as industrial/agricultural activities.

High ratios of TBT to its degradation productsmay suggest either long residence time where degradation occurs at slow pace or an evidence of new inputs from anthropogenic sources. There are bacterial and cyanobacterial communities that can facilitate biodegradation of organic pollutants [24, 25]. As Table 3 shows, apart from trivially fish that hada TBT/DBT ratio of 2.153, all other biota species had very low ratios (0.102-0.662), suggesting, in part, the long residency that allowed for biodegradation to take place. On the other hand, high ratios of TBT/MBT may indicate the slow rate of biodegradation of DBT to MBT or of new input of TBT from external sources.

IV. Conclusions

TBT and its degradation products DBT and MBT were determined in ten species of biota that were collected from the Eastern Province of Saudi Arabia. After digesting the samples with acetonitrile, liquid-liquid extraction was performed and the extract was analyzed on GC-MS system after derivatization of the polar analytes. Calibration parameters showed that the method was linear between the concentration range of 0.05 to

10 µg/g wet weight. Limits of detection at signal-to-noise ratio of three (S/N = 3) for this analysis ranged between 9.3 and 12.9 ng/g wet weight, and precision for three determinations was demonstrated to be 10-13%. Results indicated that both TBT and DBT were found in all species while MBT was only present in Indian Mackerel, Solea and Barracuda fish.

The total of the three organotin species averaged 8.21 µg/g wet weight with trivially fish having the highest value of 11.73 µg/g wet weight, and Emperor fish recording the lowest value of 4.085µg/g wet weight. The highest individual species quantified was DBT at 8.986±1.090µg/g wet weightwhich was detected in the Barracuda fish; this shows that the principal species in the biota samples was DBT. These results indicate long residence of TBT in the biota samples and slow degradation of DBT to MBT as supported by the low ratios of TBT to DBT and high ratios of TBT to MBT. There was, therefore, strong indication for the contamination of the biota samples, generally, with the various species of organotin.

Acknowledgment

Mr. Mohsen thanks the King Fahd University of Petroleum and Minerals for the award of scholarship for his Masters studies. We appreciate the moral support from Dr. Chanbasha Basheer.

References

[1]. Harino H, Fukushima M, Yamamoto Y, Kawai S, Miyazaki N (1998) Organotin compounds in water, sediment, and biological samples from the port of Osaka, Japan. Arch. Environ. Contam. Toxicol. 35:558-564.
[2]. Ruedel HP, Lepper P, Steinhanses J, Schroeter-Kermani C (2003) Retrospective Monitoring of Organotin Compounds in Marine Biota from 1985 to 1999: Results from the German Environmental Specimen Bank. Environ. Sci. Technol. 7:1731-1738.
[3]. Harino H, O'Hara SCM, Burt GR, et al. (2003) Organotin compounds in Mersey and Thames Estuaries a decade after UK TBT legislation, Journal of the Marine Biological Association of the UK 83:11-22.
[4]. Hajjaj el Hassani L, Frenich AG, Vidal JLM, Muros MJS, Benajiba MH (2005) Study of the accumulation of tributyltin and triphenyltin compounds and their main metabolites in the sea bass, *Dicentrachuslabrax*, under laboratory condition. Sci. Total Environ 348: 191-198.
[5]. Ikeda KT, Minami M, Yamada H, Koyama J, Kagaku K (2002) Bioaccumulation of organotin compounds through the food web developed in the deep water of the Japan Sea. J. Environ. Chem. 12:105-114.
[6]. Chatani Y, Ogawa T, Kitano R, Ohfuji M, Takemae M (2001) Survey of endocrine disrupting chemicals in fish and shellfish: PCB, organochlorine pesticides, bisphenol A, and organotin compounds. Kyoto-fu Hoken KankyoKenkyushoNenpo 46:14.
[7]. Michel P, Averty B (1999) Contamination of French coastal waters by organotin compounds: 1997 update. Mar Poll Bull. 38:268-275.
[8]. Bryan GW, Gibbs PE, Hummerstone LG, Burt GR (1986) The decline of the gastropod Nucella lapillus around south-west England: evidence for the effect of tributyltin from antifouling paints. Journal of the Marine Biological Association, UK 66:611-640.
[9]. Alzieu C (1998) Tributyltin: Case study of a chronic contaminant in the coastal environment. Ocean and Coast Manage 40:23-36.
[10]. Alzieu C, Sanjuan J, Deltriel JP, Borel M (1986) Tin contamination in Arcachon Bay: Effects on oyster shell anomalies. Mar. Poll. Bull. 17:494-498.
[11]. Gibbs PE (1993) A male genital defect in the dog-whelk, *Nucella lapillus* (Neogastropoda), favouring survival in a TBT-polluted area. Journal of the Marine Biological Association, *UK* 73:667-678.
[12]. Alzieu C, Sanjuan J, Michel P, Borel M, Dreno JP (1989) Monitoring and assessment of butyltins in Atlantic coastal waters. Mar. Poll. Bull. 20:22-26
[13]. Bryan GW, Gibbs PE, Hummerstone LG, Burt GR (1987) Copper, zinc and organotin as long-term factors governing the distribution of organisms in the Fal Estuary in southwest England. Estuaries 10:208-219.
[14]. Gibbs PE, Pascoe PL, Burt GR (1988) Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. Journal of the Marine Biological Association, UK, 68:715-731.
[15]. Zabaljauregui M, Delgado A, Usobiaga A, Zuloaga O, de Diego A, Madariaga JM (2007) Fast method for routine simultaneous analysis of methylmercury and butyltins in seafood. J. Chromatogr. A 1148:78-85.
[16]. Wahlen R, Catterick T (2003) Comparison of different liquid chromatography conditions for the separation and analysis of organotin compounds in mussel and oyster tissue by liquid chromatography-inductively coupled plasma mass spectrometry. J. Chromatogr. B 783:221-229.
[17]. Bravo M, Lespes G, De Gregori I, Pinochet H, Gautier MP (2005) Determination of organotin compounds by headspace solid-phase microextraction–gas chromatography–pulsed flame-photometric detection (HS-SPME–GC–PFPD). Anal. Bioanal. Chem. 383:1082-1089.
[18]. Centineo G, Rodriguez-Gonzalez P, Gonzalez EB, et al. (2006) Isotope dilution GC-MS routine method for the determination of butyltin compounds in water. Anal. Bioanal. Chem. 384:908-914.
[19]. Panggabean AS, Amran MB, Achmad S (2009) Speciation of Organotin Compounds with Ion Pair-Reversed Phase Chromatography Technique. Eurasian J. Anal. Chem. 4:215-225.
[20]. Wasik A, Radke B, Bolalek J, Namiesnik J (2007) Optimisation of pressurized liquid extraction for elimination of sulphur interferences during determination of organotin compounds in sulphur-rich sediments by gas chromatography with flame photometric detection. Chemosphere 68:1-9.
[21]. Zuliani T, Lespes G, Milacic R, Scancar J (2010) Development of the extraction method for the simultaneous determination of butyl-, phenyl- and octyltin compounds in sewage sludge. Talanta 80:1945-1951.
[22]. Kannan K, Falandysz J (1997) Butyltin compounds in sediment, fish, fish-eating birds, harbor porpoise and human tissues from the polish coast of the Baltic Sea. Mar Poll Bull 34:203-207.
[23]. Bancon-Montigny C, Lespes G, Potin-Gautier M (2004) Organotin survey in the Adour–Garonne basin. Water Res38:933-946.
[24]. Pain A, Cooney JJ (1998) Characterization of Organotin-Resistant Bacteria from Boston Harbor Sediments. Arch. Environ. Contam. Toxicol. 35:412–416.
[25]. Kuritz T, Wolk CP (1995) Use of Filamentous Cyanobacteria for Biodegradation of Organic Pollutants. Appl Environ Microbiol. 61:234-238.

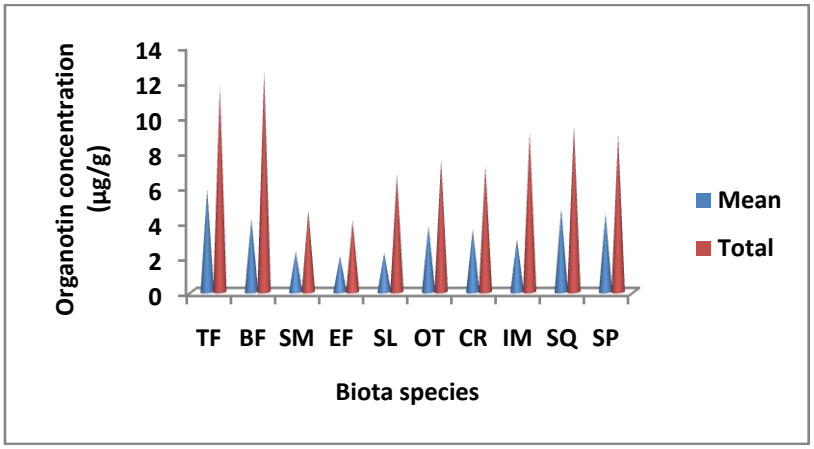

Fig 1: Mean and total concentrations of three organotin species (TBT, DBT and MBT) in biota samples: TF-trivially fish, BF-barracuda fish, SM-stripped red mullet, EF-emperor fish, SL-solea, OT-oyster, CR-crab, IM-Indian mackerel, SQ-squid, and SP-shrimp.

Table 1: Calibration parameters, analytical limits, recovery and repeatability in biota sample matrix

Analytes	Slope±SD (x 10^{-3})	Intercept±SD (x 10^{-5})	R^{2*}	LOD (ng g^{-1})	LOQ (ng g^{-1})	% R	%RSD (n=3)
MBT	1.81±0.32	3.35±1.17	0.9960	9.3	31	81	10
DBT	0.35±0.02	9.97±1.37	0.9931	12.9	43	94	12.1
TBT	0.10±0.01	12.1±1.6	0.9891	9.3	31	85	13.0

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

Table 2: Organotin concentrations in ten species of biota

Biota Species	Organotin Concentration (µg/g), Mean±SD		
	TBT	DBT	MBT
Trivially Fish	**8.008±1.041**	**3.720±0.452**	**ND**
Barracuda Fish	**3.252±0.422**	**8.986±1.090**	**0.327±0.033**
Stripped Red Mullet	**0.828±0.110**	**3.823±0.463**	**ND**
Emperor Fish	**0.543±0.071**	**3.542±0.430**	**ND**
Solea	**0.679±0.088**	**5.944±0.718**	**0.120±0.013**
Oyster	**1.493±0.090**	**6.038±0.731**	**ND**
Crab	**0.665±0.087**	**6.528±0.790**	**ND**
Indian Mackerel	**1.046±0.136**	**7.927±0.959**	**0.113±0.010**
Squid	**2.230±0.303**	**7.260±0.879**	**ND**
Shrimp	**0.889±0.116**	**8.121±0.990**	**ND**

ND: Not detected

Table 3: Ratios of TBT to its degradation products in biota samples

Biota species	TBT/DBT	TBT/MBT
Trivially Fish	2.153	-
Barracuda Fish	0.662	9.945
Stripped Red Mullet	0.217	-
Emperor Fish	0.153	-
Solea	0.114	5.658
Oyster	0.247	-
Crab	0.102	-
Indian Mackerel	0.131	9.257
Squid	0.307	-
Shrimp	0.109	-