DERIVATIZATION FOLLOWED BY GC/MS ANALYSIS OF ORGANOTINS AND HALOACETIC ACIDS IN ENVIRONMENTAL SAMPLES

BY

Mohsen. A. Al-Shatri

A Thesis Presented to the

DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

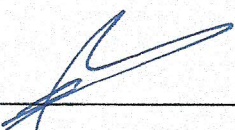# MASTER OF SCIENCE

In

CHEMISTRY

MAY, 2014

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN- 31261, SAUDI ARABIA

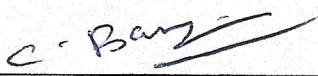**DEANSHIP OF GRADUATE STUDIES**

This thesis, written by **Mohsen Abdo Yahia Al-shatri** under the direction of his thesis

advisor and approved by his thesis committee, has been presented and accepted by the

Dean of Graduate Studies, in partial fulfillment of the requirements for the degree of

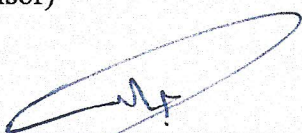**MASTER OF SCIENCE IN CHEMISTRY.**

Dr. Basher chanbasha
(Advisor)

Dr. Abdullah Jafar Al-Hamdan
Department Chairman

Dr. Dr. Khalid Al-hooshani
(Member)

Dr. Salam A. Zummo
Dean of Graduate Studies

Dr. Dr. Assad Al-Thukair
(Member)

26/6/14
Date

Dedication

Every challenging work need self effort as well as guidance of elders especial who were

very close to our heart. My humble effort I dedicate to

My sweet and loving Father , Mother and my wonderful wife

Whose affection, love, encouragement, prayers of day and night make me able to get

such success and honor

ACKNOWLEDGMENTS

Thanks and all praise be to Allah (azzawagal) who created me, blessed me with health and who grant me success.

I would like to state my deep appreciation and gratitude to my thesis advisor Dr. Basheer Chanbasha who has always been providing kindest support, continual encouragement and supervision.

I also would like to acknowledge my committee members: Dr. Assad Al-Thukair and Dr. Khalid Al-hooshani for their valuable advices and guidance.

I also acknowledge KFUPM and chemistry department as well as the all faculties who taught and helped me.

Finally, I acknowledge Kingdom of Saudi Arabia for providing me MS scholarship and for all different facilitations.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

OTs   :   Oraganotins

OTC   :   Organotin Compounds

TBT   :   TriButylTin

MBT   :   Monobutyltin

HAAs   :   Haloacetic Acids

DBPs   :   Disinfection By-Products

EPA   :   Environmental Protection Agency

USEPA   :   United States Environmental Protection Agency

IMO   :   International Maritime Organization

PVC   :   PolyVinyl Chloride

NOM   :   natural organic matter

MCL   :   Maximum contaminant levels

LLE   :   Liquid-Liquid Extraction

SFE   :   Supercritical fluid extraction

SPE   :   Solid-phase extraction

LLE   :   Liquid–liquid extraction

SPME   :   Solid-phase microextraction

SME   :   Solvent Microextraction

SDME   :   Single-Drop Microextraction

DLLME   :   Dispersive Liquid-Liqiud Microextraction

PFBBr	:	PentaFlouroBenzylBromide
THMs	:	TriHaloMethans
MTBE	:	Methyl Tertiary Butyl Ether
ETOH	:	Ethanol
MEOH	:	Mthanol
AC	:	Acetone
THF	:	TetraHydroFuran
DCM	:	DiChloroMethane
ECD	:	Electron Capture Detection
GC-MS	:	Gas Chromatography Mass Spectrometry
GC-AES	:	Gas Chromatography Atomic Amission Spectrometry
GC-ECD	:	Gas Chromatography Electron Capture Detector
HPLC-ICO-MS	:	High-performance liquid chromatography Inductively Coupled Plasma Mass Spectrometry
GC-PFP	:	Gas Chromatography with Pulsed Flame Photometric Detector
GC-EII-MS	:	Gas Chromatography Electron Impact Ionization Mass Spectrometry
IP-RPC/HG-QFASS	:	Ion-Pair Reversed Phase Chromatography with Hydride Generation Quartz Furnace Atomic Absorption Spectrometry Detection,
GC-FID	:	Gas Chromatography Flame Ionization Detector
SPM	:	Suspended Prticulate Matters .
LOD	:	Limit Of Detection
LOQ	:	Limit Of Quantitation
STD	:	Standard Deviation
RSD	:	Relative Standard Deviation

ABSTRACT

Full Name   : Mohsen Abdo Yahia Al-Shatri

Thesis Title   :Derivatization followed by GC/MS Analysis of Organotins and
                    Haloacetic Acids in Environmental Samples

Major Field   : Chemistry

Date of Degree   : May, 2014

A general feature of many extraction applications is that the final extract is organic which means that the preferred analytical method is GC or GC–MS. Thus, to convert polar or non-volatile analytes to their volatile forms, derivatization is necessary. So, derivatization followed by gas chromatography is more appropriate choice.

Haloacetic acids are one class of polar analytes present at trace levels in drinking water sample matrices. To determine these compounds, various derivatization approaches have been reported in the literature which includes etherification, acylation and silylation. Esterification is simpler when comparing to other two approaches and suitable for simultaneous extraction and derivatization of haloacetic acids. This study is to optimize the various experimental conditions affecting derivatization and extractions to achieve better analytical performance and more accurate results. In this work, *n*-Octanol has been used as extractant solvent and derivatized reagent with ethanol as dispersive solvent to determination of HAAs in drinking water sample by GC-MS.

To determine concentrations of six species of organotins in seawater, marine sediments and biota samples, a total of 17 locations around the Eastern Province of Saudi Arabia,

are identified as potential hot spots for organotins contamination. The seawater, sediment and biota samples were extracted using solid-liquid extraction and solid phase extraction method, and the analysis was performed using gas chromatography/mass spectrometry instrumentation method after Grignard reagent derivatization.

ملخص الرسالة

الاسم الكامل : محسن عبده يحيى الشاطري

عنوان الرسالة:عملية اشتقاق مركبات القصدير العضوية و هاليدات حمض الخليك في عينات من البيئة وتحليلها باستخدام الكرموتوجرافيا الغازية المتصل بمطياف الكتلة

التخصص:كيمياء

تاريخ الدرجة العلمية : مايو 2014

إن عمليات الاستخلاص في الغالب ينتج عنها مستخلص عضوي والذي يعني أن استخدام الكرموتوجرافيا الغازية المتصل بمطياف الكتلة كأفضل طريقة لعملية ألتحليل لذلك فإن عملية الاشتقاق ضرورية لتحويل هذه المركبات العضوية او الغير متطايرة الى الصورة المتطايرة لها. تعتبر هاليدات حمض الخليك من المركبات العضوية القطبية والتي يمكن ان تكون موجودة في المياه. ولتحديد هذه المركبات فأن هناك العديد من عمليات الاشتقاق والتي انجزت من قبل قد تم نشرها وتتضمن الأسترة والأسيلة والسليلة.وتعتبر الأسترة هي ابسطهم وأكثر ملائمة لعملية الاشتقاق والاستخلاص في آن واحد. والهدف الرئيسي من دراستنا هو تحسين عملية الاشتقاق والاستخلاص للحصول على عملية تحليل ذات كفاءة وأكثر دقة. وفي دراستنا هذه فقد استخدمنا الاوكتانول العادي كمذيب لعملية الاستخلاص والاشتقاق وقد خلط مع الايثانول كمذيب مبعثر.

أما فيما يخص تحديد تركيزات مركبات القصدير العضوية في عينات بيئية مختلفة من الماء والرواسب والكائنات الحية فأنه قد تم تحديد 17 موقع حول المنطقة الشرقية بالمملكة العربية السعودية كمناطق لدراسة التلوث بمركبات القصدير العضوية . هذه العينات تم استخلاصها بطريقة التحليل استخلاص صلب –سائل (SLE) وبطريقة استخلاص الطور الصلب (SPE) ومن ثم اشتقاقها بواسطة كاشف جريجنارد ثم تليها عملية التحليل باستخدام الكروماتوجرافيا الغازية المتصلة بجهاز مطياف الكتلة.

CHAPTER 1

INTRODUCTION

1.1　Background of the Study

1.1.1　Haloacetic Acid (HAA)

For the analysis of HAAs by GC, a prior derivatization step is necessary because of their low volatility and high polarity. In recent study, *n*-Octanol was used as extractant and derivatization reagent for the determination of six haloacetic acids (HAAs) in water samples. HAAs were derivatized simultaneously during the extraction in the solvent microdrop, and after extraction, inside a glass microvial A new microextraction technique termed as Dispersive Liquid–Liquid Microextraction (DLLME), was used to enhance the extraction of number of polar organic compounds DLLME is a very simple and rapid method for extraction and preconcentration of organic compounds from water samples. In this proposal work, DLLME is being used for simultaneous extraction and derivatization of HAAs.

Disinfected by chlorine or other chlorine-related reagents are common in water treatment process, though it has been found that HAAs are among Disinfection By-Products (DBPs) that are produced during chlorination of water containing natural organic matter and bromide, which were firstly found as a nonvolatile and high carcinogenic risk chemical in the middle of 1980s [3]. Monochloroacetic acid (MCAA), Dichloroacetic

acid (DCAA), and Trichloroacetic acid (TCAA) are known to be formed from dissolved humic matter during disinfection of water with chlorine. Bromochloroacetic acid (BCAA), Monobromoacetic acid (MBAA), and Dibromoacetic acid (DBAA) may be formed by the reaction of bromide ion with MCAA [4]. The US Environmental Protection Agency (EPA) has set a Maximum Contaminant Level (MCL) of 0.060 mg/L for the sum of concentrations of five haloacetic acids (MCAA, DCAA, TCAA, MBAA and DBAA) in water [5].

1.1.2   Organotins

Organotin compounds (OTs) are a large class of synthetic compounds with widely varying chemical properties [6]. The organotin compounds especially Tributyltin (TBT) is effective as an anti-fouling agent, and has therefore been added to many ship-paint formulations to keep ship hulls free from algae, barnacles and other fouling organisms. Fish and fishery products are considered as the main source of Organotin Compounds (OTC)[7]. Trace determination of organotin compounds in environmental samples is complicated by the fact that organotins with one to three substituents are polar, in volatile substances due to their ionic characterization. The preparation of volatile derivatives makes the ionic organotin compounds amenable to evaporative separation techniques like GC. Derivatization of OTs was performed using Grignard reagent, propyl magnesium chloride. Without derivatization, lead to poor peak separation and low accuracy.

The Kingdom of Saudi Arabia has an oil-based economy and it possesses one of the largest proven petroleum reservoirs in the world. It ranks as the largest exporter of petroleum products in the world. In addition to petroleum, Saudi Arabia is also known for its huge petrochemical and mining industries. For that, Saudi Arabia has been rated

among the economically most competitive country. Saudi Arabia imports many types of products from abroad and has an excellent import-export record with many countries. Therefore, the intensive trading activities necessitate good infrastructures to handle such activities through the borders of the country. Saudi Arabia has built many international highways, airports and seaports for the shipment of various goods across the borders of the country.

Unfortunately, most of the time, the intense industrial and trading activities are accompanied by negative impacts on environment. The marine pollution is a result of the intense export-import activities. In the last few decades, millions of trips were made by tankers and ships across the Arabian Gulf for export-import activities of the country. In order to protect these tankers and ships from algal growth, their bodies are coated by paints that include chemicals that are turned out to be hazardous for the marine life. Among these chemicals, Organotin compounds that were found to be hazardous and their occurrence in the Gulf waters have to be investigated. Organotin compounds are highly versatile groups of organometallic chemicals used for various purposes. Dibutyltin (DBT) compounds are mainly used as stabilizers in polyvinylchloride plastics. Tributyltin (TBT) compounds and Triphenyltin (TPT) compounds are used as biocides, fungicides, antifouling agents for ship bottoms and fishery. Organotin compounds comprise a group of organometallic moieties characterized by a Sn atom covalently bound to one or more organic substituents (e.g. methyl, ethyl, butyl, propyl and phenyl). Organotin compounds conform to the following general formula: $(n\text{-}C_4H_9) SnX_2$, chemically represented as by the formulas $RSnX_3$, R_2SnX_2, R_3SnX and R_4Sn in which R is any alkyl or aryl group and X is anionic species[41]. The nature of X influences the physicochemical properties,

notably the relative solubility in water and non-polar solvent and the vapor pressure. They are reported to be stable at a temperature up to 200ºC, thermal decomposition has no significance under environmental conditions, UV-radiation, strong acids and electrophilic agents readily cleave the Sn-C bonds.

Organotin compounds are highly toxic towards living organisms. Although deformities of marine organisms, especially near marinas, ports and harbors, were observed in the early seventies, they were not linked to TBT until eighties. Since then, many countries have banned the use of boats less than 25 m long. Larger sea-going vessels, however, continued using TBT containing anti-fouling paints and it is now considered a global pollutant. International Maritime Organization (IMO) has banned the application of these paints effective January 1st, 2003 with a complete ban on the presence of TBT containing paints on ships and boats effective January 1st, 2008.

In addition to the use of TPT and TBT as pesticides, DBT is used as a heat stabilizer in PVC pipes. Most of these compounds are extremely toxic with a threshold of 1 ng/L (parts per trillion). TBT is known to cause toxic effects for some marine organisms. For example, TBT concentrations, as low as 3–5 ng/L, are known to cause sterilization in some female marine organisms.

Although, imposex (the development of male reproductive structures in females) in marine organisms, was observed in the early 1970s [6, 7], It was not linked to TBT until early 1980s which made governments around the world to take action to curb the use of organotin-based anti-fouling paints. Two landmark cases drew the attention of the world to this growing environmental problem.

The first was in France, which introduced legislation prohibiting the application of Tributyltin (TBT) paints to small (< 25 meter) vessels in 1982, after the near collapse of the oyster-farming industry at Arcachon Bay. The second was in the United Kingdom where, upon establishing the link between TBT and widespread imposex in marine organisms in coastal areas [8, 9], the government introduced legislation in 1985 to restrict the application of TBT-containing paints to small boats.

By the late 1980s, TBT was recognized as a global pollutant and prohibitions addressing small vessels were introduced in the United States (1988), Canada, New Zealand & Australia (1989) and the European Union (1991). It was also shown that organotin compounds were present in deep-sea marine organisms [3, 9]. In a review article presented in 2001, Donard et al.[4] stated that organotin compounds affect all compartments of the ecosystem and should be considered as global pollutants like polychlorinated biphenyl, Hg and polychlorinated dibenzodioxins. They also stated that trisubstituted organotin compounds should be at the top of the priority list of pollutants considering their efficiency as endocrine disrupters, even at very low concentrations.

Recognizing the well-documented toxicity of organotin compounds to marine life and their widespread presence in the marine environment, the International Maritime Organization, the United Nations agency responsible for safety of shipping and cleaner oceans, has prohibited the application of organotin compounds which acts as biocides in anti-fouling systems on ships, by January 1st, 2003. It has also promulgated a complete prohibition of the presence of organotin compounds, which acts as biocides in anti-fouling systems on ships, by January 1st, 2008.

Considering the amount of worldwide research on organotin compounds in the environment, relatively little work has been carried out in the Gulf region. A literature survey found two published reports on the topic [5, 10]. Hasan and Juma [11] measured the TBT concentrations of marine water and sediment collected from four sites. They found that the TBT levels in marine water to be above the threshold value of 1 ng/L. Mora et al. [12] measured Butyltin (BT) and Phenyltin (PT) species in sediments and biota from coastal locations in Bahrain, the United Arab Emirates, Oman and Qatar. The level of TBT concentrations in sediment in Bahrain, Oman and Qatar were found to be above the level required for sediment to be considered as contaminated.

Many studies conducted worldwide indicate that organotin compounds are present in biota, marine water and sediments including those in deep-sea environments. Only a few studies, aimed at quantifying these global pollutants, have been conducted in the gulf region with none of them conducted near Saudi Arabian ports and coastline. In the proposed research project, it is intended to quantify the concentration of TBT and TPT and their degradation products (Dibutyltin (DBT), Monobutyltin (MBT), Diphenyltin (DPT) and Monophenyltin (MPT)) in biota, marine water and sediments collected from sampling stations close to the possible sources such as ports, sea lanes and sewage discharge points in Dammam area.

Due to the intense activities in these seaports, they were considered as the study area in which the levels of the organotins were identified and measured to assess whether these levels are high and if they pose any potential health hazards for humans and marine life.

1.2 Problem Statement

Development of sensitive and selective analytical methods is required for trace polar compounds required appropriate derivatization and preconcentration prior to instrumental analyses. After analytes derivatization, the polar analytes converted to volatile which is suitable for GC application. The most common derivatization reactions are alkylation, acylation, etherification and silylation. In this study, two classes of polar analytes (organotins and haloacetic acids) will be selected; conditions influencing extraction and derivatrization will be investigated to achieve higher extraction efficiency.

1.3 Objective of the Study

The major objectives of the proposed work are as follows:

1- Investigation of extraction and derivatization of organotins and haloacetic acids in environmental water samples.

2- Optimization of derivatization and extraction conditions to achieve better recoveries.

3- Application of developed method of extraction:

- Determination of organotins in the seawater, sediment and biota samples.

- Determination of haloacetic acids in drinking water samples.

1.4   Derivatization Process

The preparation of appropriate derivatives is required for the application of GC or GC–MS. Derivatization is a chemical process for modifying compounds in order to generate new products with better chromatographic properties [13]. Thus, derivatization is a very useful tool for detecting compounds in complex samples. It is widely used in many other fields such as, forensic, medical and environmental chemistry [14]. Derivatization is usually done by substitutions on the polar function where the most common reactions are alkylation, acylation and silylation [9, 13]. Alkylation reagents reduce the polarity of the compounds by substituting labile hydrogens for an aliphatic or aliphatic–aromatic (e.g., benzyl) group. This technique is often used to modify compounds containing acidic hydrogens, such as carboxylic acids and phenols, which are converted either into esters or ethers.

1.5   Dispersive Liquid-Liquid Microextraction (DLLME)

A new mode of microextraction technique was described as a Dispersive Liquid–Liquid Microextraction (DLLME) which developed in 2006 by Rezaee [2]. DLLME technique has become a popular environmentally benign sample-preparation technique because of its fast, inexpensive, and easy to operate with a high enrichment factor and low volume consumption of organic solvent. In DLLME technique, the appropriate mixture of extraction solvent and disperser solvent are quickly injected into the aqueous sample by a syringe. Therefore, cloudy solution is formed. In fact, it is consisted of fine particles of

extraction solvent which is dispersed entirely into aqueous phase[15]. In this case, the

contact area between the extraction solvent and the sample solution is extremely large.

After centrifuging, the fine particles of extraction solvent are sedimented in the bottom of

the test tube if the organic solvents are selected on the basis of higher density rather than

water.

CHAPTER 2

DETERMINATION OF HALOACETIC ACIDS IN DRINKING WATER SAMPLES

2.1   LITERATURE REVIEW

A main objective of drinking water treatment is to provide microbiologically safe drinking water. In most of the countries, chlorine is often the final disinfectant added to the treated water for microbiological protection before it is discharged into a drinking water distribution system. If the source waters contain Natural Organic Matter (NOM) and bromide, the treatment by chlorination leads to formation haloacetic acids as Disinfection By-Products (DBPs) when chlorine reacts with bromide and NOM in source waters. Haloacetic Acids (HAAs) are found in industrial wastes as by-products of water chlorination [14]. They are also found in other fields such as drugs, dyes and chemicals [15].They are highly soluble in water and toxic to humans, animals, plants and algae[16].

Toxicology studies have shown some HAAs (especially TCA and DCA) and other DBPs to be carcinogenic or causing adverse effects in laboratory animals [17, 18]. Because of this serious health risk, regulatory action has been taken to control the levels of these DBPs in finished drinking water. Maximum Contaminant Levels (MCL) for the sum of concentrations of five Haloacetic Acids (HAAs) have been set as follows: 60 μg/L by

US Environmental Protection Agency [19] and for individual HAAs: MCA, DCA and TCA: 20, 50 and 200 μg/L respectively, by World Health Organization (WHO) [20].

Determination of HAAs without derivatization is possible by using liquid chromatography [21, 22] especially ion chromatography [23] or capillary zone electrophoresis [24]. Most of the methods used to determine HAAs, involve Gas Chromatography (GC) with Electron Capture Detection (ECD) or coupled with Mass Spectrometry (GC/MS). Among these techniques, GC is the most widely used method due to its inherent advantages of high resolution, rapid separation, low cost and easy linkage with sensitive and selective detectors [25-27]. For the analysis of these compounds by GC, a prior derivatization step is necessary because of their low volatility and high polarity [28]. The sample preparation step in an analytical process using GC typically consists of an extraction followed by derivatization or simultaneously in one step. The extraction step can be achieved by Liquid–Liquid Extraction (LLE) [29]. Solid-Phase Extraction (SPE) and Supercritical Fluid Extraction (SFE) have been used for HAAs extraction [30]. Solid-Phase Microextraction (SPME) [31] and Solvent Microextraction (SME) [32] have been developed. Single-Drop Microextraction (SDME) and GC-MS were applied to the analysis of HAAs in water samples [33]. A new mode of microextraction technique was described as a dispersive Liquid–Liquid Microextraction (DLLME) which has been developed [2].

After an extraction step, the derivatization of HAAs to short-chain esters using different reagents, such as diazomethane [23], acid–alcohol[34, 35], dimethyl sulfate [26] or BF3–methanol [36], is performed. Pentafluorobenzyl Bromide (PFBBr) is widely used reagent for derivatization of different compounds. In 2003, Jia et al used (PFBBr) as a new and

11

suitable reagent for derivatization of nine HAAs[37-39]. HAAs were derivatized both simultaneously during the extraction in the solvent micro drop, by using *n*-Octanol as extractant and derivatization reagent [1].
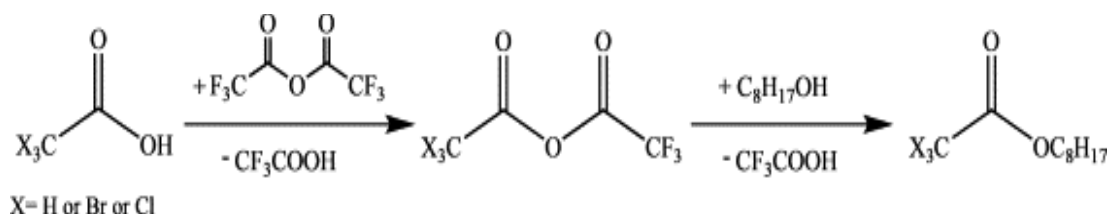
Proposed etherification mechanism (Journal of Chromatography A, 1216 (2009) 1059–1066)

However, the proposed method is very tedious and applicable for small volume of clean samples but not suitable for environmental samples which contains interfering matrices. Thus our objectives are set to develop more reliable method for the simultaneous extraction and derivatization of haloacetic acids.

HAAs are more difficult to determine than THMs, and the analytical chemistry has been recently re-viewed elsewhere (Urbansky 2000e). This is a result of the acidic nature of these contaminants which cause them not to be amenable to direct GC analysis like the THMs. To solve this problem, EPA Method 552.0 (Hodgeson et al. 1988) provides for the analysis of 5 HAAs using diazomethane to esterify the analytes after extraction into tert-butyl methyl ether. The methyl esters are then injected into a GC and detected by

electron capture. Advice for using this procedure was provided (Ulmer et al. 1988). Method 552.1 followed, replacing the diazomethane with acidified methanol. In Method 552.1, the analytes were extracted by running the tap water through a solid phase anion exchange resin. The current version of the method, Method 552.2 (Munch et al. 1995b)[19], eliminates the use of explosive diazomethane, which is the most carcinogenic substance known to man (on a base pair methylation basis). Method 552.2 was designed with the preferred steps from both 552 and 552.1. Method 552.2 combines an MTBE extraction with acidified methanol esterification (Pawlecki-Vonderheide et al. - 1997). Method 552.2 was verified for all 9 HAAs. Although EPA promulgated Method 552.2 to monitor HAA9 under the Information Collection Rule, many laboratories have continued using Method 552. More care is required with Method 552 because diazomethane used in Method 552 degrades the -brominated trihaloacetic acids, especially in white light (Rubio et al. 2000). Following the promulga tion of the Information Collection Rule, EPA attempted to discern how well labs were doing using EPA-approved methods for DBP quantification [40]. The performance of Method 552.2 is dependent on both the specific water used and the skill of the analyst, particularly for the brominated trihaloacetic acids. As an alternative, complexation electrospray mass spectrometry was recently used to determine HAA9 in drinking water. Since, it does not have the acidic methanol step, problems with the brominated trihaloacetic acids are reduced [40].

2.2   EXPERIMENTAL WORK

2.2.1   Reagents and Chemicals

The molecular structures and molar masses of the six species of haloacetic acids determined in this research work are given in **Figure 1**. Analytical grade standard mixture of six haloacetic acids containing Monochloroacetic Acid (MCAA), Dichloroacetic Acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), dibromoacetic acid (DBAA) and bromochloroacetic acid (BCAA) (2000µg/ml each in methyl tert-butyl ether (MTBE)). HAAs were extracted and derivatized using octanol. Dispersive solvent, ethanol (ETOH), methanol (MEOH), acetone (AC), acetonitryl (CAN) and tetrahydrofuran (THF) were mixed individual with octanol; all of these solvents in HPLC graded for analysis. Sulfuric acid (98%, HPLC-grade for analysis) And Sodium sulfate anhydrous were supplied by Riedel-de-Haen, AG, Switzerland.

2.2.2   Apparatus and Materials:

Gas chromatographic analysis of six HAAs, namely MCAA, DCAA, TCAA, MBAA, DBAA and BCAA were separated and detected using gas chromatography-mass spectrometry (Agilent, 6890 GC-MS) system. Chromatographic separation of the six HAAs was accomplished with a HP 5MS 5% Phynylmethyl siloxan (30 m x 0.25 mm x .25 mm nominal) from J&W Scientific. The column was initially maintained at 40°C for 1minute; subsequently, the temperature was increased to 180°C at a rate of 25°C/minute (11minutes hold) and then was increased to 250°C (30°C/minute, 2 minutes hold). The

GC–MS interface and the ion source temperatures were set at 200°C. Helium (99.999%) at a head pressure of 50 kPa was used as carrier gas with flow rate 2.0 ml/min. The split/splitless injector temperature was set at 250°C. The injector volume was 0.2 µL by using a 10 µL GC microsyringe. These chromatographic conditions are presented in Table 1. A 5mL sample solution (acidified with concentrated H_2SO_4, 10% (v/v)) glass vial (Supelco, Bellefonte, PA, USA). The vial was placed in an ultrasonic water bath .

Table 1:GAS CHROMATOGRAPHIC CONDITIONS for PAHs

Instrument	Agilent, 6890 GC-MS
Column	HP 5MS 5% Phynylmethyl siloxan (30 m x 0.25 mm x .25 mm nominal)
He flow rate	2.0 ml/min
Injection mode	split (1:10)
Injection volume	2μl
Oven temperature program	40 ∘C for 1min; subsequently, increased to 180 ∘C at a rate of 25∘C/min (11min hold) then was increased to 250 ∘C (30 ∘C/min, 2min hold).
Injection port temperature	250 °C
MS temperature	200 °C

2.2.3   Preparation of Standards

The standard solution of six HAAs prepared at a concentration of 80 ppm by diluting the standard solution (1ml) into 25ml by using acetone solvent. The concentration of HAAs compound became the standard solution and was stored at 4^oC and warmed to ambient temperature before use.
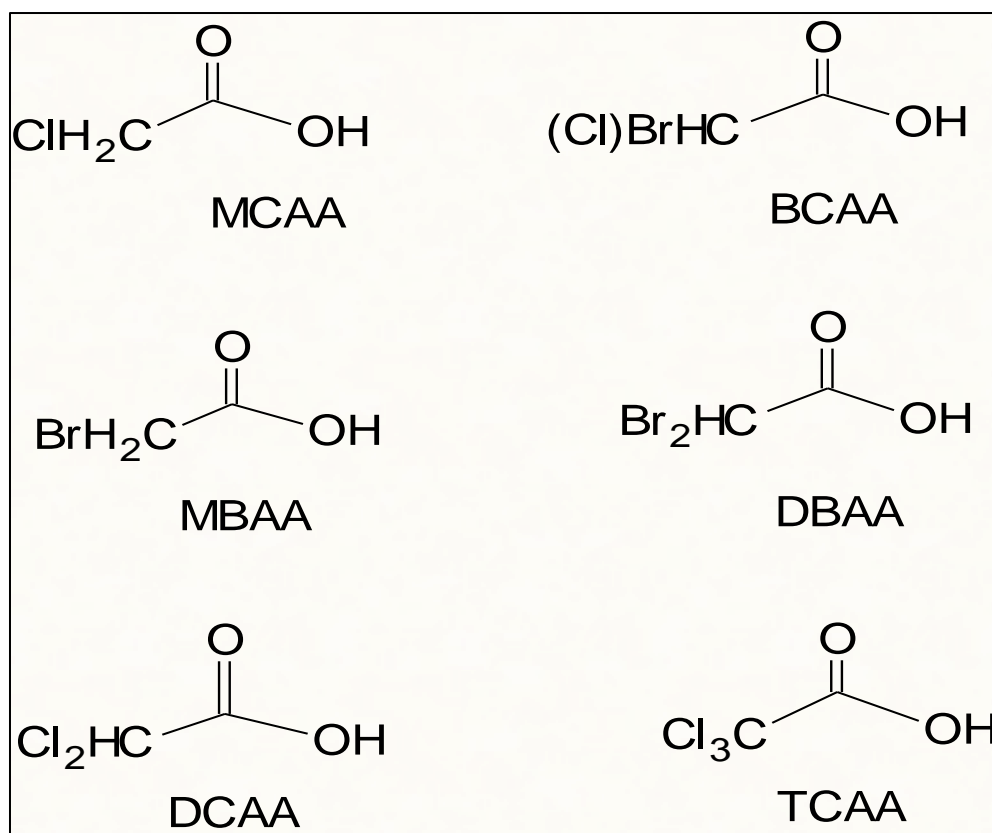
Figure 1: the structure of six HAAs

2.2.4 Sampling Locations and Samples Collection

Seven water samples were collected and purchased from the different places in Al-Khobar city. Three bottled drinking water samples were purchased from local market in Al-Khobar city. Four Spigot water samples were collected from different places. Two samples collected from students housing buildings and water distribution station in King Fahd University of Petroleum and Minerals (KFUPM) campus. The other two samples from water distribution station in Al-Aqrabia and 23rd street in Al-Thouqba in Al-Khobar city. The chosen tape was used most frequently. Any external fittings such as filters and any contaminants around the spout must be removed and clean with a cloth before collecting the samples. The water samples were collected in 1 L screw cap plastic bottles with no headspace after flushing for at least 2 min. The samples were collected with as little agitation or disturbance as possible. The analysis was preformed immediately after sampling.

2.2.5 Extraction and Derivatization Process

Dispersive Liquid–Liquid Microextraction (DLLME) technique has been used for extraction by using *n*-Octanol as extracting solvent and derivatization reagent simultaneously with another appropriate solvent as dispersive solvent (aceton (AC), acetonitryl (CAN), *tetrahydrofuran* (THF), ethanol (ET), methanol (ME)), and we will study the parameters that effect the derivatization reaction yield and the extraction efficiency (temperature, quantity of catalyst trifluoroacetic anhydride (TFAA), kind quantity of dispersive solvent, extraction time. The Gas Chromatography–Mass

Spectrometry (GC-MS) is the technique that will be used for determination of HAAs compounds.

A volume of 100 µl of standard solution (HAAs mixture) (80 ppm) was added into 10 ml vial which contained a (5ml) of distilled water acidified with concentrated H_2SO_4, 10% (v/v) containing 12.4% (w/v) Na_2SO_4 and add 1ml octanol as the extracted and derivatized solvent was mixed and then injected quickly by a syringe into analyte vial and put it in an ultrasonic water bath. To optimize the derivatization parameter, the influence of temperature (25^oC, 60^oC and 100^oC), the reaction time is 2, 5, 10, 20 and 25 minutes), TFAA amount is 2µl, 5µl, 10µl, 15µl, 30µl, 50µl, and the type of dispersive solvent is 1ml (and ethanol, methanol, acetone, acetonitryl, *tetrahydrofuran* will be investigated.

2.2.6 Effect of pH

Many studies have investigated the effect of sample acidity for the determination of HAAs. Mohammad Saraji et al [1] have studied the effect of acidity of sample on the extraction efficiency by adding different amount of concentrated sulphuric acid (1, 3, 5, 7, 10 %v/v) into sample solution. The results showed the higher response was observed with 10% H_2SO_4. Sarrion et al (1999) [26] have studied the effect of pH of reaction. They reported that the reaction obtained good results when pH was less than 0.5 (pH< 0.5). Approximately, 10% H_2SO_4 was chosen according to these studies.

2.2.7 Effect of Salt Addition

The addition of salt into the sample solution leads to improve the extraction efficiency of the analytes by increasing the ionic strength of the aqueous sample. Generally, NaCl is used to study the effect of ionic strength of the solution. However, in the extraction of HAAs from water samples, use of NaCl is not allowed [9, 30, 31] . Sodium chloride can contain trace levels of bromide, and as mentioned before, it can promote the formation of brominated HAAs [31]. Many studies have been done to investigate the effect of salt addition. Mohammed Saraji, et al, studied the effect of salt on the extraction efficiency by adding Na_2SO_4 to 3mL water samples. Based on the results they obtained, 12.4% salt concentration produced higher extraction efficiency. In this study, anhydrous sodium sulfate up to 0.6g was added into the 5ml water sample.

2.3 RESULTS AND DISCUSSION

2.3.1 Method Development

This method was achieved to determine the six haloacetic acids (HAAs) in water samples. The extractant and derivatization that used in this method was *n*-Octanol. HAAs were extracted and derivatized simultaneously. DLLME technique was used by mixed *n*-Octanol with suitable dispersive solvent. The parameters, extraction time, temperature, amount of HFAA and the type of dispersive solvent were investigated.

Figure 2 shows the influence of change of temperature in the extraction and derivatization reaction of the six HAAs. Increase in the temperature of the reaction up to

20

100°C lead to decrease the peak area of analytes due to the loss of analytes. So, a lower reaction temperature (25°C) was chosen for the experiment. In order to examine the effect of reaction time on the extraction and derivatization reaction, reaction time 2, 5, 10, 20, 25 minutes were tested at 25°C with addition of 30μl of TFAA. **Figure 3** shows the peak area for the effect of reaction time on the derivatization reaction. The results show that there is no significant effect with the reaction time. So, the moderate extraction and derivatization time (10 min) was chosen. The effect of TFAA amount on the derivatization reaction between haloacetic acid and *n*-Octanol tested by adding different amount of TFAA (2μl, 5μl, 10μl, 15μl, 30μl, 50μl) into the reaction at 25°C. **Figure 4** shows the peak area of six HAAs compounds at different amount of TFAA. The results show that there is no significant difference in the peak area of HAAs. So, 30μl was chosen. To examine the effect of dispersive solvent on derivatization reaction, the different dispersive solvents used ethanol (ETOH), methanol (MEOH), acetone (AC), acetonitryl (CAN), tetrahydrofuran (THF) at 25°C with 30μl of TFAA for 10 minutes. The influence of the type of dispersive solvent on the derivatization reaction is shown in **Figure 5**. Ethanol gave the best result with higher yield. As the result of the experimental work, the extraction and derivatization reaction will be achieved by using 1ml 1-octanol as extracted solvent mixed with 1ml ethanol as dispersive solvent by adding 30μl TFAA at 25°C for 10 minutes.

2.3.2 Extracting Solvent

From the previous studies, the solvent used for extraction must have good affinity for target compounds, low solubility in water, enough stability over the extraction time, and has an excellent gas chromatographic behavior [36]. In our study, we used *n*-Octanol because it has low-density rather than water and easy to mix it with dispersive solvent for all these reasons 1-octanol used in our study as extraction solvent.

2.3.3 Effect of Extraction Temperature

The influence of the temperature in the extraction process was examined at different temperature (25, 60 and $100^{o}C$) (see **Figure. 2**). Increase in temperature means an increase in kinetic energy and thermodynamic efficiency of the diffusion process during the extraction. This leads to reduce the time require reaching the equilibrium and extraction time by enhancing the diffusion of the analytes in solution. On the other side, increasing temperature leads to decrease partition coefficient in the organic solvent, thus decreasing the extraction yield [36]. The results show a higher peak area for all compounds of analytes were obtained at lower reaction temperature ($25^{o}C$).
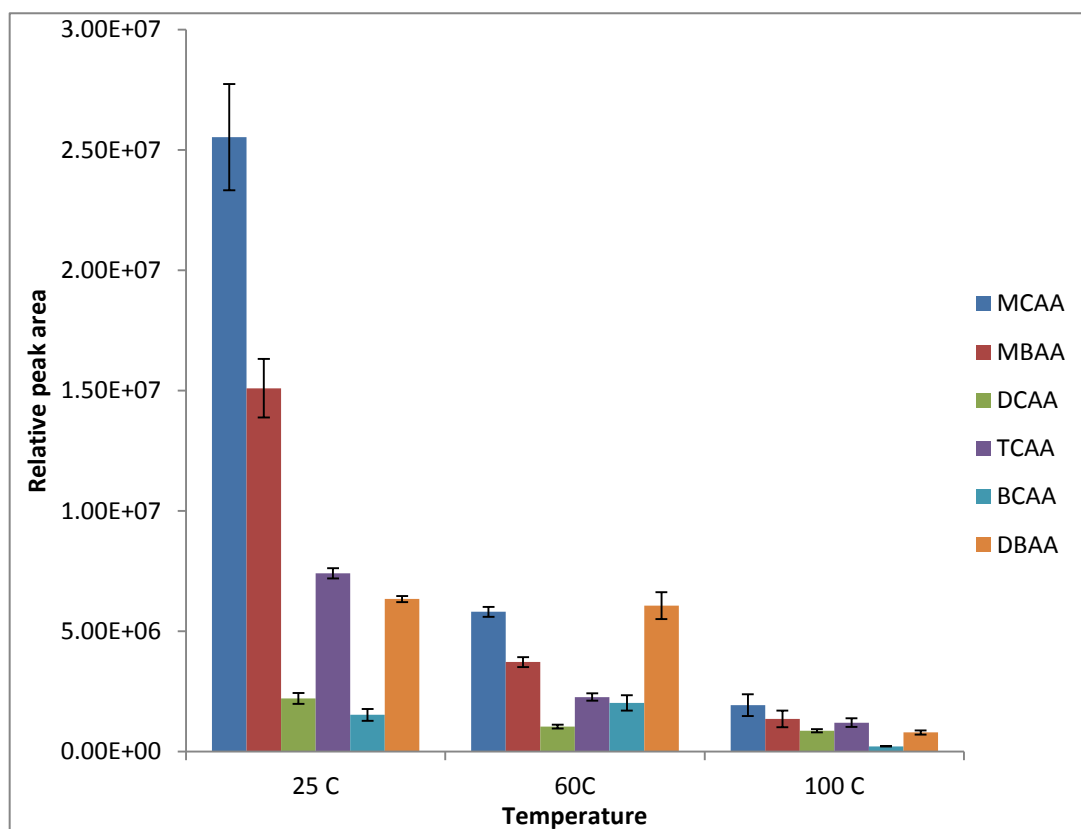
Figure 2: Effect of temperature in the derivatization reaction.

2.3.4 Effect of Extraction Time

Extraction time is one of the most important parameter to be optimized even in order to minimize the energy cost of the process. The influence of extraction time on the efficiency was tested by determination of peak areas of compounds at various exposure time 2, 5, 10, 20 and 25 min. The results of peak areas that obtained for all compounds gradually increase in the peak areas with increasing the time extraction from 2 to 10 minutes. However, there is no significant increase in the peak areas of compounds with increasing the extraction time up to 25 minutes. In this study, an extraction time of 10 minutes was used for all subsequent experiments (**Figure 3**).
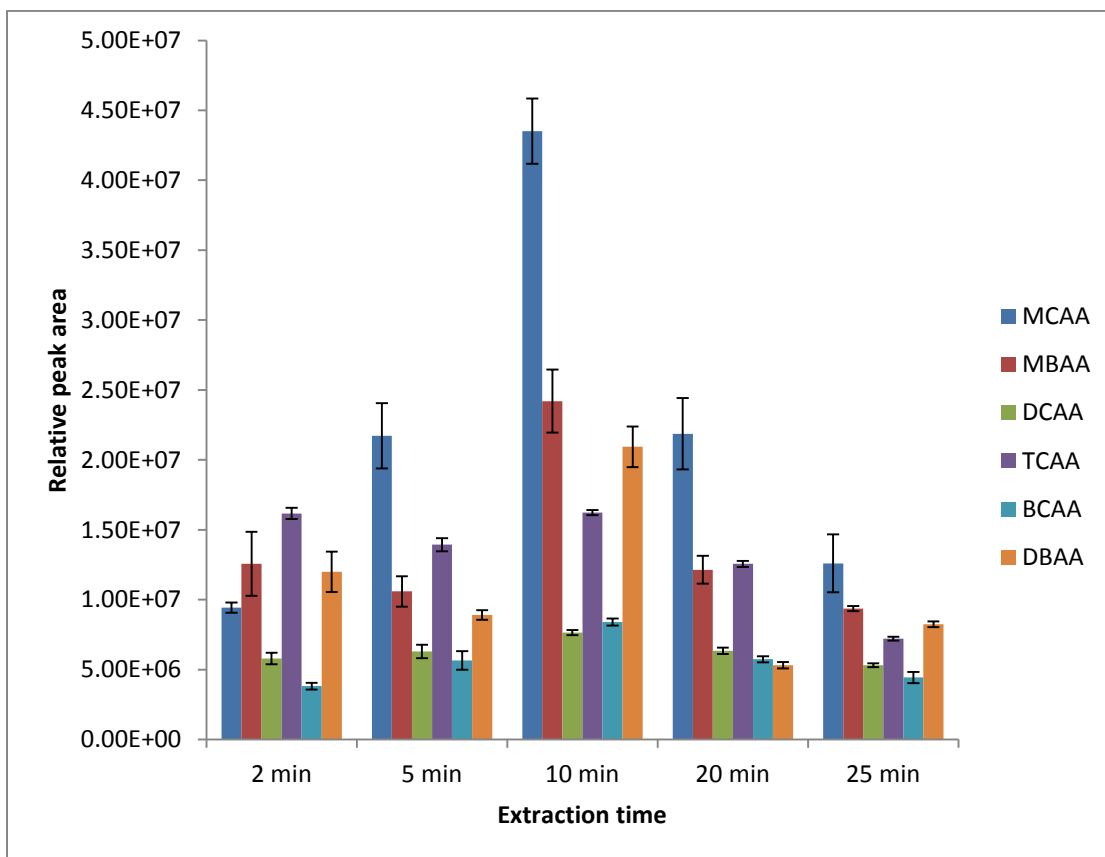
Figure 3: Effect of extraction time for the derivatization reaction of HAAs with n-octanol.

2.3.5 Effect of Amount of TFAA

The effect of TFAA amount on the reaction yield was evaluated by adding different volume of TFAA (2, 5, 10, 30 and 50 µL) into the reaction vial. The results showed that there was no significant difference in the yield when 2, 5, 10 µL were added. The results in **Figure 4** showed that 30 µL of TFAA gave the best response. The pervious study showed that a higher amount did not produce higher yield [36]. Increasing of the concentration of TFAA leads to increase concentrations of trifluoroacetic acid liberated during reaction, the acylating power, rather than trifluoroacetylating power of the acyl trifluoroacetates is enhanced [41].

$$C_8H_{17}OH + CCl_3COOH + (CF3CO)_2O \longrightarrow CCl_3COOR + 2CF_3COOH$$
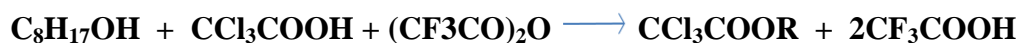
From the above results, chosen amount of TFAA in our study is 30 µL.
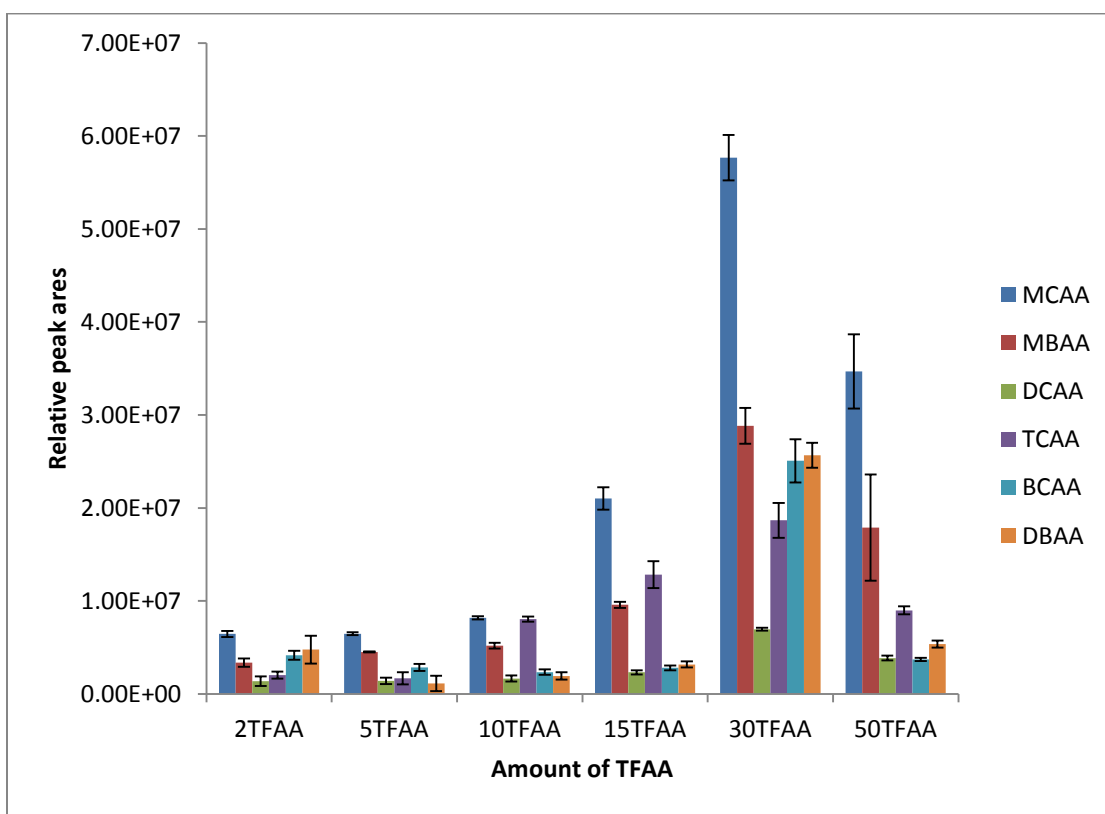
Figure 4: Effect of trifluoroacetic anhydride amount in derivatization reaction yield

2.3.6 Effect of Type of Dispersive Solvent

Acetone, methanol, acetonitrile, ethanol and *tetrahydrofuran* are normally used as disperser solvents. The mixture of extraction solvent (1ml of 1-octanol) and disperser solvent (1.00mL) was tested by inject this mixture into the aqueous sample (5.00mL) by syringe, quickly. The result showed that there was no significant difference between the efficiency of extraction when the acetone, methanol, acetonitrile and *tetrahydrofuran* were used. But, ethanol showed the high efficiency of extraction **Figure 5**.
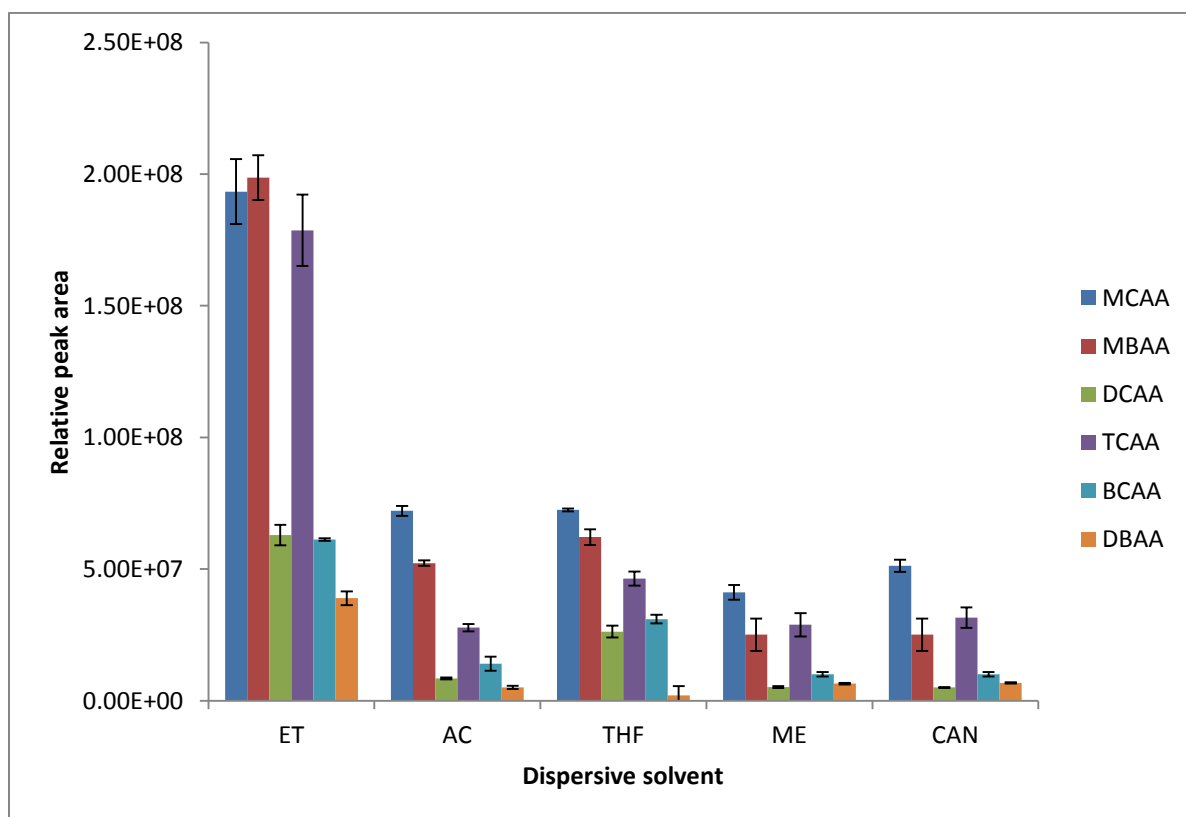
Figure 5 :effect of dispersive solvent volume on derivatization reaction

2.3.7 Real Water Samples Analysis

As a result of experimental work, the procedures of proposed method that applied to determine the concentration of haloacetic acids in real drinking water samples, were by adding a mixture of 1 ml *n*-Octanol as extractant solvent and derivatized reagent with 1ml of ethanol as dispersive solvent into 5 ml of water sample (10% H_2SO_4, 12.4% Na_2SO_4) contain 30µl of trifloroacetic anhydrous TFAA at room temperature for 10 minutes.

In order to investigate the applicability of the proposed method to the real water sample, the current method has been applied to determination of HAAs in drinking water and tap water samples. Seven water samples were collected from different places in Al-Khobar city. Three bottled drinking water samples were also purchased from the local market in Al-Khobar city. Four Spigot water samples were collected from other different places. Two of them from students housing buildings and water distribution station in King Fahd University of Petroleum and Minerals (KFUPM) campus. The other two samples from water distribution station in Al-Aqrabia and 23rd street in Al-Thouqba in Al-Khobar city.
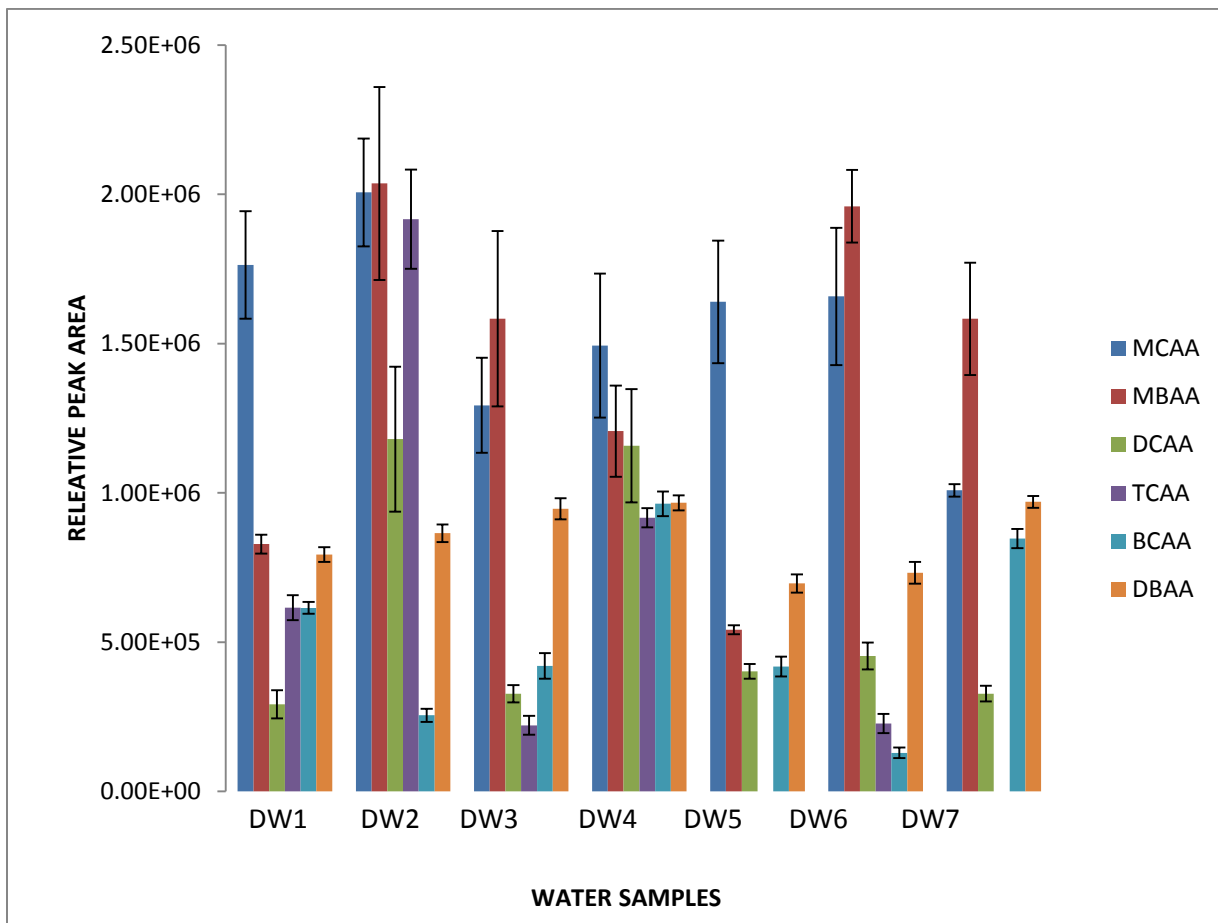
Figure 6: the relative peak area for real drinking water samples

Table 2: HAAs concentrations in drinking water from local sources and local tap water

	DW1	DW2	DW3	DW4	DW5	DW6	DW7	Mean Conc.(µg/L)
MCAA	4.29	4.89	3.15	3.64	3.99	4.04	2.46	3.78
MBAA	1.31	3.12	2.5	1.9	8.53	3.09	2.5	3.28
DCAA	2.18	8.82	2.45	8.65	3.39	3.39	2.45	4.48
TCAA	0.54	1.68	0.2	0.8	Nd	0.2	Nd	0.684
BCAA	3.15	1.31	2.15	4.94	2.14	6.62	4.34	3.52
DBAA	3.19	3.48	3.81	3.89	2.8	2.95	3.95	3.44

Table 3: Comparison of analytical data of proposed method and other techniques in determination of HAAs in water

Method	RSD(%)	D.R[a]	D.T[b] (min)	Sample volume(ml)	Reference
LLE-GC-ECD EPA 552-1	7-59	Diazomethane	30	100	[40]
E-GC-ECD EPA 552-3	0.36-4.0	Acidic methanol	120	40	[41]
LLE-CZE	1.1-4.2	-	15	30	[42]
LLE-GC-MS-MS	0.9-19.9	PFBBR	180	1	[43]
LLE-ESI-MS	-	-	-	188	[45]
SPE-LC-ED	-	-	-	-	[46]
SLME-LC-UV	1.5-10.8	-	-	20	[47]
HSHFLPME-GC-MS-μECD	5-12	Acidic methanol	-	10	[48]
HS-SPMS GC-MS	4.1-8.9	Acidic ethanol	1O		[39]
EV-SPME-GC-MS	6.3-7.9	Acidic ethanol	10	30	[49]
HS-SPME-GC-MS	6.3-10.9	Dimethyl sulphate	5	10	[11]
SDME-GC-MS	5.1-8.5	Octanol	20	3	[12]
DLLME-GC-MC	0.4 - 11.4	n-octanol	10	5	Proposed method

2.4 Conclusion

Dispersive liquid-liquid micro-extraction followed by GC-MS analysis was employed for the determination of six species of haloacetic acid in water matrix. This method utilized n-octanol as both extractant solvent and reagent for the simultaneous derivatization of these polar analytes for compatibility with GC-MS determination. The method was relatively fast as the derivatization and extraction procedure was completed in 10 min. Both intra-day and inter-day precision showed that the method had good repeatability. This and other parameters tested showed that the proposed method compared favorably with methods reported in the literature. The method was successfully applied to the quantification of the analytes in real samples of bottled water and tap water sources. Concentrations determined in these sources showed that the different sources contained HAAs at concentrations lower than the maximum contaminant levels prescribed by both WHO and USEPA

CHAPTER 3

DETERMINATION OF ORGANOTINS IN

ENVIRONMENTAL SAMPLES

3.1   LITERATURE REVIEW

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 3-6 ng/g [42]. 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 [33].

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 [43]. 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 [44]. Also in water matrix, 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-QFAAS. Ion pairs for the organotin compounds were generated by reaction with decane sulfonate [45]. Analysis of organotin compounds in the marine environment has involved the separation by Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC) with detection by Atomic Emission Detector (AED), Atomic Absorption Detector (AAD), Flame Photometry Detector (FPD) or Mass Spectrometry (MS) [46]. Current methods of analysis include GC/MS, GC/AED, GC/ICPMS (Inductively Coupled Plasma Mass Spectrometry) and HPLC/ICPMS [44]. GC/MS currently enjoys a pre-eminent position in the environmental analysis of organic and organometallic compounds. However, ICPMS has emerged as one of the most important tools for the analysis of trace elements in environmental samples. The recent development of hyphenated techniques that provide on-line pre-concentration, matrix elimination and speciation has significantly enhanced the capabilities of atomic spectrometric analysis of environmental samples, especially those with complex matrices such as marine water [47, 48].

An elaborate sample preparation has always been required before GC analysis can be carried out. This involved acidification with HCl or HBr followed by extraction into an organic solvent. The compound is then derivatized using Grignard reagent, usually alkyl magnesium bromide [46, 49]. Some researchers have used only hydride generation (using NaBH4) for GC/AAD quantification of organotin species in water samples [50]. The

derivatization is time consuming, costly and yields may vary between species and in terms of efficiency, depending on matrix components.

Analysis of mixed organotin standard solutions by GC/ICPMS and HPLC/ICPMS has shown that the GC method could separate a greater number (n = 10 - 12) of compounds in a single run compared to the HPLC method (n = 5 − 6) [48]. The injection-to-injection time was 40 % shorter for HPLC/ICPMS because of the temperature profile used for GC separations and the reagent cost per sample is approximately double for GC sample preparation owing to the cost of the derivatization agent. Organotin separations by HPLC offer the advantage that derivatization is not required, which eliminates a potential source of uncertainty in the final result and reduce the analysis time significantly. The United States Environmental Protection Agency recently approved a standard method (Method 8323) for the separation of BT and PT compounds using HPLC [51]

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 [52]. 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. 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 [53].

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. The proposed mechanism of derivatization is shown in the following equation.

$$R'MgCl + R_3SnX \longrightarrow R_3SnR' + MgClX$$

_____
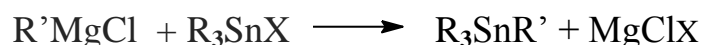
R' = Isopropyl magnesium chloride

R = alkyl radical or aryl radical

Ethylation using Sodium Tetraethyl Borate, STEB, is another class of derivatization employed in the gas chromatographic determination of organotin compounds [42, 54]. However, if 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 [55]

Ruedel et al. [55] determined the concentration levels of organotin compounds including TBT and TPT as well as their 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. While the TPT concentration in North Sea mussels decreased from 98 to 7 ng/g, 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 another study by Harino and his group [56], organotin compounds were detected in surface sediments and mussels Mytilus edulis 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 showed that TBT was the predominant BT species in sediments (approx. 50%) and that only 4% of the total tin in sediments was made up of BTs. They showed that the 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.

The occurrence of volatile organotin compounds (butylmethyltin) in three European estuaries was investigated by Tessier et al. [57]. They found that the most ubiquitous species in surface water to be methylated forms of butyl-tin derivatives. (Bu_nSnMe_{4-n}, n = 0-3). The presence of volatile organotin compounds is thought to result from the natural methylation processes of both inorganic tin and anthropogenic derivatives accumulated in the sediments (i.e. tributyltin released from ship antifouling paintings and wastewater discharges).

Ikeda et al.[58], who studied bioaccumulation of organotin compounds through the food web in the deep water of Japan Sea, found that TBT is present in all samples but at a

lower concentration compared to coastal areas: seawater 0.3-0.8 ng/L, sediment 4.4-16 ng/g-dry and marine organisms 1.8-240 ng/g-dry. They also determined the concentration of TPT; seawater (less than 0.9 ng/L), sediment (3.9 -12 ng/g-dry) and marine organisms (5.0- 460 ng/g-dry).

A survey of endocrine disrupting chemicals in fish and shellfish conducted by Chatani [59] and his group in Japan found TBT in the range of 10 to 30 ppb in 3 of 24 samples analyzed and 10 ppb of TPT in 3 samples. Michel and Averty [60] 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. Concentrations in marine water measured in the coastal areas outside port facilities very often exceeded recognized toxic levels and seventy-five percent of the measurements were above the threshold of 1 ng/L, which is known to cause toxic effects for some marine species. The average tributyltin concentration was 4.6 ng/L, but for ten stations the contamination level exceeded 100 ng/L.

Although the use of organotin compounds as anti-fouling agents, due to their known toxicity to fouling organisms, was intentional, the wider impact of this class of compounds in the marine environment was grossly underestimated. TBT is considered to be the most toxic of all organotin compounds and is the main constituent in antifouling paints. 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[61], [62]. TBT at these concentrations is also known to cause shell deformity and larval mortality [63]. Several studies have shown the effects of TBT

compounds, such as shell malformations of oysters, imposex in marine snails, reduced resistance to infection (e.g. of flounder), effects on the human immune system etc.

First effects of organotin compounds on higher marine organisms were observed in the form of imposex in early 1970s in female dog whelks (Nucella lapillus) [10] in the United Kingdom and in American mud-snails [64] in the United States. Subsequently imposex has been observed in over 140 species worldwide. It has been shown that the sterilization of female dogwhelks occurs at TBT concentrations as low as 3 -5 ng/L [65], with almost all females affected at 10 ng/L. In Arcachon Bay imposex was first observed in the predatory gastropod Ocenebra erinacea (oyster drill) in 1970, which was attributed to TBT in early 1980s and led to its near extinction[66]. 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 [67]. More fundamental changes including testes development and suppression of egg production occurred at higher concentrations.

Workers handling dibutyl- and tributyltin have reported eye irritation and skin lesions [68] and mucus irritation after exposure to interior paints containing tin [69]. Toxicity of organotin compounds in humans is most frequently reported as loss of memory and insomnia as well as other effects including death [70]. Neurotoxicity has also been reported with trimethyltin exposure in humans. Liver damage has been reported in people spraying triphenyltin acetate. Among six workers who were exposed to a solution of 75%

dimethyltin and 25 % trimethyltin for a total of 90 minutes over three days, one worker died, one remained hospitalized and only three were able to return to work [70].

In general there is a lack of rodent subchronic and chronic studies for organotin compounds but developmental toxicity studies have been conducted for butyltins [71]. Some short-term studies identify immune [72] and nervous [73] systems as being sensitive to organotins with hepatic and renal effects being less frequently reported.

All Wistar rats administered with a dose of 2,000 mg/kg monobutyltin during gestation days 7 and 8 died and weight loss of dams and fetuses were observed at lesser doses [71]. Decreased thymic weights for all doses and decreased maternal weights for the highest dose were observed in pregnant rats exposed to 1.7 to 50 mg/kg/day dibutyltin diacetate during days 7 through 17 of gestation. Dibutyltin caused increased fetal mortality and increased malformations compared to controls [74]. Significant decreases in maternal weight gain, decreased fetal weights, higher post-implantation loss and increased fetal malformations compared to controls were observed in Wistar rats administered with doses of 10 or 15 mg/kg of dibutyltin dichloride during gestation days 7 and 8 with sacrifice on gestation day 20 [73].

For the series of diorganotins, dibutyltin is the most cytotoxic for brain derived cells while dimethyltin is the least toxic [75]. Monobutyltin and dibutyltin were shown to be g using the chromotest, while the mono- and dimethyltins were not genotoxic [76]. Monobutyltin and dibutyltin were mutagenic in Salmonella typhimurium TA100 while only dibutyltin was positive in Salmonella typhimurium TA98. In these assays, monomethyltin and dimethyltin failed to show mutagenicity [77].

In a more recent studies conducted in China, Zhang et al.[78, 79] showed that the amount of butyltins (BTs) were higher than phenyltins (PTs) in surface sediments of fishing areas of China Sea. In the surface sediments, the concentration of TBT is higher than dibutyltin (DBT) but lower than monobutyltin (MBT). Higher amounts of BTs have been reported in other coasts such as North coast in Kyoto (Japan) [80], Kochi Harbor in India [81], Sao Vicente Estuary of Brazil [82] and Adriatic Sea in Slovenia[83].

Recent study by Harino et al. [84] on coastal waters of Japan showed that the previously observed drastic reductions in TBT concentrations stopped and no significant change can be observed in later years during the period of the study. However, they observed high amount of TBT and TPT in areas with shallow waters and poor flushing. Generally, mussels from coasts with many fishing ports were reported to contain high organotin concentrations. They also noted variation in concentration of TBT with depth, which is constant in water to a depth of 20 m. Though in sediment, top 10 cm have higher concentrations. It was concluded that TBT contamination persist for a very long time.

In an attempt to further understand environmental risks of OTs through the study of their behaviors and transport, Santos et. al.[8] explored a previously unexplored matrix, suspended particulate matters (SPM). The study showed that surface samples are of lower concentrations of Organotin than bottom samples of SPM. OTs tend to accumulate in the bottom of water bodies. Earlier report by Felizzola et. al. [85] had already shown that the finer the sediments, the higher the concentration of OT found in them. This feature, like other features of Organotin compounds enhances their toxicity and environmental risks. As pointed out by Roberts [86] in his review on re-suspension of contaminants in sediments, that toxicity and bioaccumulation of contaminants are enhanced by suspension

of the contaminated sediments. This was proven in various laboratory experiments. However, it is still not clear whether there is potential for the ecological effect of this enhancement in the field.

Yi et. al. [87] also ranked TPT concentration in marine environment as water < sediment < invertebrates and fish. The highest concentration in marine organisms is evidence of bioaccumulation. Blue mussel (Mytilus edulis) was reported to TPT accumulator containing 5.9 µg g-1 dw. It noted that in Europe and Japan, higher animals like birds and mammals have lower concentrations of TPT than lower animals like invertebrates and fish. Pattern of bioaccumulation is clearly not regular. In their (Yi et. al.) attempts attempt to see the trends of TPT concentration in birds, fish, invertebrates and mammals, no significant difference was observed. This shows that no meaningful comparison can be made. The review also summarized information on bio-concentration and bio-magnification of OT compounds in fish through in algae (phytoplanktons) and marine invertebrates (zooplanktons). Literatures show that TPT hardly bio-accumulate in mammal and birds because of their ability to bio-transform it to excretable forms. In water and sediments, TPT was found to be easily photodegradable. Because OTs such as tributyltin is photodegradable, studies have been conducted to see how N-doped TiO2 photocatalyst can be used to treat TBT under natural light. In a particular study by Bangkedphol et. al. [88], N-doped TiO2 proved to hold a good potential, by showing highest photocatalytic degradation of up to 28% in 3 hours, whereas commercial and undoped types degrade only 18% and 14% respectively in during similar time frame.

In Turkey, from Eastern part of Aegean Sea, Kucuksezgin et. al. [89] measured mean concentrations of TBT to be 116, 235 and 635 ng Sn g-1 in mussels, fish and barnacles.

Samples collected from harbor and marinas are richer in Organotin compounds. The authors implicated barnacles as a good indicator to bio-monitor Organotin contamination in the Sea. Furdek et. al. [90] however used mussel species (Mytilus galloprovincialis) to evaluate the OT contamination in Croatia coast. In the study, majority of the OTs collected were butyltins (TBT, DBT, MBT) and in mussels, as much as 1675 ng Sn g-1 was recorded, while in seawater, the maximum amount of butyltins was27.98 ng Sn L^{-1}.

More recent study of spatio-temporal variation of OT concentrations in sediments confirmed that seasonal effects on impact the concentrations of organotin compounds in sediments. Yozukmaz et. al. [91]collected samples in from designated stations in winter and then in summer. Winter average of total butyltin in sediments was 1091.5 ng Sn g^{-1} while summer average was 2691.2 ng Sn g-1. Concentrations of OT compounds in sediments are higher in summer than in winter. This is not surprising as it may be due to lower volume of water in the coast while the OT amount remains constant, leading to stronger concentration of total butyltins.

Similarly, in India, Garg et. al. [92] found total butyltin in Indian coast water to be between 1.7 to 342 ng Sn g-1 while in sediment, the concentration was from below detection limit to as high as 14681 ng Sn g-1. They found total butyltins in sediments to be well correlated with that in surface water and with organic carbon. They therefore advocated for the use of adsorption/desorption methods to control TBT in the ports. They also called for the regulation of organotin pollution in the coast.

3.2 EXPERIMENTAL

3.2.1 Chemicals and Reagents

Analytical grade standards of monobutyltin (MBT) as monobutyltin trichloride, dibutyltin (DBT) as dibutyltin dichloride, monophenyltin (MPT) as monophenyltin trichloride and diphenyltin (DPT) as diphenyltin dichloride were supplied by Sigma-Aldrich (St. Louis, MO), while standards of both TBT and triphenyltin (TPT) as tributyltin chloride and triphenyltin chloride respectively were purchased from Fluka (Buchs, Swizerland) (fig 7). 1000 mg/ml solutions were prepared in acetone and stored as stock from which necessary dilutions were made when needed. OTs were derivatized using the Grignard reagent, isopropyl magnesium chloride (Sigma-Aldrich, St. Louis, MO). 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 (Barnstead, Dubuque, IA, USA) system.
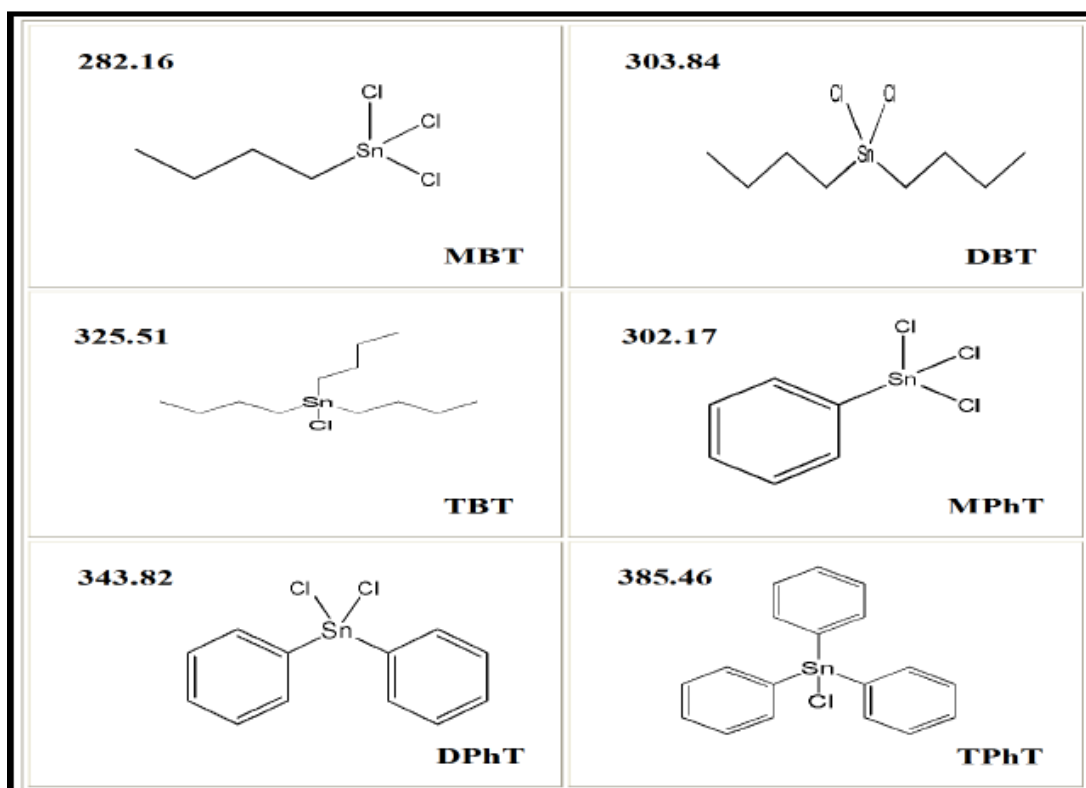
Figure 7: Molecular structures and molar masses of six species of organotin

3.2.2   Sampling Area and Sample Collection

The study area is located in southern coast of the Arabian Gulf which is situated in the Eastern province of Saudi Arabia, including King Abdul Aziz Port in Dammam and surrounding areas of Dammam (fig 8). We determined the organotins in the Dammam area samples (most of the samples). The area is home to commercial fishing activities with small to medium, as well as big boats birthing the sea. Seventeen stations were sampled from four locations within the area: King Abdulaziz Port, Inside (KAPI), King Abdulaziz Port, Outskirts (KAPO), Shipping Channel, Dammam (SCD), Near Dammam Corniche (NDC). King AbdulAziz Port is the main gateway through which cargoes from all over the world enter the Eastern and Central Provinces of the Kingdom. The Port has fully functional, self-sufficient mechanical and marine workshops, water treatment plants and about 39 berths. It is one of the busiest ports in the Kingdom of Saudi Arabia. While Dammam Corniche area is considered to be highly polluted due to inputs from nearby industrial plants and   agricultural and runoff waters.

Water samples were collected in 1 L Teflon jars in accordance with USEPA surface water sampling SOP (EPA, 1991). Sampling was done from subsurface, midwater and above seabed depths ranging between 0.4 m and 14.5 m. Similarly, sediments were sampled following the USEPA procedure for soil, water and solid waste sampling (EPA, 2004). 300 g grab sediment samples were collected in Teflon bottles from each sampled location above. Site I.D., location, coordinates, date of sampling are given in Table A1(in the appendix) while site quality indicators including temperature (^{o}C), dissolved oxygen (DO, mg/L), salinity (sal, ppt), total dissolved solids (TDS, ppt), turbidity (tur, NTU), pH and chlorophyll-a (chl-a, µg/L) were collected as reported in Table A2 (in the appendix).

All the samples were returned in ice to the laboratory immediately and analyzed pronto. Seafood samples were purchased from local super market and fisher mongers form Dammam (fig 9). The specimens were frozen at -20C until the analysis of samples. The samples were thawed and 5 g from each sample was used for extraction.
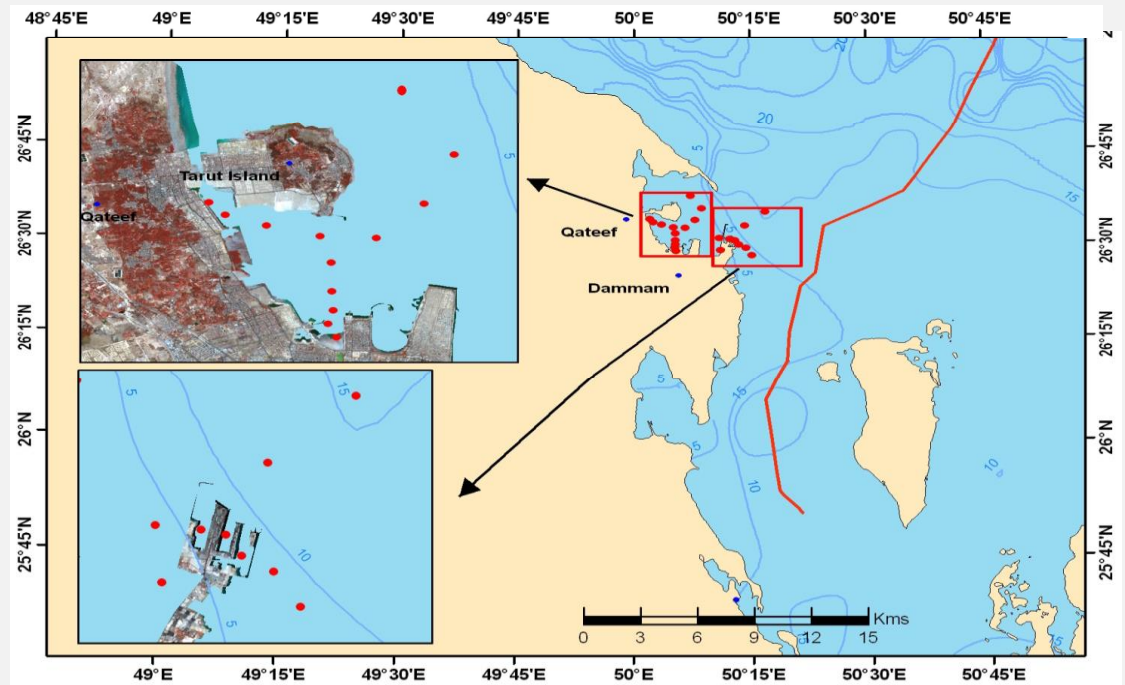
Figure 8: map of organotins sampling locations
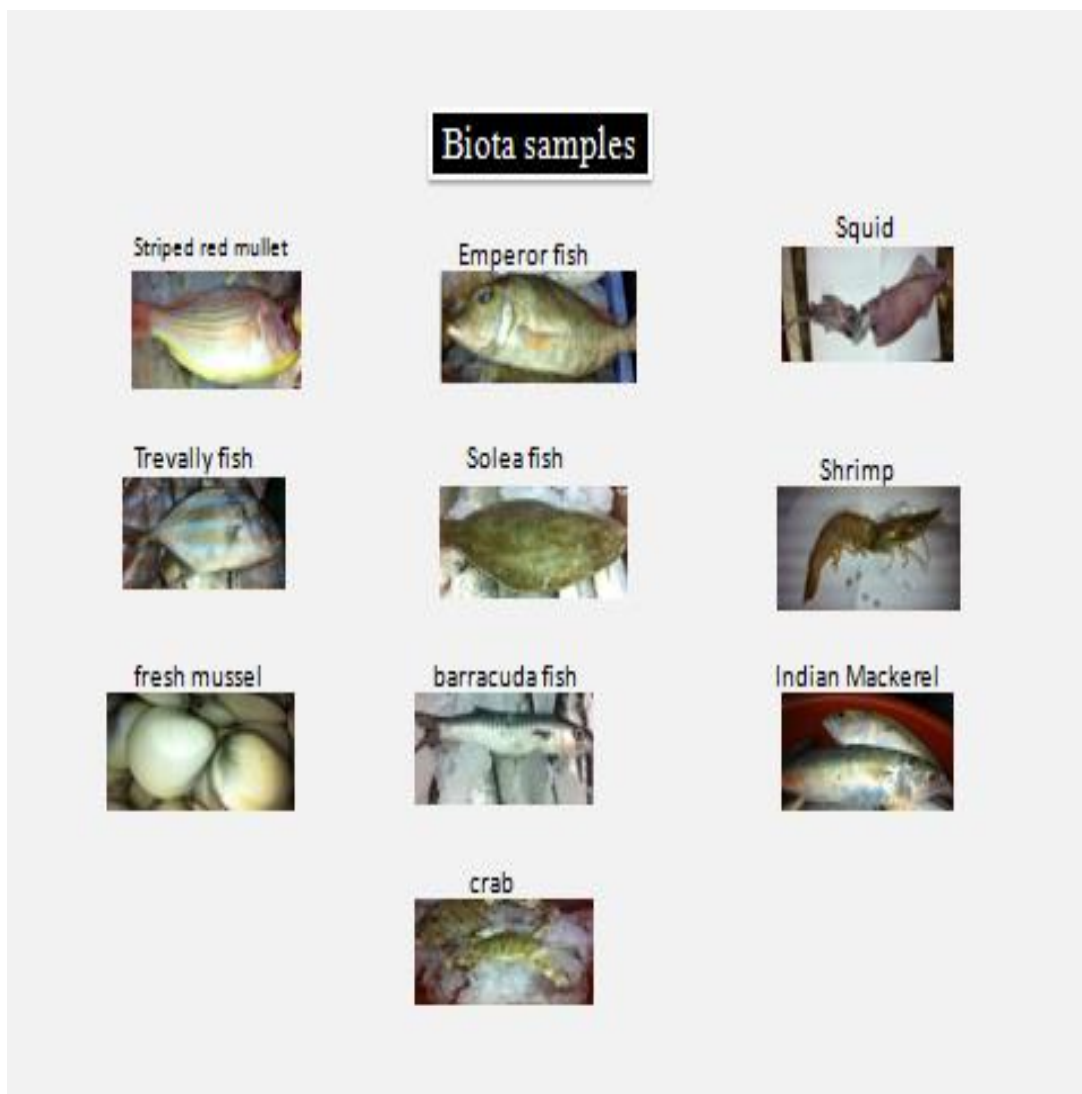
Figure 9: **Selected biota samples for organotin analysis**

3.2.3   Extraction and derivatization of organotins

3.2.3.1   Sample Preparation and Analysis

3.2.3.1.1   Seawater Samples

All glassware used for the extraction and derivation 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, removed and rinsed with ultrapure water after methanol rinsing and subsequently dried in oven at $50^{\circ}C$. C_{18} SPE discs with 47mm Nu-phase fibers (CPI International, USA) were conditioned with deionized water and employed for the extraction of water samples. One liter of each water sample was washed through the discs via an Ultra ware glass cup. The set was powered by Edwards High Vacuum (B.O.C. Ltd., Crawley, UK). Adsorbed compounds were then eluted with 25 mL of DCM. The SPE disc was used only once and then discarded.

3.2.3.1.2 Sediment Samples

For sediments, 50 g of each sediment sample was taken in Erlenmeyer flask and 50 mL of DCM added and stoppered. This was agitated for 30 min at 150 rpm on a Lab Companion Shaker (model SK-600, GEOL Tech, Korea) to effect extraction. After the extraction, traces of moisture were removed by addition of pinches of anhydrous sodium sulfate. Extracts were further pre-concentrated to 1 mL by a combination of Buchi Rotavapor R-200 equipped with heating bath B-490, and by slow stream of dry liquid nitrogen. Derivatization of OTs was performed using the Grignard reagent, isopropyl

magnesium chloride. From the pre-concentrated extract, 500 µL was taken in a 10 mL vial and 1 mL n-hexane added. This was shaken for about 1 min, followed by the addition of 500 µL of the derivatization reagent. The mixture was vortexed for further 1min and left to stand at room temperature for 15 min. The reaction was then quenched by the addition of 0.05 M sulfuric acid. The vial was centrifuged, and the upper layer was analyzed in GC-MS.

3.2.3.1.3   Biota Samples

Biota samples were extracted using liquid-liquid extraction method. The 5 g wet wt samples were weighted and minced. The minced sample was digested with 20 ml of acetonitrile for 15 min and decanted. Repeat the digestion with 20 ml of acetonitrile and decanted. Organotins were 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 remove 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. 2 ml of organic extracts was concentrated to 1 ml under a gentle stream of nitrogen. Concentrated organic extract 1 ml was derivatized with 500 µl of 2M n-propylmagnesium bromide for 20 min. The derivatized extracts were then filtered out. Finally the clean extract was analyzed for quantitation.

3.2.4 Determination of Organotins

The six species of OTs namely, MBT, DBT, TBT, MPT, DPT and TPT 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. The column temperature was initially set at 40°C which was held for 5 min, and then ramped to 300°C at the rate of 12°C/min. It was held at this final temperature for 4 min. Total ion current (TIC) in SCAN mode for ions of masses between 50 and 550 was used for acquisition. Selected ion monitoring (SIM) mode was employed for quantitation using m/z of 246.8 (MBT), 277 (DBT), 291.1 (TBT), 283 (MPT), 361 (DPT) and 351 (TPT). For the quantitative estimation of the OTs, a five-point calibration curve was constructed and found linear between the concentration range of 0.05-1000 μg L^{-1} for water samples (R^2, 0.9914-0.9997) (Table 4) and 0.05-1000 ng g^{-1} for sediment (R^2, 0.9861-0.9998) (Table 5). Limit of detection (LOD) was calculated from signal-noise ratio of three (S/N = 3) and limit of quantitation (LOQ) from S/N = 10. Repeatability of analysis was investigated using percent relative standard deviation (%RSD) estimated from triplicate determinations.

3.3 RESULTS AND DISCUSSION

Seawater, sediment and biota samples were tested for recoveries with known concentration of spiked organotins. Extraction recoveries were in the range between 80-

109% for most of the analytes. Biota and sediment samples were lower when compared to water samples this could be due to the matrix effect and multi steps extraction procedure was used (USEPA protocols). Overall the recoveries were acceptable levels.

3.3.1 Concentration of Organotins in Sea Water Samples

Seawater samples were analysed from 17 locations in and around Dammam port areas. Each location 3 to 4 seawater samples were collected tested for organotin concentrations. Each sampling areas organotins were detected and the concentrations are reported in the Table 6 and 7. Total mean concentrations of organotins are reported in Figure 10. Results clearly indicates that sampling location 6 (outskirt of port) has low total organotins (1.97 µg/L) and highest concentration (5.446 µg/L) at shipping lane site (location 5). The mean concentrations of individual organotin concentration at Dammam port in and outskirt areas tributyltin and triphenyltins were detected at relatively higher concentration (0.94 and 1.06 µg L^{-1}, respectively). The high values of tributyl and triphenyltins were indicating that fresh input of organotins in these locations. Whereas, in the shipping lanes of Dammam port and Dammam Corniche locations, high concentration of monobutyl and diphyltin (i.e. 1.05 and 1.01 µg L^{-1}, respectively) were detected. This could be due to higher degradation rate of tributyltin and tripheyltin and lower fresh input of these compounds. Overall, the reported concentration is not high when comparing with previously published results[93]. Higher frequency of organotins are present at Dammam areas this could be due to shipping and other fishing activities in this region.

3.3.2 Concentration of Organotins in Sediment Samples

In sediments from were collected from same locations or closer location from where the water samples collected. Total 17 sediment samples collected and analyzed based on the above mentioned extraction methodology. The total mean concentration of organotins were relatively higher at shipping lanes of Dammam when compared to the port areas (See Figure 11). The reason for high concentrations at shipping lane could be due to some fresh input from the small boats and other fishing activities. Sampling location at closer to port area and shipping lanes has highest total concentration 1640 ng/g. (Table 8 and 9). Overall, the total mean concentration of organotins in sediment samples were higher at shipping lanes (Figure 4), this could be due to shipping lanes are shared by Bahrain and Saudi Arabia.

Being one the busiest ports in Saudi Arabia where both commercial and recreational activities happen regularly, one would have expected greater than unity for these ratios if new inputs were recorded. As reported earlier, environmental factors and physiochemical properties of water and sediments plays a major role on the degradation of organotins in the environment [84]. However, some industrial and other fishing activities may contribute large amounts of organotins into the maritime systems, amounts determined may not always reflect the physico-chemical properties of sampling location [8, 78]. Previously, high concentrations of organotins have been reported in Bahrain [11] with maximum value of 1.930 µg/g. Our values are comparable with previously reported values in this region, this indicating that organotins are persistent in sediment environment.

3.3.3 Concentration of Organotins in Biota Samples

Total 10 types of samples were extracted for the analysis of organotins in biota samples, total concentration detected in the biota were in the rage of 5.1 and 16.7 ng/g. All samples were contaminated with organotins. Barracuda fish show higher accumulation of organotins (16.7 ng/g) and next highest concentration was detected in the oyster samples (14.6 ng/g). Reason for higher level might be due to high fat and bioaccumulation properties in both samples. Additionally the former has ability to migrate long range in the seawater. However, more studies are required to conclude the bioaccumulation studies.

Table 10 shows the results obtained for organotins in biota samples (all of the purchased directly from fisher man at Damman and its closer proximity). The total of the 10 species of organotins present in all samples, in most of the samples DBT was detected in relatively higher concentration than other organotin compounds (average values was 6.1±1.8 ng/g). MBT and TPT were detected lower than other organotin compounds (See Table 11). The ratio between TBT/DBT for Barracuda fish was 9.9, mackerel fish 9.2 and sole fish was 5.6; these values indicates the fresh input or fresh bioaccumulation of TBT in these fishes. As expected these three species are higher mobility in the marine waters
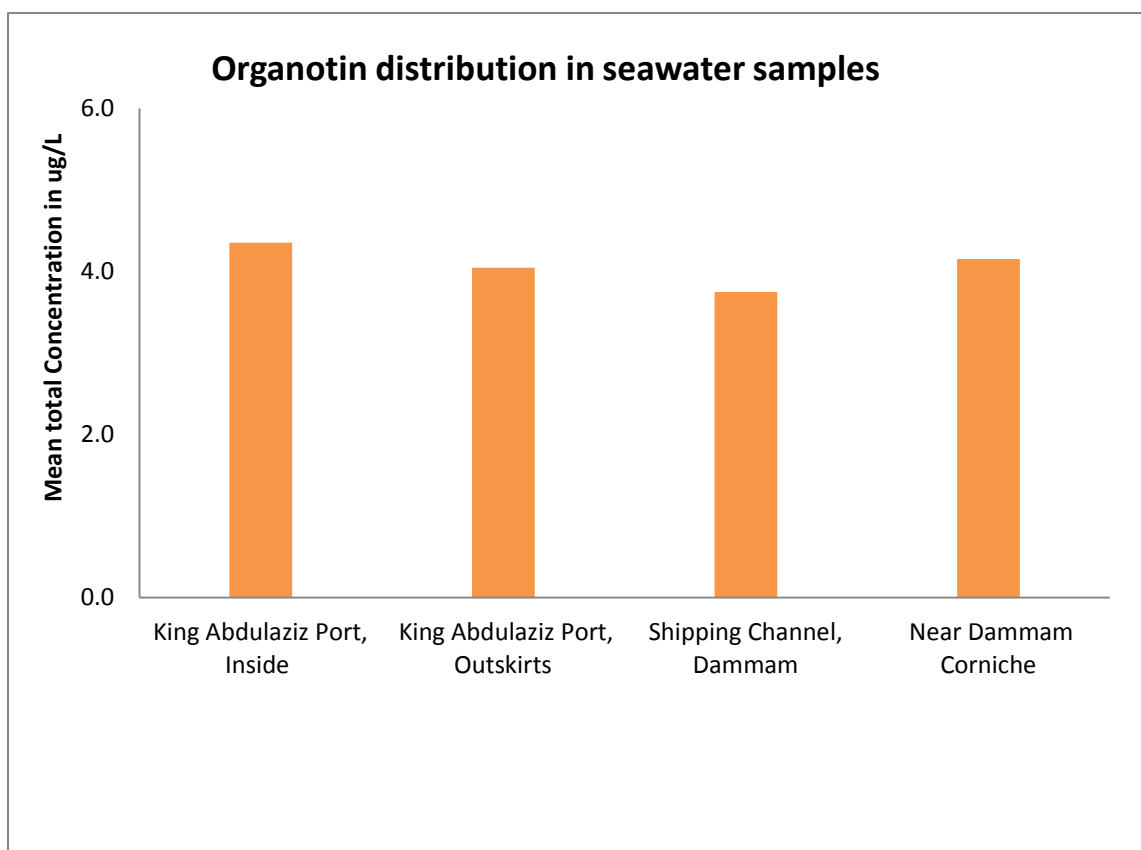
Figure 10: Overall total mean concentration of Organotins in seawater samples

Table 4: Calibration parameters, analytical limits, recovery and repeatability in water matrix.

Analytes	Slope±SD (x 10^{-3})	Intercept±SD (x 10^{-5})	R^{2*}	LOD (ng L^{-1})	LOQ (ng L^{-1})	% Mean recovery	%RSD (n=3)
MBT	24.2±0.14	8.7±0.51	0.9914	7.8	26	82	6.1
DBT	0.8±0.06	6.3±0.52	0.993	11	36.7	86	8.2
TBT	0.1±0.01	0.9±0.07	0.9997	7.5	25	89	7.5
MPT	4.9±0.49	5.1±0.64	0.9978	5.2	17.3	82	10
DPT	0.07±0.01	0.05±0.01	0.998	10	33.3	81	11.5
TPT	17.4±1.57	0.22±0.18	0.9965	8	26.7	85	9

- **For the concentration range of 0.05-1000 µg L^{-1}**

.

Table 5: Calibration parameters, analytical limits, recovery and repeatability in sediment matrix.

Analytes	Slope±SD (x 10⁻³)	Intercept±SD (x 10⁻⁵)	R^{2*}	LOD (ng g⁻¹)	LOQ (ng g⁻¹)	% Mean recovery	%RSD (n=3)
MBT	2.28±0.32	8.35±1.17	0.9996	8.5	28.3	70	14
DBT	0.13±0.02	9.97±1.37	0.9861	12.2	40.7	74	13.7
TBT	0.1±0.01	10.1±1.12	0.9992	8.1	27	81	11.5
MPT	0.09±0.01	0.07±0.01	0.9982	6.6	22	84	11

[*] For the concentration range of 0.05-1000 ng g⁻¹

Table 6: Concentration of organotins in the seawater samples collected in the vicinity of Dammam.

	Water samples (µg/L)										
	1	15	16	17	2	3	6	7	12	13	14
	[a] King Abdulaziz Port, Inside (Site ID: 1, 25-27)				[b] King Abdulaziz Port, Outskirts (Site ID: 2, 3, 6, 7, 18-20)						
MBT	0.257	0.37	0.209	0.382	0.208	0.489	0.274	0.132	0.773	0.193	0.244
DBT	0.322	0.461	0.376	0.573	0.256	0.702	0.227	0.228	0.231	0.221	0.158
TBT	1.07	0.29	1.31	0.67	0.98	0.79	0.74	1.97	1.53	0.53	0.45
MPT	0.45	0.2	0.21	0.27	0.12	0.25	0.1	0.29	0.31	0.12	0.23
DPT	0.987	0.762	0.286	0.34	0.77	1.863	0.23	0.21	0.385	0.29	0.764
TPT	1.87	0.335	0.624	1.78	1.077	1.142	0.4	1.2	1.01	1.285	0.977

Table 7: Concentration of organotins in the seawater samples collected in the vicinity of Dammam.

	Water samples (µg/L)					
	4	5	8	9	10	11
	[c] Shipping Channel, Dammam (Site ID: 4, 5)		Near Dammam Corniche (Site ID: 8-11)			
MBT	0.461	0.804	0.746	1.467	1.519	1.297
DBT	0.448	0.405	0.109	0.447	0.613	0.449
TBT	0.14	1.86	0.39	0.83	0.2	1.29
MPT	0.22	0.29	0.21	0.62	0.26	0.5
DPT	0.192	1.157	1.284	1.313	1.793	0.312
TPT	0.582	0.93	0.119	0.116	0.21	0.53

Total mean concentrations of organotins in sediment samples
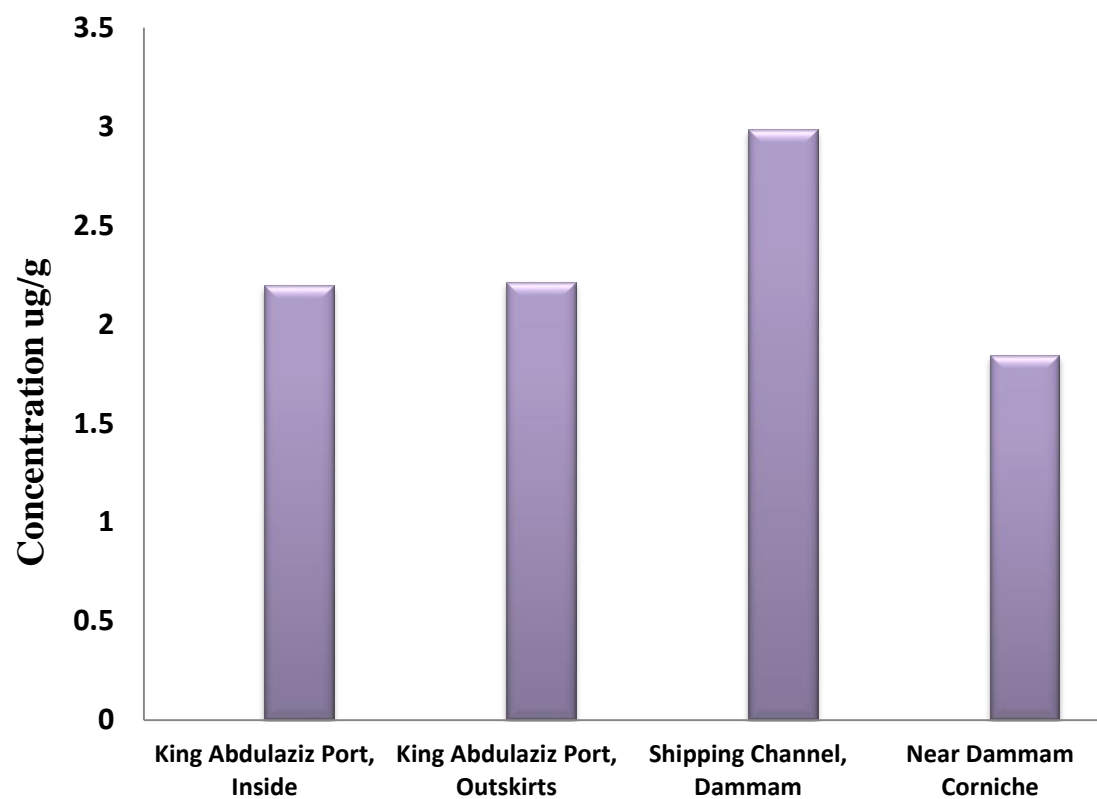
Figure 11: Overall mean concentrations of organotins in sediment samples

Table 8: Organotin species in sediment matrix at different locations.

	Concentrations in µg/g*										
	1	15	16	17	2	3	6	7	12	13	14
	[a] King Abdulaziz Port, Inside (Site ID: 1, 25-27)				[b] King Abdulaziz Port, Outskirts (Site ID: 2, 3, 6, 7, 18-20)						
MBT	0.19	nd	0.18	nd	0.19	nd	0.19	0.19	nd	nd	nd
DBT	1.49	0.14	0.50	0.19	0.68	0.83	1.07	0.75	0.39	0.91	0.66
TBT	1.07	0.29	0.75	0.67	0.98	0.79	0.74	0.50	0.15	0.53	0.45
MPT	0.45	0.20	0.21	0.27	0.12	0.25	0.10	0.29	0.31	0.12	0.23
DPT	0.59	0.19	0.33	0.12	0.12	0.20	0.32	0.47	0.41	0.30	nd
TPT	0.28	0.35	0.18	0.18	0.15	0.44	0.21	0.82	0.26	0.27	0.14

Table 9: Organotin species in sediment matrix at different locations.

	Sediment samples (µg/g)					
	4	5	8	9	10	11
	[c] Shipping Channel, Dammam (Site ID: 4, 5)		Near Dammam Corniche (Site ID: 8-11)			
MBT	nd	nd	0.17	0.17	0.09	0.17
DBT	0.67	1.64	0.45	0.86	0.87	0.43
TBT	1.14	0.85	0.39	0.11	0.10	0.06
MPT	0.22	0.29	0.21	0.62	0.26	0.50
DPT	0.22	0.39	0.15	0.41	0.07	0.30
TPT	0.25	0.30	0.14	0.06	0.30	0.49

Generally, in water and sediment matrices, the areas around NDC possess higher concentrations of OTs. This is not surprising since this area is highly polluted with inputs from both agricultural and industrial discharges. Some agricultural and industrial activities may contribute large amounts of OTs into the maritime systems [94]. High ratios of TBT or TPT to respective degradation species may suggest either long residence time where degradation occurs at slow pace or an evidence of new inputs from anthropogenic sources. The process and mechanism controlling the degradation of these analytes may determine which species is ultimately present and at what concentration. High ratios of TBT/DBT in sediments may suggest slower rate of degradation in this microenvironment . In sea water around NDC where turbidity is high (up to 8.1 NTU), biodegradation was expected to be high which would lead to low TBT/DBT ratios. Therefore, the high TBT/DBT ratios here may strengthen the indication of recent discharges from anthropogenic sources. In the study areas, sea water appears to have high concentration of *chl-a* which may reach up to 19.7 μg L^{-1} at NDC. Although it was earlier reported that *chl-a* content of freshwater algae *Scenedesmus quadricauda* was impacted negatively by OTs [95], some bacterial and cyanobacterial communities known to carry out biodegradation of organic pollutants may be resistant to OTs and can facilitate their biodegradation [96, 97]. The not very high ratios of TPT/DPT may suggest rapid degradation of TPT or limited inputs from new sources. We did not detect MPT in any of our samples which may indicate high rate of photolytic dephenylation or its presence is below our method detection limits. Previously, we have reported the absence of MPT in Singapore coastal waters.[98].

Table 10: Organotin species in sediment matrix at different locations.

Analytes	Slope±SD (x 10^{-3})	Intercept±SD (x 10^{-5})	R^{2*}	LOD (ng g^{-1})	LOQ (ng g^{-1})	% Mean recovery	%RSD (n=3)
MBT	1.81±0.32	3.35±1.17	0.9960	9.3	30.1	81	10
DBT	0.35±0.02	9.97±1.37	0.9931	12.9	38.2	94	12.1
TBT	0.1±0.01	12.1±1.6	0.9891	9.3	30.1	85	13.0
MPT	0.19±0.01	0.09±0.01	0.9892	7.7	23.1	88	9.3
DPT	0.53±0.6	1.02±0.17	0.9992	9.0	27.0	90	11.0
TPT	1.6±0.5	0.36±0.03	0.9986	14.1	42.3	81	14.3

[*] For the concentration range of 0.05-1000 μg g^{-1}

According to United Kingdom Environmental Quality Standard, 20 ng/L of TBT is high enough to cause toxicological complications [99]. From our study, sea water samples and sediments from the southern part of the Arabian Gulf could be termed as highly contaminated with OTs. With the heavy ship and boat traffic at the King AbdulAziz Port, in particular, as well as agricultural and industrial activities onshore, the level of OTs in our study locations would not be surprising. High concentrations of OTs have been reported earlier in the waters and sediment samples of neighboring Bahrain [11] with maximum value of 1930 ng/g. Elsewhere, a whopping 43mg/kg was recorded at port of Antwerp, Belgium and near ship repair station [53].

Table 11: Concentrations organotin in seafood samples collected from local environment.

	Trivially fish	Barracuda fish	Stripped red mullet	Emperor fish	Solea	Oyster	Crab	Indian mackerel	Squid	Shrimp
				Biota Concentrations in ng/g						
MBT	nd	0.327	nd	nd	0.120	nd	nd	0.113	nd	nd
DBT	3.720	8.986	3.823	3.542	5.944	6.038	6.528	7.927	7.260	8.121
TBT	8.008	3.252	0.828	0.543	0.679	1.493	0.665	1.046	2.230	0.889
MPT	0.875	3.472	0.928	0.887	0.680	5.870	1.420	1.307	3.234	4.054
DPT	nd	0.419	nd	nd	0.261	0.427	nd	0.297	0.439	0.140
TPT	nd	0.243	0.097	0.150	0.267	0.815	nd	nd	0.735	0.221

3.4   CONCLUSIONS

In this study, we have determined six different species of OTs in sediment and sea waters of the southern coast of Arabian Gulf using propylative derivatization in combination with solid-liquid and solid-phase extraction techniques and GC-MS analysis. These methods have low analytical limits which were adequate for the determination and quantitation of these analytes. All species, with the exception of MPT, were found at various sites within the study area. Concentrations in sediments are higher than in surface waters. Concentrations determined indicate that these locations are highly contaminated with these organotins species in addition to other possible contaminants. There is high possibility of exposure of the general populace to some health risks considering the potential as well as established toxic and anti-androgenic effects of these compounds. It is worth to mention that regular monitoring organotins concentration at the shipping zone of Saudi Arabia is need to completely understand the fate of thee organotin pollution in our local marine environment.

.

Reference:

[1].   Saraji, M. and A.A.H. Bidgoli, *Single-drop microextraction with in-microvial derivatization for the determination of haloacetic acids in water sample by gas chromatography–mass spectrometry.* Journal of Chromatography A, 2009. **1216**(7): p. 1059-1066.

[2].   Rezaee, M., et al., *Determination of organic compounds in water using dispersive liquid–liquid microextraction.* Journal of Chromatography A, 2006. **1116**(1–2): p. 1-9.

[3].   H.Zhou, e.a., *Occurrnce of Haloacetic in Drinking Water in Certain Cities of Chaina.* Biomedical and Environmental Sciences, 2004. **17**: p. 299-308.

[4].   R. Al-Horr, e.a., *measurement of Haloacetic Acids in Drinking Water by IC-MS and IC-MS/MS Dionex Corporation.* Sunnyvale, CA USA.

[5].   Agency, U.E.P., *Disinfectants and disinfection byproducts rule.* 2006. **EPA-HQ-OW-2002-0043.Washington**
.

[6].   Champ, M.A., Seligman, Peter F, *Organotin: environmental fate and effects*. Vol. 20. 1996: Springer.

[7].   Belfroid, A.C., M. Purperhart, and F. Ariese, *Organotin Levels in Seafood.* Marine Pollution Bulletin, 2000. **40**(3): p. 226-232.

[8].   Sheikh, M.A., et al., *Detection and ecological threats of PSII herbicide diuron on coral reefs around the Ryukyu Archipelago, Japan.* Marine Pollution Bulletin, 2009. **58**(12): p. 1922-1926.

[9].   Harris, D.C., in *Quantitative Chemical Analysis.* 2011, Michelson Laboratory: China Lake p. 74.

[10].   BLABER, S.J., *The occurrence of a penis-like outgrowth behind the right tentacle in spent females of Nucella lapillus (L.).* Journal of Molluscan Studies, 1970. **39**(2-3): p. 231-233.

[11].   Ali Hasan, M. and H. Ahmed Juma, *Assessment of tributyltin in the marine environment of Bahrain.* Marine Pollution Bulletin, 1992. **24**(8): p. 408-410.

[12].   De Mora, S., S. Fowler, R. Cassi, and I.Tolosa   *Assessment of organotin contamination in marine sediments and biota from the Gulf and adjacent region.* Marine Pollution Bulletin, Tolosa (2003). **46**: p. 401.

[13]. Schummer, C., et al., *Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis.* Talanta, 2009. **77**(4): p. 1473-1482.

[14]. Reimann, S., K. Grob, and H. Frank, *Chloroacetic Acids in Rainwater.* Environmental Science & Technology, 1996. **30**(7): p. 2340-2344.

[15]. Brashear, W.T., C.T. Bishop, and R. Abbas, *Electrospray Analysis of Biological Samples for Trace Amounts of Trichloroacetic Acid, Dichloroacetic Acid, and Monochloroacetic Acid.* Journal of Analytical Toxicology, 1997. **21**(5): p. 330-334.

[16]. Cantor, K., *Drinking water and cancer.* Cancer Causes & Control, 1997. **8**(3): p. 292-308.

[17]. USEPA, *Controlling Disinflection By-Products and Microbial Contaminants in Drinking Water,.* Office of Research and Development, Washington DC 20460, EPA/600/R-01/110, 2001.

[18]. USEPA, *National Primary Drinking Water Regulations: Disinfectants and Disinfection By-Products Notice of Data Availability.* Office of Ground Water and Drinking Water,, 1998.

[19]. D.J. Munch, J.W.M., A.M. Pawlecki, , *Determination of haloacetic acids and dalapon in drinking water by liquid–liquid extraction, derivatization and gas chromatography with electron capture detection, Methods for the Determination of Organic Compounds in DrinkingWater.* EPA method 552.2, Revision 1.0,, 1995(EPA/600/R-95/131 (Suppl. III), Cincinnati, OH).

[20]. USEPA, a., *National Primary Drinking Water Regulations: Disinfectants and Disinfection By-Products Notice of Data Availability.* Office of Ground Water and Drinking Water, 1998a.

[21]. Hashimoto, S. and A. Otsuki, *Simultaneous Determination of Haloacetic Acids in Environmental Waters using Electrospray Ionization Liquid Chromatography Mass Spectrometry.* Journal of High Resolution Chromatography, 1998. **21**(1): p. 55-58.

[22]. Sarzanini, C., M.C. Bruzzoniti, and E. Mentasti, *Preconcentration and separation of haloacetic acids by ion chromatography.* Journal of Chromatography A, 1999. **850**(1–2): p. 197-211.

[23]. Heller-Grossman, L., et al., *Formation and distribution of haloacetic acids, THM and TOX in chlorination of bromide-rich lake water.* Water research, 1993. **27**(8): p. 1323-1331.

[24]. Martínez, D., F. Borrull, and M. Calull, *Evaluation of different electrolyte systems and on-line preconcentrations for the analysis of haloacetic acids by capillary zone electrophoresis.* Journal of Chromatography A, 1999. **835**(1–2): p. 187-196.

[25]. WHO, l.a., *Desinfection de l'eau.* health and environment briefing pamphlet series, 1995. **3**.

[26]. Sarrion, M., F. Santos, and M. Galceran, *Solid-phase microextraction coupled with gas chromatography–ion trap mass spectrometry for the analysis of haloacetic acids in water.* Journal of Chromatography A, 1999. **859**(2): p. 159-171.

[27]. Domino, M.M., et al., *Optimizing the determination of haloacetic acids in drinking waters.* Journal of Chromatography A, 2004. **1035**(1): p. 9-16.

[28]. Sarrión, M., F. Santos, and M. Galceran, *In situ derivatization/solid-phase microextraction for the determination of haloacetic acids in water.* Analytical chemistry, 2000. **72**(20): p. 4865-4873.

[29]. H. Liu, P.K.D., *Analytical hemistry in a Drop. Solvent Extraction in a Microdrop.* Anal. Chem, 1996. **68**: p. 1817-1821.

[30]. Chester, T., J. Pinkston, and D. Raynie, *Supercritical fluid chromatography and extraction.* Analytical chemistry, 1994. **66**(12): p. 106R-130R.

[31]. Djozan, D. and Y. Assadi, *Modified Pencil Lead as a New Fiber for Solid-Phase Microextraction.* Chromatographia, 2004. **60**(5-6): p. 313-317.

[32]. Cacho, J., V. Ferreira, and P. Fernández, *Microextraction by demixing for the determination of volatile compounds in aqueous solutions.* Analytica Chimica Acta, 1992. **264**(2): p. 311-317.

[33]. Wahlen, R. and T. Catterick, *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.* Journal of Chromatography B, 2003. **783**(1): p. 221-229.

[34]. Hodgeson, J.W.a.D.B., *Methods for the Determination of Organic Compounds in Drinking Water, Supplement II.* US Environmental Protection Agency, Cincinnati,OH, 1992.

[35]. A.D. Eaton, e.a., (Eds.), Standard method 6251B, 19th ed.Washington, DC,, 1995.

[36]. M.M. Domino, B.V.P., D.J. Munch, Y. Xie, , EPA method 52.3,Revision1.0,EPA Document EPA-815-B-03-002, Cincinnati, OH,, 2003.

[37]. Mateo-Vivaracho, L., J. Cacho, and V. Ferreira, *Quantitative determination of wine polyfunctional mercaptans at nanogram per liter level by gas chromatography–negative ion mass spectrometric analysis of their pentafluorobenzyl derivatives.* Journal of Chromatography A, 2007. **1146**(2): p. 242-250.

[38]. Mateo-Vivaracho, L., V. Ferreira, and J. Cacho, *Automated analysis of 2-methyl-3-furanthiol and 3-mercaptohexyl acetate at ng L-1 level by headspace solid-phase microextracion with on-fibre derivatisation and gas chromatography-negative chemical ionization mass spectrometric determination.* Journal of Chromatography A, 2006. **1121**(1): p. 1-9.

[39]. Jia, M., et al., *Simultaneous determination of trace levels of nine haloacetic acids in biological samples as their pentafluorobenzyl derivatives by gas chromatography/tandem mass spectrometry in electron capture negative ion chemical ionization mode.* Analytical chemistry, 2003. **75**(16): p. 4065-4080.

[40]. Magnuson, M.L. and C.A. Kelty, *Microextraction of nine haloacetic acids in drinking water at microgram per liter levels with electrospray-mass spectrometry of stable association complexes.* Analytical chemistry, 2000. **72**(10): p. 2308-2312.

[41]. P. C. ARNI, e.a., *Use of trifluoroacetic anhydride in the esterification of wood by carboxylic acids.* J. appl. Chem., 1961: p. 157.

[42]. Zabaljauregui, M., et al., *Fast method for routine simultaneous analysis of methylmercury and butyltins in seafood.* Journal of chromatography A, 2007. **1148**(1): p. 78-85.

[43]. Bravo, M., et al., *Determination of organotin compounds by headspace solid-phase microextraction–gas chromatography–pulsed flame-photometric detection (HS-SPME–GC–PFPD).* Analytical and bioanalytical chemistry, 2005. **383**(7-8): p. 1082-1089.

[44]. Centineo, G., et al., *Isotope dilution GC-MS routine method for the determination of butyltin compounds in water.* Analytical and bioanalytical chemistry, 2006. **384**(4): p. 908-914.

[45]. Panggabean, A.S., M.B. Amran, and S. Achmad, *Speciation of Organotin Compounds with Ion Pair-Reversed Phase Chromatography Technique.* Eurasian Journal of Analytical Chemistry, 2009. **4**(2): p. 215-225.

[46]. Dirkx, W.M., R. Lobiński, and F.C. Adams, *Speciation analysis of organotin in water and sediments by gas chromatography with optical spectrometric detection after extraction separation.* Analytica chimica acta, 1994. **286**(3): p. 309-318.

[47]. Fairman, B. and R. Wahlen, *Speciation analysis of organotin compounds by HPLC-ICP-MS.* Spectroscopy Europe, 2001. **13**(5): p. 16-23.

[48]. Wahlen, R., *A comparison of GC-ICP-MS and HPLC-ICP-MS for the analysis of organotin compounds.* LC GC EUROPE, 2002. **15**(10): p. 670-677.

[49]. Higashiyama, T., et al., *Concentrations of organotin compounds in blue mussels from the wharves of Tokyo Bay.* Marine Pollution Bulletin, 1991. **22**(12): p. 585-587.

[50]. Ritsema, R., *Dissolved Butyltins in Marine Waters of the Netherlands Three Years After the Ban.* APPLIED ORGANOMETALLIC CHEMISTRY, 1994. **8**: p. 5-10.

[51]. ., U.E.P.A.m., *Determination of Organotins by Micro-Liquid Chromatography-Electrospray Ion Trap Mass Spectrometry* 2003(0).

[52]. Arambarri, I., R. Garcia, and E. Millan, *Assessment of tin and butyltin species in estuarine superficial sediments from Gipuzkoa, Spain.* Chemosphere, 2003. **51**(8): p. 643-649.

[53]. Devos, C., et al., *Automated headspace-solid-phase micro extraction–retention time locked-isotope dilution gas chromatography–mass spectrometry for the analysis of organotin compounds in water and sediment samples.* Journal of Chromatography A, 2005. **1079**(1): p. 408-414.

[54]. Wasik, A., et al., *Optimisation of pressurised liquid extraction for elimination of sulphur interferences during determination of organotin compounds in sulphur-rich sediments by gas chromatography with flame photometric detection.* Chemosphere, 2007. **68**(1): p. 1-9.

[55]. Ruedel, H., P. Lepper, J. Steinhanses, and C. Schroeter-Kermani *Retrospective Monitoring of Organotin Compounds in Marine Biota from 1985 to 1999: Results from the German Environmental Specimen Bank.* Environmental Science and Technology, 2003. **37**(9): p. 1731.

[56]. Harino, H., et al., *Organotin compounds in Mersey and Thames Estuaries a decade after UK TBT legislation.* Journal of the Marine Biological Association of the UK, 2003. **83**(01): p. 11-22.

[57]. Tessier, E., D. Amouroux, and O.F. Donard, *Volatile organotin compounds (butylmethyltin) in three European estuaries (Gironde, Rhine, Scheldt).* Biogeochemistry, 2002. **59**(1-2): p. 161-181.

[58]. Ikeda, e.a., *bioaccumulation of organotin compounds through the food web in the deep water of Japan Sea.* J. Koyama, Kankyo Kagaku,, 2002. **12**: p. 105.

[59]. Chatani, Y., T. Ogawa, R. Kitano, M. Ohfuji, and M. Takemae, *Survey of endocrine disrupting chemicals in fish and shellfish: PCB, organochlorine pesticides, bisphenol A, and organotin compounds* Kyoto-fu Hoken Kankyo Kenkyusho Nenpo,, 2001. **46**: p. 14.

[60]. Michel, P. and B. Averty, *Contamination of French coastal waters by organotin compounds: 1997 update.* Marine Pollution Bulletin, 1999. **38**(4): p. 268-275.

[61]. Bryan, G.W., et al., *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 of the United Kingdom, 1986. **66**(03): p. 611-640.

[62]. Alizieu, C., *Tributyltin: Case study of a chronic contaminant in the coastal environment* Ocean and Coastal Management, , 1998. **40**: p. 23.

[63]. Alizieu, C., J. Sanjuan, J. P. Deltriel and M. Borel *Tin contamination in Arcachon Bay: Effects on oyster shell anomalies* Marine Pollution Bulletin,, 1986. **17**: p. 494.

[64]. SMITH, B.S., *Sexuality in the American mud snail, Nassarius obsoletus Say.* Journal of Molluscan Studies, 1971. **39**(5): p. 377-378.

[65]. Gibbs, P., P. Pascoe, and G. Burt, *Sex change in the female dog-whelk, Nucella lapillus, induced by tributyltin from antifouling paints.* Journal of the Marine Biological Association of the United Kingdom, 1988. **68**(04): p. 715-731.

[66]. Gibbs, P.E., *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,, 1993. **73**: p. 667.

[67]. Alizieu, C., J. Sanjuan, P. Michel, M. Borel and J. P. Dreno *Monitoring and assessment of butyltins in Atlantic coastal waters* Marine Pollution Bulletin,, 1989. **20**: p. 22.

[68]. Sadiki, A.-D. and D.T. Williams, *A study on organotin levels in Canadian drinking water distributed through PVC pipesa.* Chemosphere, 1999. **38**(7): p. 1541-1548.

[69]. Wax, P.M., and L. Dockstader *Tributyltin use in interior paints: a continuing health hazar.* J. Toxicol. Clin. Toxicol., 1995. **33**: p. 239.

[70]. Rey, C.H., H. J. Reinecke, and R. Besser *Methyltin intoxication in six men,.* Toxicologic and clinical aspects Vet. Hum. Toxicol. , 1984. **26**: p. 121.

[71].   Kurisaka, H.A., and Y. Ogawa *Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats.* J. Appl. Toxicol. , 1995. **15**: p. 297.

[72].   Whalen, M.M., B.G. Loganathan, and K. Kannan, *Immunotoxicity of Environmentally Relevant Concentrations of Butyltins on Human Natural Killer Cells< i> in Vitro</i>.* Environmental research, 1999. **81**(2): p. 108-116.

[73].   Xiao, Y., G. J. Harry, and K. R. Pennypacker *Expression of AP-1 transcription factors in rat hippocampus and cerebellum after trimethyltin neurotoxicity* . Neurotoxicology 1999. **20**: p. 761.

[74].   Noda, T., et al., *Comparative teratogenicity of di-n-butyltin diacetate with n-butyltin trichloride in rats.* Archives of environmental contamination and toxicology, 1992. **23**(2): p. 216-222.

[75].   Borenfreund, E. and H. Babich, *In vitro cytotoxicity of heavy metals, acrylamide, and organotin salts to neural cells and fibroblasts.* Cell biology and toxicology, 1987. **3**(1): p. 63-73.

[76].   Hamasaki, T., et al., *The genotoxicity of organotin compounds in SOS chromotest and rec-assay.* Mutation Research/Genetic Toxicology, 1992. **280**(3): p. 195-203.

[77].   Hamasaki, T., et al., *The mutagenicity of organotin compounds as environmental pollutants.* Mutation Research/Genetic Toxicology, 1993. **300**(3): p. 265-271.

[78].   Santos, M., et al., *Organotin levels in seafood from Portuguese markets and the risk for consumers.* Chemosphere, 2009. **75**(5): p. 661-666.

[79].   Zhang, K., et al., *Organotin compounds in surface sediments from selected fishing ports along the Chinese coast.* Chinese Science Bulletin, 2013. **58**(2): p. 231-237.

[80].   Ohji, M., et al., *Distribution and fate of organotin compounds in Japanese coastal waters.* Water, air, and soil pollution, 2007. **178**(1-4): p. 255-265.

[81].   Bhosle, N.B., et al., *Butyltins in the sediments of Kochi and Mumbai harbours, west coast of India.* Environment International, 2006. **32**(2): p. 252-258.

[82].   de Oliveira, C.R., et al., *Speciation of butyltin derivatives in surface sediments of three southern Brazilian harbors.* Journal of hazardous materials, 2010. **181**(1): p. 851-856.

[83].   Milivojevič Nemanič, T., R. Milačič, and J. Ščančar, *A Survey of Organotin Compounds in the Northern Adriatic Sea.* Water, Air, and Soil Pollution, 2009. **196**(1-4): p. 211-224.

[84]. Harino, H., et al., *Distribution of organotin compounds in representative coastal areas from Japan: a review.* 2008.

[85]. Felizzola, J.F., et al., *Butyltin speciation in sediments from Todos os Santos Bay (Bahia, Brazil) by GC-PFPD.* Química Nova, 2008. **31**(1): p. 89-93.

[86]. Roberts, D.A., *Causes and ecological effects of resuspended contaminated sediments (RCS) in marine environments.* Environment international, 2012. **40**: p. 230-243.

[87]. Yi, A.X., et al., *Review of measured concentrations of triphenyltin compounds in marine ecosystems and meta-analysis of their risks to humans and the environment.* Chemosphere, 2012. **89**(9): p. 1015-1025.

[88]. Bangkedphol, S., et al., *Enhancement of tributyltin degradation under natural light by N-doped TiO2 photocatalyst.* Journal of Hazardous Materials, 2010. **184**(1–3): p. 533-537.

[89]. Kucuksezgin, F., et al., *Assessment of organotin (butyltin species) contamination in marine biota from the Eastern Aegean Sea, Turkey.* Marine pollution bulletin, 2011. **62**(9): p. 1984-1988.

[90]. Furdek, M., et al., *Organotin compounds in seawater and< i> Mytilus galloprovincialis</i> mussels along the Croatian Adriatic Coast.* Marine pollution bulletin, 2012. **64**(2): p. 189-199.

[91]. Yozukmaz, A., et al., *The Determination of Organotin Compounds Levels in Sediment Samples from Turkish Aegean Sea Coast.* Turkish Journal of Fisheries and Aquatic Sciences, 2011. **11**: p. 649-660.

[92]. Garg, A., et al., *Distribution of butyltins in the waters and sediments along the coast of India.* Marine pollution bulletin, 2011. **62**(2): p. 423-431.

[93]. Nemanič, T.M., et al., *Organotin compounds in the marine environment of the Bay of Piran, Northern Adriatic Sea.* Journal of Environmental Monitoring, 2002. **4**(3): p. 426-430.

[94]. Bancon-Montigny, C., et al., *High-yield synthesis of milligram amounts of isotopically enriched methylmercury (CH3198HgCl).* Applied organometallic chemistry, 2004. **18**(2): p. 57-64.

[95]. Fargašová, A., *Sensitivity of Chironomus plumosus larvae to V 5+, Mo 6+, Mn 2+, Ni 2+, Cu 2+, and Cu+ metal ions and their combinations.* Bulletin of environmental contamination and toxicology, 1997. **59**(6): p. 956-962.

[96].   Kuritz, T. and C.P. Wolk, *Use of filamentous cyanobacteria for biodegradation of organic pollutants.* Applied and environmental microbiology, 1995. **61**(1): p. 234-238.

[97].   Pain, A. and J. Cooney, *Characterization of organotin-resistant bacteria from Boston Harbor sediments.* Archives of environmental contamination and toxicology, 1998. **35**(3): p. 412-416.

[98].   Basheer, C., K. Tan, and H. Lee, *Organotin and Irgarol-1051 contamination in Singapore coastal waters.* Marine Pollution Bulletin, 2002. **44**(7): p. 697-703.

[99].   Cleary, J. and A. Stebbing, *Organotin in the surface microlayer and subsurface waters of southwest England.* Marine Pollution Bulletin, 1987. **18**(5): p. 238-246.

APPENDIX:

Table A1: Site ID, Station, Coordinates and Sampling Date

Site ID	Station	Coordinates		Date
		X	Y	
1	King AbdulAziz Port, inside	50.19837	26.52295	13/12/10
2	King AbdulAziz Port, outskirts	50.17113	26.50161	20/02/11
3	King AbdulAziz Port, outskirts	50.17426	26.47031	20/02/11
4	Shipping Channel, Dammam	50.22756	26.47632	13/12/10
5	Shipping Channel, Dammam	50.24008	26.45690	13/12/10
6	King AbdulAziz Port, outskirts	50.22474	26.53596	20/02/11
7	King AbdulAziz Port, outskirts	50.26665	26.57264	20/02/11
8	Near Dammam Corniche	50.08182	26.46707	13/02/11
9	Near Dammam Corniche	50.07827	26.47526	13/02/11
10	Near Dammam Corniche	50.08036	26.48379	13/02/11
11	Near Dammam Corniche	50.07976	26.49575	13/02/11
12	King AbdulAziz Port, outskirts	50.12070	26.55055	20/02/11
13	King AbdulAziz Port, outskirts	50.13410	26.58127	20/02/11
14	King AbdulAziz Port, outskirts	50.11090	26.61474	20/02/11
15	King AbdulAziz Port, inside	50.19113	26.51172	08/02/11
16	King AbdulAziz Port, inside	50.21303	26.50175	08/02/11
17	King AbdulAziz Port, inside	50.22078	26.50185	08/02/11

TableA2: : Physico-chemical characteristics of the sampling locations

Station	Temp Tur	DO Chl-a		TDS	Sal		pH
1	17.93	7.27	41.22	39.83	8.48	0.4	1
2	17.41	7.2	41.25	39.85	8.53	1.2	2.4
3	17.88	8.28	40.74	39.41	8.63	0.3	1.8
4	18.39	7.22	41.31	39.9	8.55	1.3	1.2
5	17.95	7.37	41.23	39.87	8.38	0.7	1.6
6	17.41	7.2	41.25	39.85	8.53	1.2	2.4
7	17.75	7.31	41.24	39.84	8.57	1.7	1.1
8	17.8	7.17	41.05	39.68	8.56	0.5	1.6
9	18.39	9.67	31.01	30.91	8.62	8.1	19.7
10	16.79	9.78	38.35	37.37	8.76	3.3	19.6
11	16.46	8.91	39.45	38.33	8.73	2.3	13.6
12	19.61	5.53	40.28	39.01	8.6	2.8	5.8
13	20.75	7.59	39.24	38.08	8.67	1.7	12.5
14	19.63	6.23	40.14	38.08	8.62	1.3	6
15	17.71	7.4	41.3	39.9	8.54	2.3	0.8
16	17.8	7.39	41.37	39.96	8.54	0.6	7
17	17.54	7.29	41.37	39.95	8.54	1.8	1.8
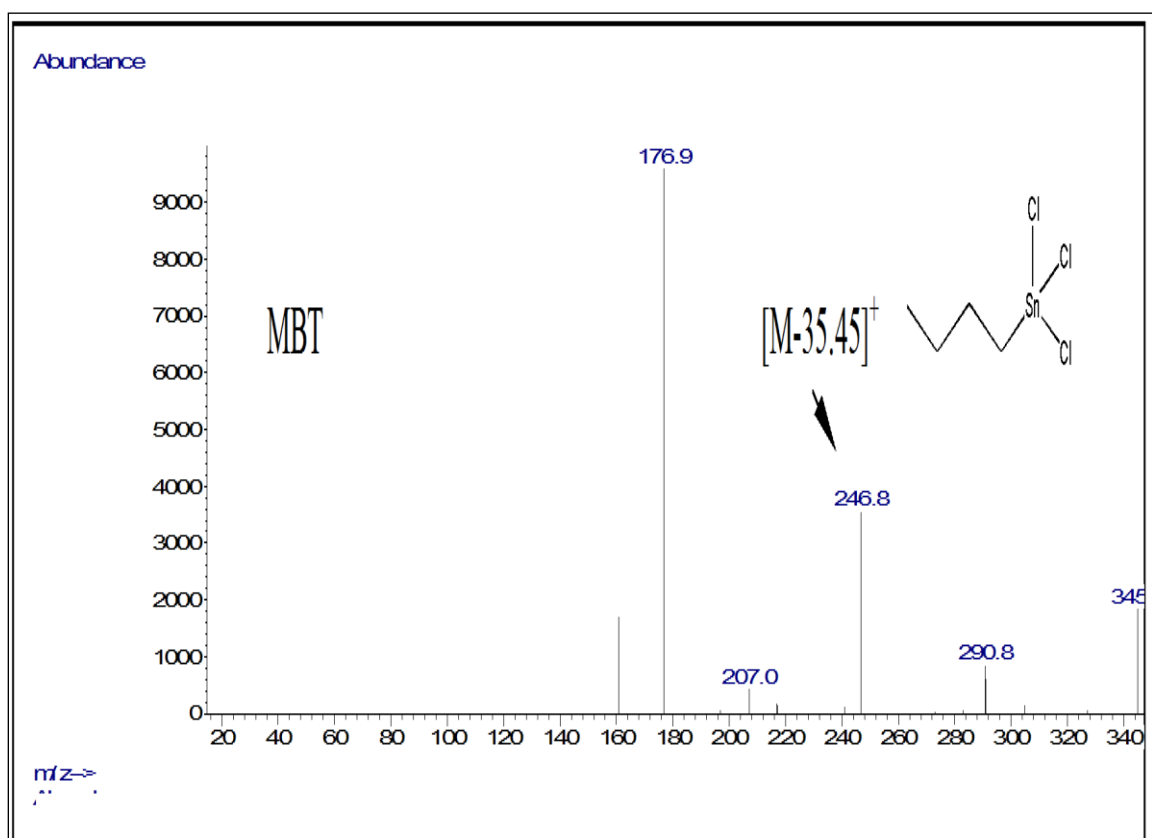
Figure A1: Characteristic ions of the analytes
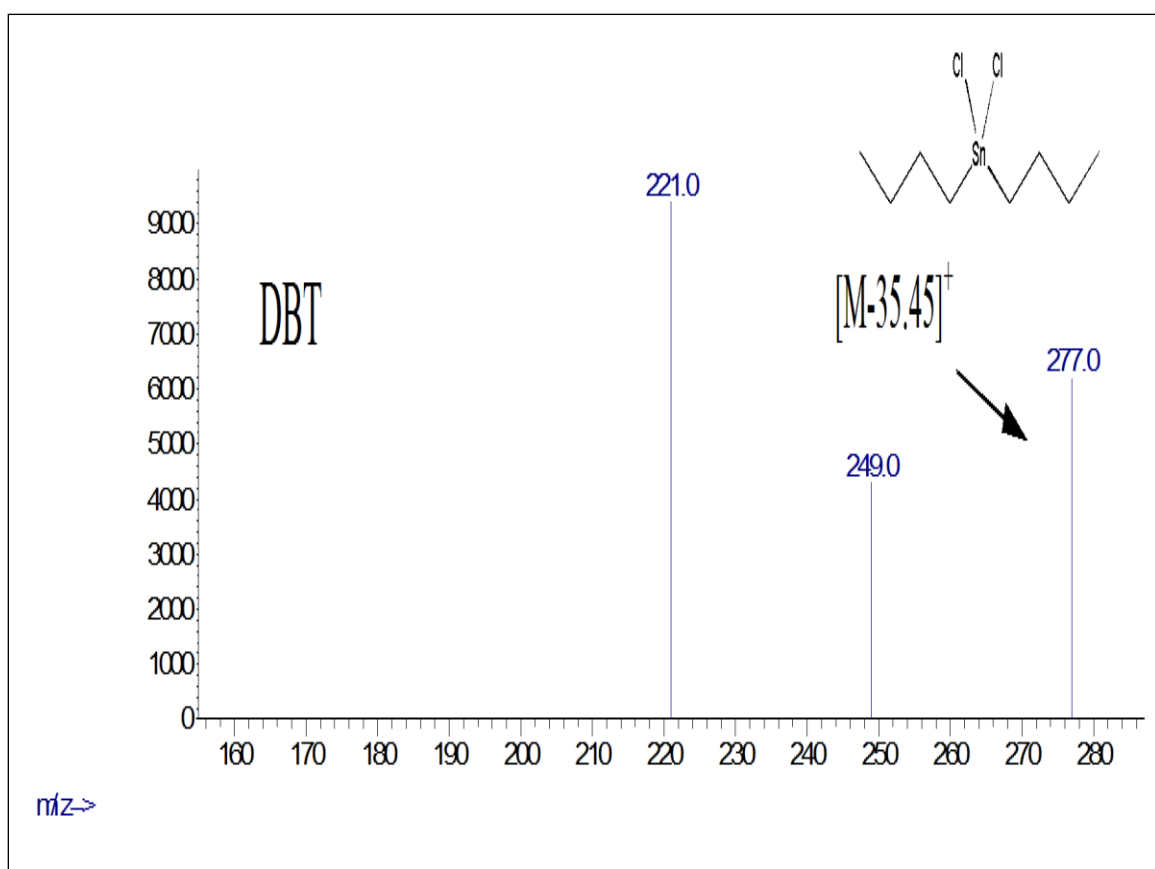
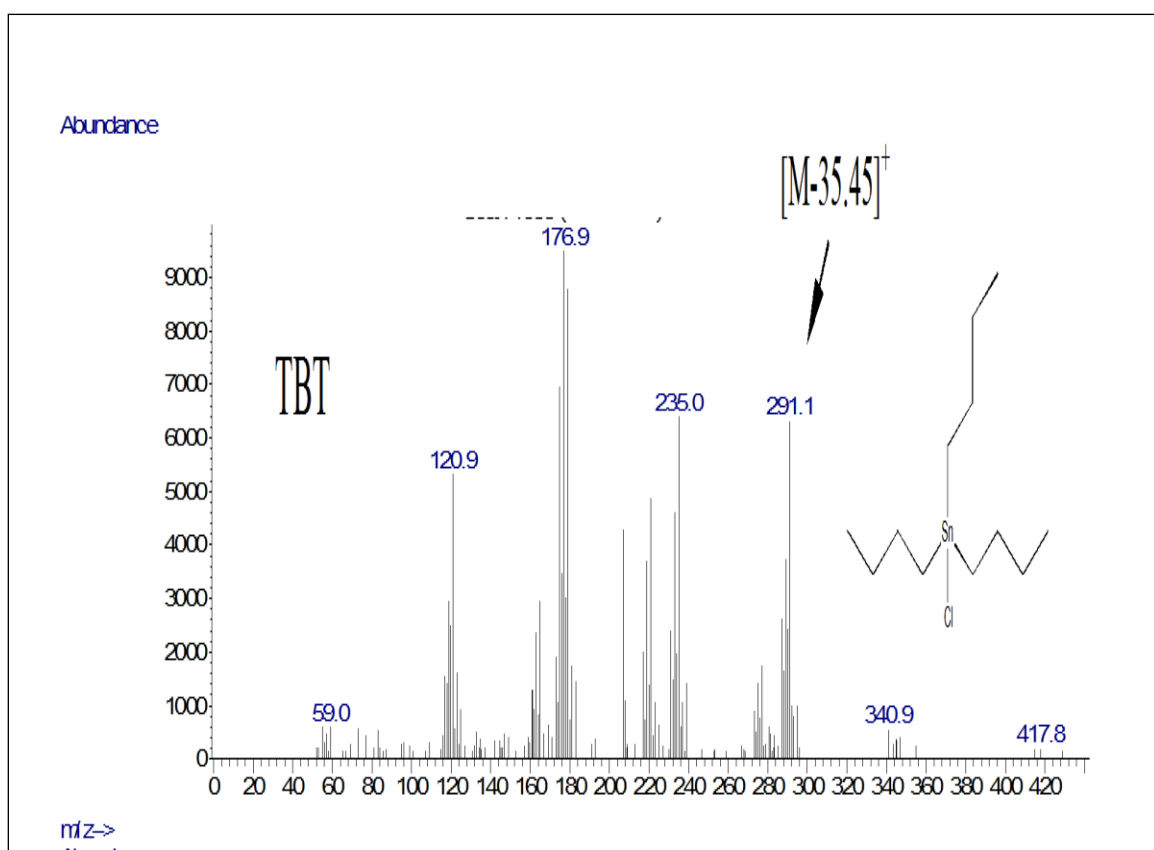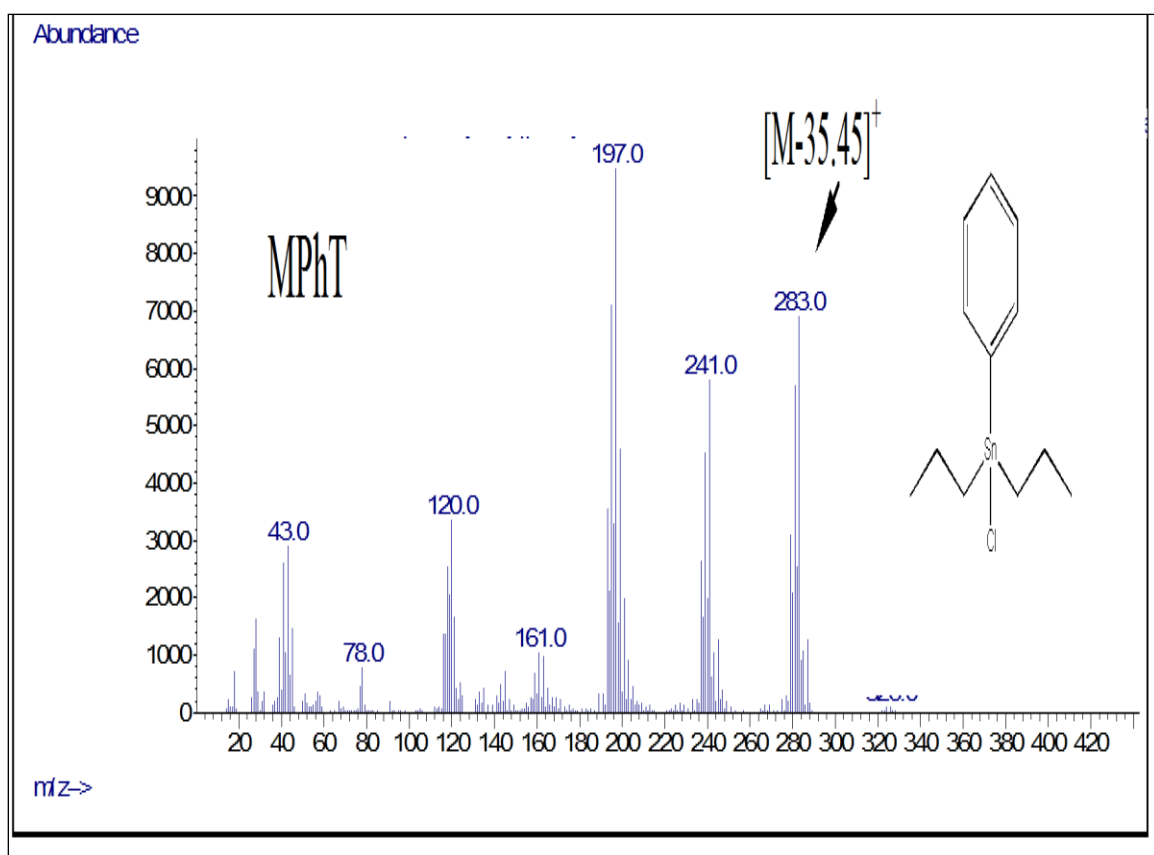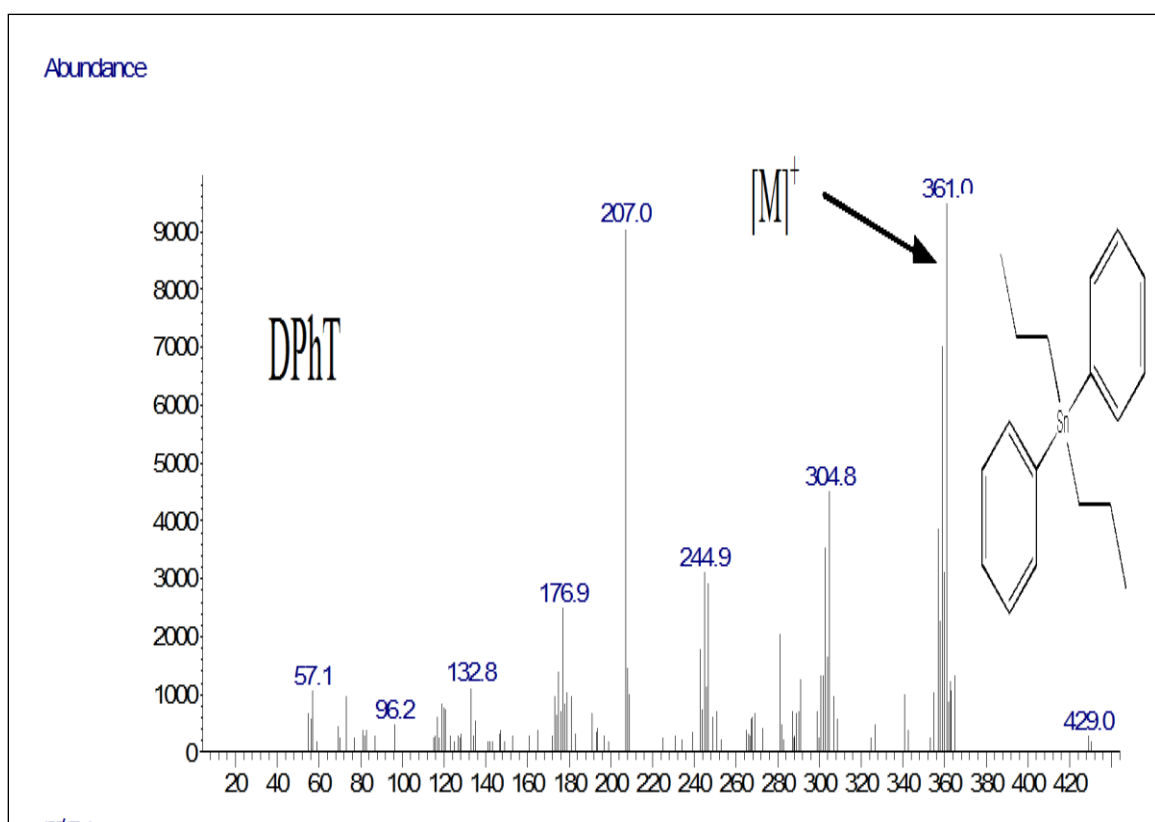Figure A2: Characteristic ions of the analytes

Figure A3: Characteristic ions of the analytes

FigureA4: Characteristic ions of the analytes
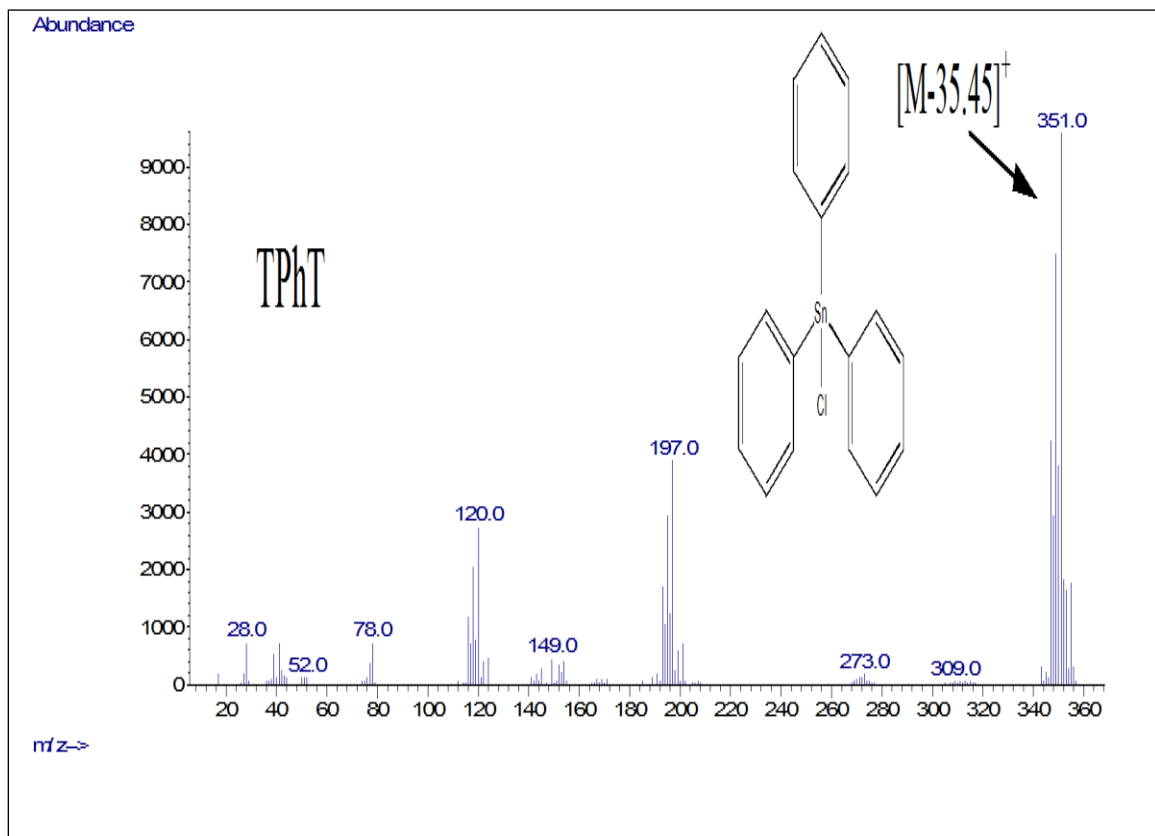
FigureA5: Characteristic ions of the analytes

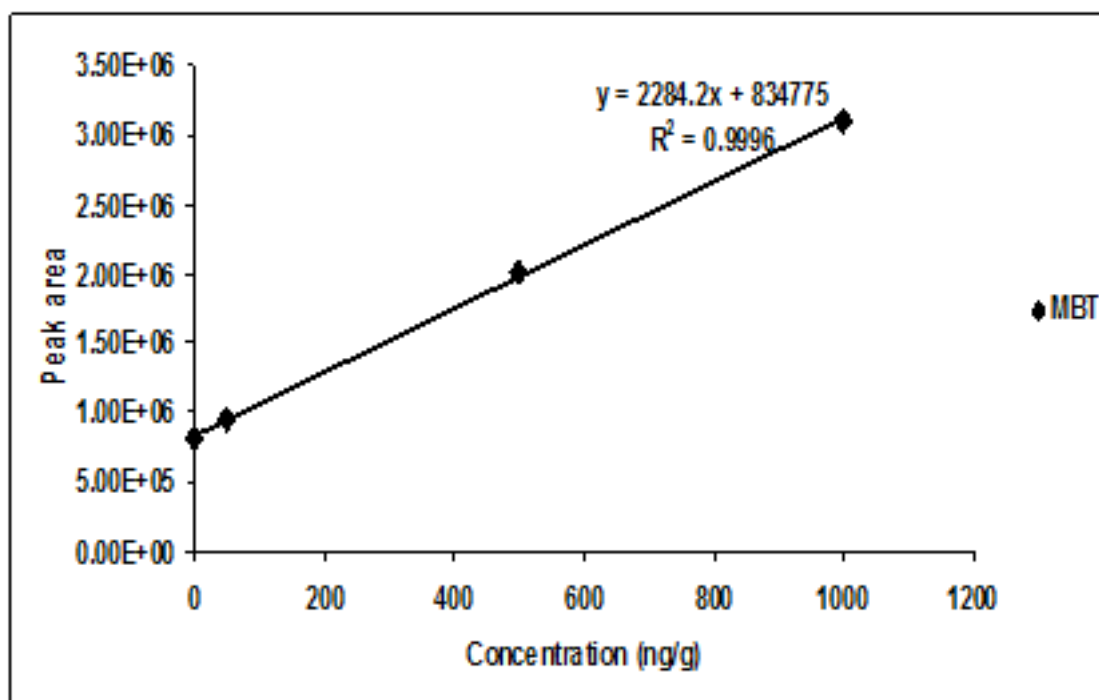Figure A6: Characteristic ions of the analytes

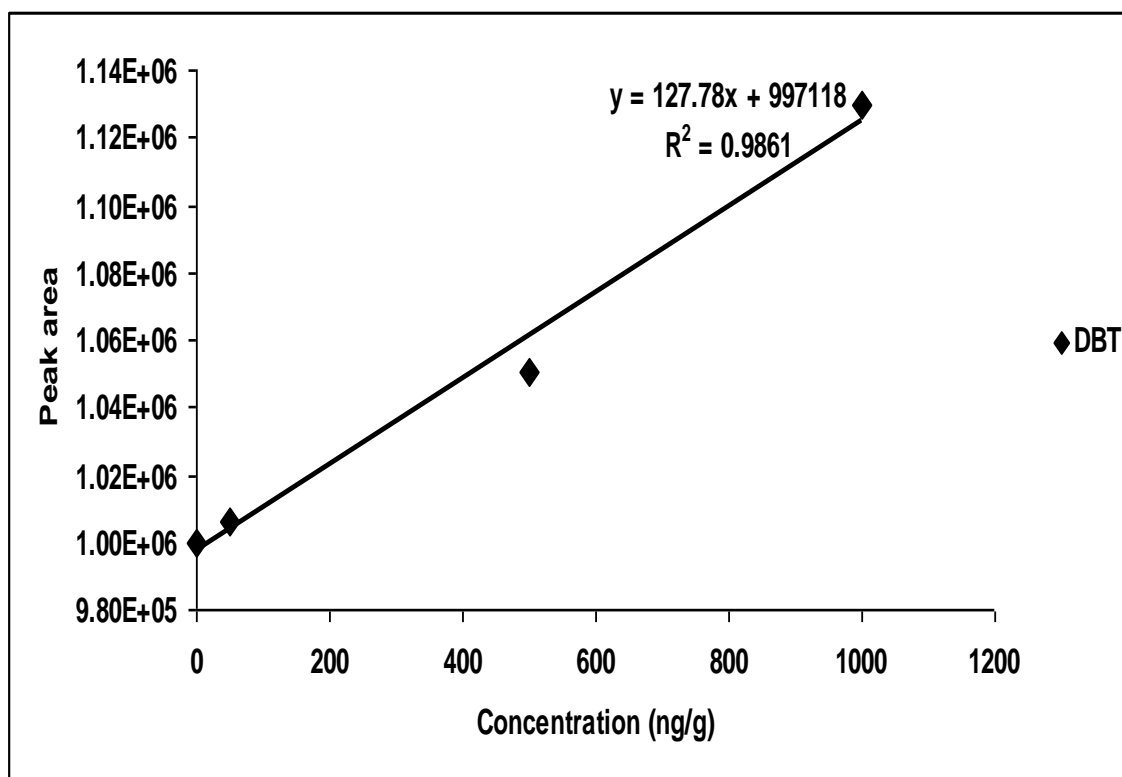Figure B1: Calibration plots for the different organotin species

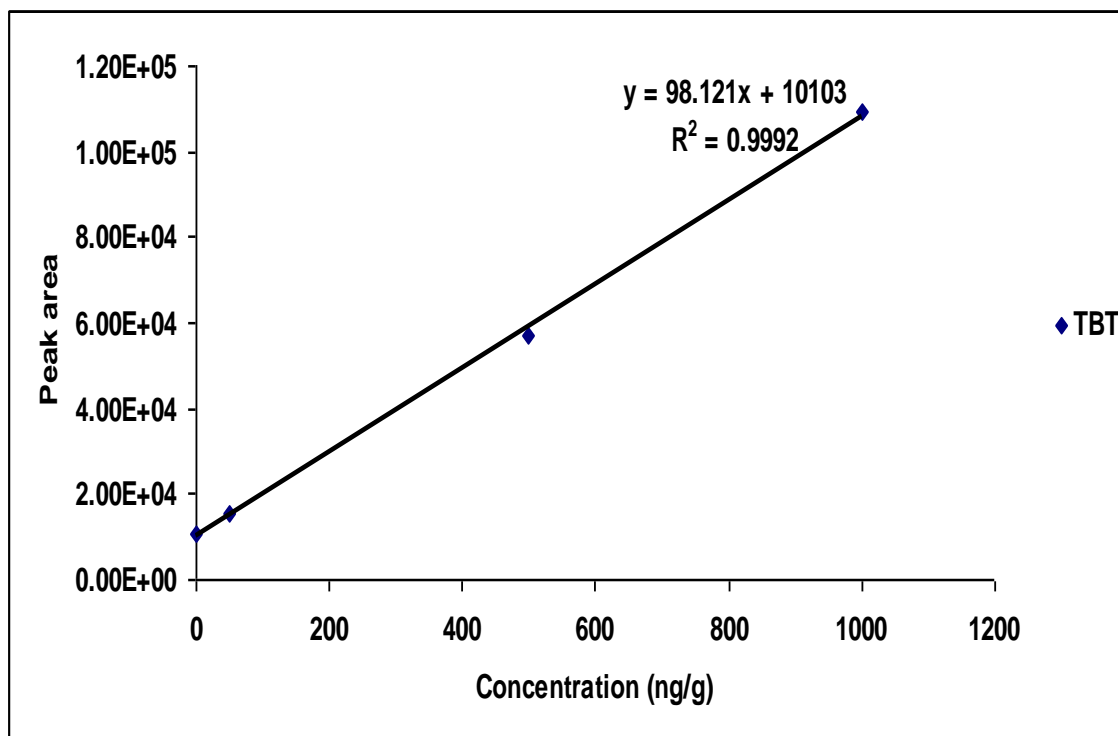Figure B2: Calibration plots for the different organotin species

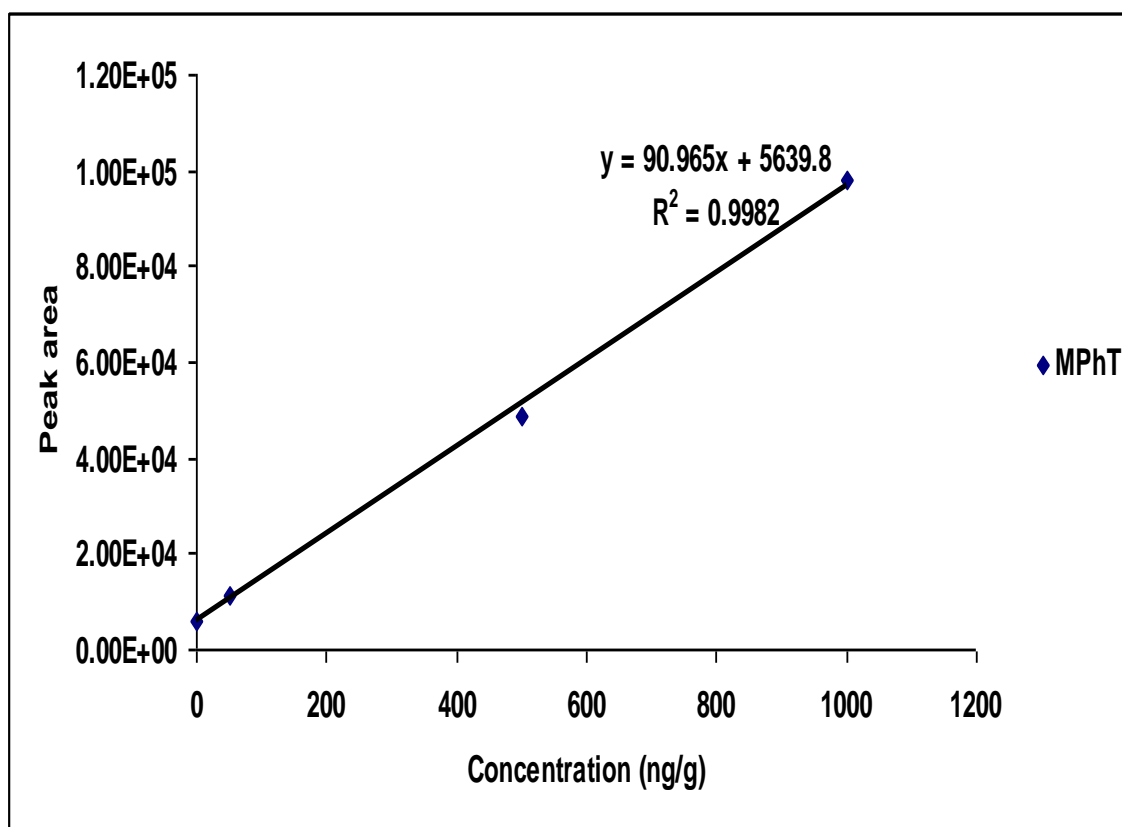Figure B3: Calibration plots for the different organotin species

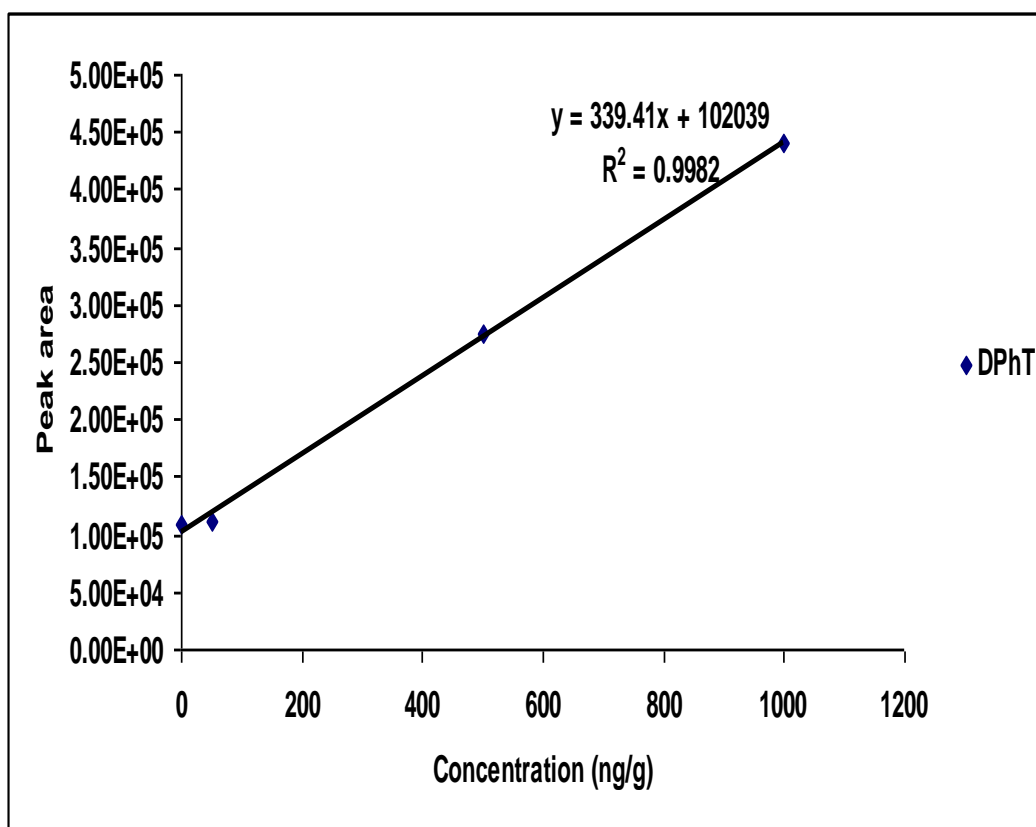Figure B4: Calibration plots for the different organotin species

The chart shows a calibration plot with the following annotations:

- y-axis label: **Peak area**, ranging from 0.00E+00 to 5.00E+05
- x-axis label: **Concentration (ng/g)**, ranging from 0 to 1200
- equation: y = 339.41x + 102039
- $R^2 = 0.9982$
- legend: DPhT

Figure B5: Calibration plots for the different organotin species
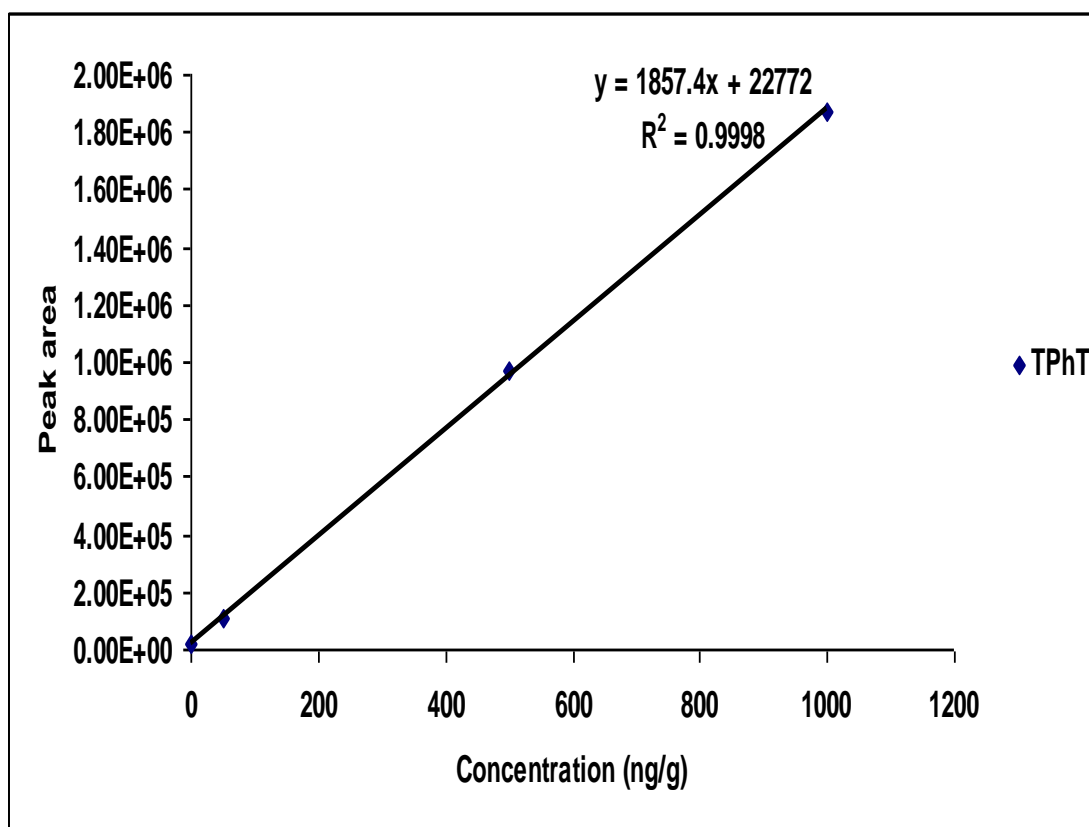
Figure C6: Calibration plots for the different organotin species.

Vitae

Name   :Mohsen Abdo Yahia Al-Shatri

Nationality   :Yemeni

Date of Birth   :12/7/1972

 Email   :malshatri@ymail.com

Address   :Department of chemistry, Aden University -Yemen

Education:

- Bachelor of Science in Chemistry, University of Aden, Aden, Yemen (June 1997).

Work Experience:

- I joined Aden University to work as a Teaching Assistant in the Chemistry Department (2002)

- I have given labs to sophomore, junior, and senior students (2002-2008).

- I was part of a team to correct final exams for high school (2000-2002)

- I work as teacher in the high school (1997-2002)

Publications

a) Mohsen Al-shatri, Abdulmumin A. Nuhu, Chanbasha Basheer, Abdulrahman Al-Arfaj and Bassam Tawabini, "Determination of tributyltin and triphenyltin compounds, and their main catabolic metabolites in Saudi coastal waters by GC-MS analysis". (have been submitted to the Scientific World Journal).

b) Mohsen Al-shatri, Abdulmumin A. Nuhu, Chanbasha Basheer, " Determination of haloacetic acids in bottled and tap water sources by dispersive liquid-liquid microextraction and GC-MS analysis". (submitted to the Scientific World Journal).