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GAS CHROMATOGRAPHIC ANALYSIS OF PHENYLTIN COMPOUNDS

ADJANA PAERUNGRUANG

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
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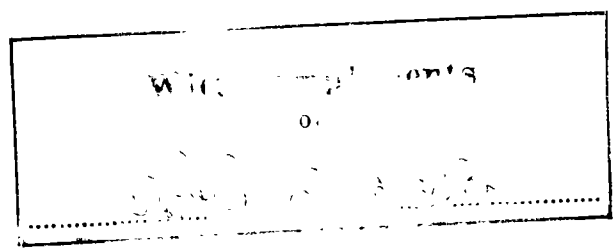

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Adjana Paerungruang

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ABSTRACT

Organotin compounds have been widely used in the plastic industry and in agriculture. The increased production and consumption of organotin chemicals are due primarily to their wide range of industrial application. However, these compounds have wide ranging toxicological properties, and environmental pollution from some organotin derivatives has become a serious problem. The need for accurate and reliable analytical techniques is in highest demand in order to monitor such compounds. This work presents the gas chromatographic data (i.e. detection limit, reproducibility) obtained from different kinds of GC columns. Both capillary and packed column were employed in this study. The optimum

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conditions for GC separation as well as the derivatization steps of phenyltin compounds were examined. The technique developed gave a detection limit of 10.45, 26.55 and 40 ppb for MPT, DPT and TPT, respectively. For real samples, a suitable handling and pretreatment technique was necessary which has led to a successful analytical procedure. Therefore, this work also presents details of the extraction steps prior to introduction to GC. Comparative study between liquid-liquid extraction (LLE) and solid-phase extraction (SPE) techniques was made. Obviously, SPE technique was superior, due to its less consumption of solvent and the ability to include clean-up step in one single process. SPE technique followed by GC detection was applied to the determination of diphenyltin and triphenyltin in seawater and pond water.

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CHAPTER I

INTRODUCTION

Organotin compounds have one or more carbon-tin covalent bonds that are responsible for the specific properties of such molecules. Essentiallly all organotin compounds are of the Sn(IV) type. The only well established compound with tin in the oxidation state +2 is the tin(II) cyclopentadienyl, $C_{10}H_{10}Sn$. There are four series of organotin compounds with the general structure: R_nSnX_{4-n}

where R = an alkyl or aryl group

Sn = the central tin atom in the oxidation state +4

X = a singly charged anion or an ionic organic group

n = 1 - 4

In the organotin compounds of practical importance, R is usually a butyl, octyl, or phenyl group and X is commonly chloride, fluoride, oxide, hydroxide, carboxylate or thiolate.

1.1 APPLICATION OF ORGANOTIN COMPOUNDS

Organotin compounds were first used as stabilizers for polyvinyl chloride (PVC) in 1940. Since then organotin compounds have found a variety of uses. However, their use as stabilizers for PVC is still the most important application of organotin compounds until now [1].

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Organotin compounds are used mainly by applying the following two characteristics. The first is the strong affinity of the tin atom for a donor ligand atom such as sulfur or oxygen. The second is their physiological activities such as biocidal activity (e.g., bactericidal, fungicidal, acarcidal, insecticidal and molluscidal) and repellent activity against wood-destroying organism, marine animals, marine plants, rodents, etc [1].

The industrial uses of organotin compounds which employ the first characteristics are stabilizers for PVC and the other vinyl polymers, and catalysts. The industrial uses which apply the second characteristic are wood preservatives, antifouling agents, agrochemicals, pharmaceuticals, disinfectants, rodent repellents, protection of masonry and stone work, and slime prevention, etc. The other minor uses of organotin compounds are glass applications (as easy handling raw materials for inorganic tin compounds (SnO_2)), and flame retardants [1].

Monoorganotin compounds, $RSnX_3$, are less known; so far have found only limited application, for example, butyltin sulfide is used as a stabilizers in PVC film [2].

Diorganotin compounds, R_2SnX_2, are chemically reactive and most of their applications are based on this property. They are used as stabilizers of PVC, as catalysts in the production of polyurethane foams, and in the cold-curing of silicon elastomers.

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Triorganotin compounds, R_3SnX, are the most important class of organotin chemicals. They are biologically very active and are widely

used as biocides. The chemical nature of the R group has a strong influence on the biological properties of these compounds. The X group, on the other hand, influences their solubility and volatility. The two most important groups of triorganotin compounds are tributyltin and triphenyltin derivatives.

Tetraorganotin compounds, R_4Sn, are primarily used as intermediates in the preparation of other organotin compounds such as tetraalkyl- and tetraaryltin compounds [2].

Among the four categories of organotin compounds, dialkyltin derivatives are the most important commercially. They are used as heat and light stabilizers for PVC plastics to prevent degradation of the polymer during the melting and forming of the resin into its final product. In addition, dialkyltin derivatives have the unique property of protecting the plastic product from degradation during use. Other important commercial uses of dialkyltin derivatives are as catalysts in the production of polyurethane foam products and as vulcanizing agents for silicone rubbers. The trialkyl-tin derivatives, which account for approximately 10% of the total production of organotin compounds, are used in agriculture as non-systemetic fungicides and acarcides. The tetraalkyltin derivatives are used as intermediates in the manufacture of other organotin compounds. The monoalkyltin derivatives have a limited commercial use as heat stabilizers for PVC plastic films [2].

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Organotin compounds have many other applications: (a) as antioxidants and anticracking agents to retard rubber deterioration and to stabilize chlorinated rubbers in chlorinated paints; (b) in transformers,

capacitators, and cables as hydrochloric acid scavengers to prevent corrosion when chlorinated diphenyls are present (e.g., tetraphenyltin); (c) as anti-oxidants for textile oils; (d) as activator, stabilizers, and catalysts for the polymerization of olefins; (e) in the treatment of glass to increase crack resistance; (f) in the treatment of fibreglass (with alkyl- and aryltin compounds) for adhesion to resins; and (g) as curing catalysts in the application of silicone to textiles and paper.

Organotin compounds have biocidal properties and are used as; (a) agricultural fungicides (triphenyltin acetate, triphenyltin hydroxide); (b) general biocides (bis(tributyltin) oxide = TBTO) in paints, in the preservation of manila and sisal ropes, leather, textiles, to make fabrics mildew-resistant; for the protection of jute and jute bags; in wood preservatives, slimicides and in the production of paper; (c) bactericides and biostats such as disinfectants for use in hospitals and stable (tributyltin benzoate); (d) helminticides in poultry (dibutyltin dilaurate, tetraisobutyltin); (e) nematocides (p-bromophenoxy triethyltin); (f) as herbicides (vinyltin compounds, e.g. trivinyltin chloride); (g) rodent repellents (tributyltin chloride, triphenyltin chlorides and acetate); (h) molluscicides (triphenyl- and tributyltin compounds); (i) ovicides (trialkyl- and triaryltin chlorides in combination with DDT or pyrethrins); (j) antifoulants in ship paints and underwater coatings (triphenyl- and tributyltin compounds); and (k) miticides have been as insect chemosterilants [2].

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A schematic presentation of the various uses of organotin compounds is given in Fig. 1.1 [2].

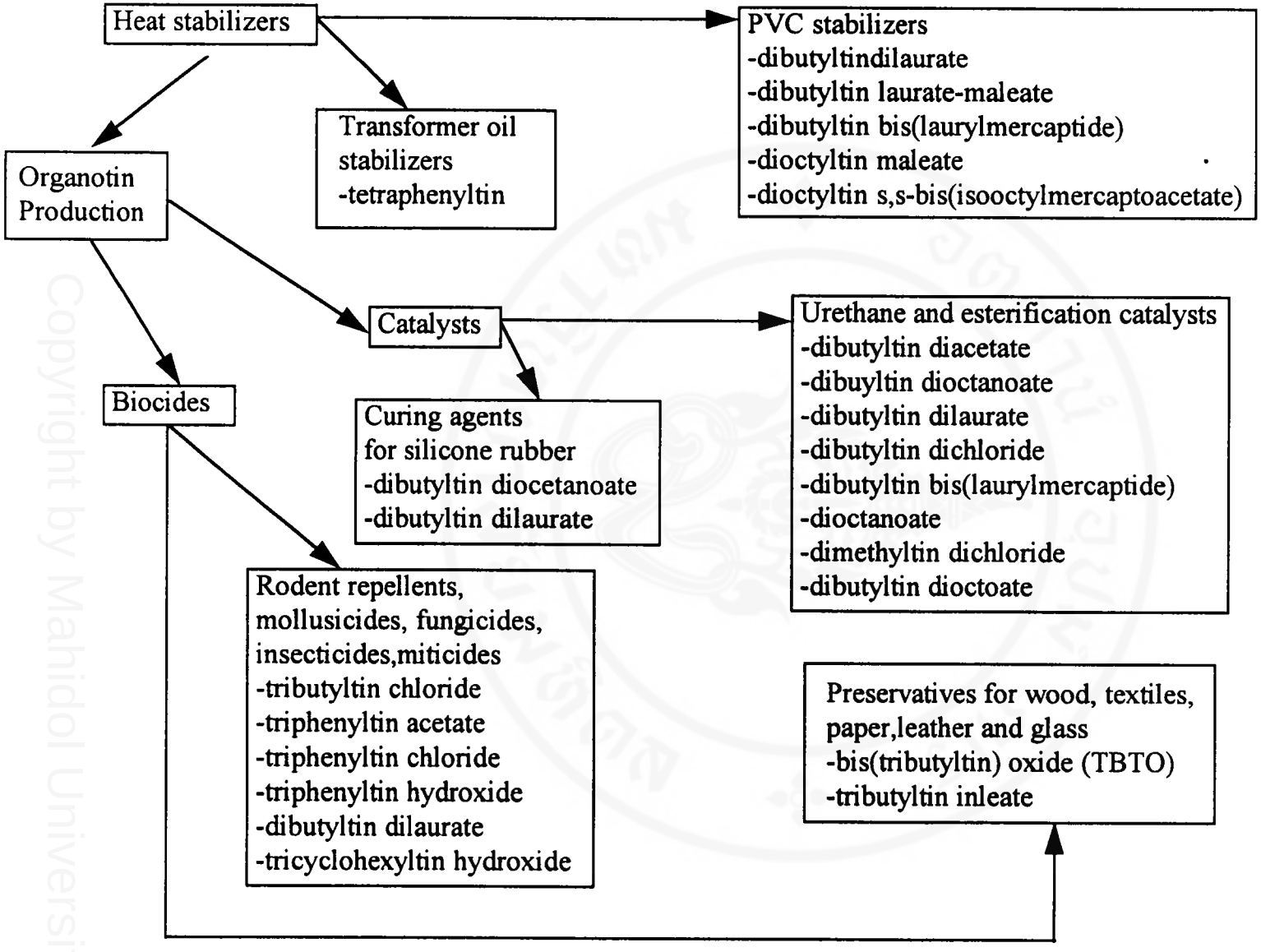

Figure 1.1 A schematic presentation of the various uses of organotin compounds [2].

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1.2 TOXICOLOGY AND ENVIRONMENTAL POLLUTION OF ORGANOTIN COMPOUNDS

The industrial output of organotin compounds has increased over the last decade as a result of the use of organotins in a large variety of applications (i.e. catalysts, polymer additives, agricultural biocides, antifouling paints). Because of their manner of usage, a significant portion of these compounds are released directly into the environment and cause environmental pollution.

A summary of the major possible direct modes of entry of organotins into the environment is presented in Table 1.1 [3].

The toxic properties of organotin are related to the number and nature of organic groups in an $R_nSnX_{(4-n)}$ compound (R and X denoting alkyl and anion, respectively). Within this class toxicity is strongly dependent on the nature of the organic substituent but very little on the anion [4]. Toxicity is apparently related to the transport of anions through cell membrane destroying important biological gradients (e.g. pH, salinity) [5].

The species towards which the R_3SnX compound is most active is primarily determined by the nature of the three organic groups. For example, in the tri-n-alkyltin series, the trimethyltin compounds have a high toxicity to insects and mammals, the triethyltin are most toxic to mammals, the tripropyltins to Gram-negative bacteria and the tributyltins to Gram-positive bacteria and fungi. Further increase in the n-alkyl chain

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Table 1.1 Possible direct modes of entry of organotin compounds into the environment [3]

Medium	Species	Source
Air	R_3SnX	Argricultural spraying
		Volatilisation from biocidal treatment
		Antifouling paint sprays
	R_3SnX, R_2SnX_2 and $RSnX_3$	Incineration of organotin treated or stabilised waste materials
	R_2SnX_2 and $RSnX_3$	Glass coating operation-spraying of organotins on to glass at high temperatures to give SnO_2 films
Soil	R_3SnX	Agricultural applications
		Wood preservation
	R_3SnX, R_2SnX and $RSnX_3$	Burial of organotin containing waste materials
Water	R_3SnX	Antifouling coatings
		Molluscicides
		Over spray from agricultural applications
		Land run-off from agricultural usage
		Industrial processes, e.g. slimicides in paper manufacture
	R_2SnX_2 and $RSnX_3$	Leaching from organotin stabilized PVC

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length produce a sharp drop in the biological activity and the trioctyltin compounds are essentially nontoxic to all living organism. A biological effect of particular importance is the high toxicity of tributyl-, triphenyl- and tricyclohexyltin derivatives to fish and other marine creatures [3].

The tetra-organotins, R_4Sn, have been found to exhibit a delayed toxic action, which can be explained by their conversion in the organism to the corresponding and more toxic trialkyltin compound since in vitro and in vivo studies have shown this process to occur, particularly in the liver [3]. Toxicology reports have confirmed that tetraalkyltins produced the same effect in animals as trialkyltins, which are the most toxic organotin compounds towards the central nervous system [6].

The biological effects of the diorganotin compounds are due to inhibition of oxygen uptake in mitochondria. However, the mechanism of their toxic action differs, since the diorganotins have been shown to inhibit α-ketoacid oxidation, probably by combining with enzymes, possesing vicinal dithiol groups. The monoorganotins do not appear to have any important toxic action in mammals and show the same pattern of decreasing toxicity with increasing chain length [3].

The numerous studies regarding the toxic impact of orgonotin compounds to the aquatic ecosystem have shown that these chemicals cause the reduction of growth on marine microalgae [7], abnormal shell thickening in the Pacific oyster [8], and the imposex in mud snail [9], and whelks [10]. However, most of the investigations have focused on their residues in aquatic organisms of lower trophic levels such as plankton,

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algae, molluscs and fish, and no analytical data are available on the higher organisms like dolphins, whales and seals.

It has been well documented that marine mammals such as cetaceans and pinnipeds, a top predator in the aquatic ecosystem, highly accumulate hydrophobic persistent organochlorines such as PCB and DDT through the food chain due to the low capacity to decompose these compounds [11]. Considering the specific metabolic capacity in these animals and similar hydrophobicity of butyltin compounds that can be predicted by octanol-water partition coefficients, it is thought that this group of contaminants may also be amplified in the marine mammals. During the late 1980s and early 1990s, mass mortalities, accounting for about 18,000 harbour seals in Europe [12], several thousands of Baikal seals [13] and Mediterranean striped dolphins [14] have been documented. Although the direct cause of these animal deaths are due to morbilli virus, the triggering mechanisms behind these infections still remained obscure [12]. It has been noted that organotin compounds affect the immune system of rat [13]. This fact imposes a possible impact of these contaminants to marine mammals affected by virus.

Because of the great differences in toxicity of the various organotin compounds depending on their structure, it is important to develop an analytical technique capable of separating of these compounds in environmental samples.

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1.3 GAS CHROMATOGRAPHIC METHOD FOR ANALYSIS OF ORGANOTIN COMPOUNDS

Organotin speciation is of paramount importance in environmental studies in order to distinguish the most toxic species from the less toxic degradation products. Until now most organotin speciation techniques have been based on the GC resolution of volatile derivatives (i.e. hydrides or alkyl) coupled to elemental detection techniques [electron capture detection (ECD), atomic absorption spectrometry (AAS), flame photometric detection (FPD) and atomic emission detection (AED)].

GC in combination with electron capture detector has also been proposed. It was used for the determination of organotin compounds in biological materials [6, 16, 17], environmental water [18] and sediments [17, 18]. Evans *et al.* [19] described an analytical method based on a liquid-solid extraction (LSE) procedure followed by GC-ECD for determination of tributyltin chloride (TBT) in fresh and marine waters. The LSE of TBT from aqueous solution offers simplicity in extracting, concentrating, preserving and storing an important environmental pollutant of great concern. The LSE discs were found to have a low background, good collection efficiency and no observable breakthrough. However, it was noted that an ECD did not have the specificity to allow measurement of TBT at trace concentrations in environmental samples.

Flame Photometric detection (FPD) has been employed in the gas chromatographic determination of organotin compounds. Aue and Flinn [20] reported the use of FPD in the open mode (no filter or mask) for the selective detection of volatile tin species. An analytical method for

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organotin compounds was developed by using capillary GC with a sulphur-mode (S-mode) (393nm) filter [21]. However, the determination of organotin compounds using an S-mode detectors requires relatively complex and mathematical analyses, because concentration were given by exponential responses of these compounds [22]. Tin-mode (Sn-mode) (610 nm) FPD for the GC determination was examined by Ohhira and Matsui [22], obtained a linear correlation between the peak heights and the amount of tin. Many authors applied Tin mode FPD for the simultaneous GC-determination of organotin compounds in fish [22], rat urine [23], biological materials [24], aquatic environment [25], marine product [26] and seawater [27]. Maguire and Hunealt [28] described a method for the determination of butyltin species including bis(tri-n-butyltin) oxide and some of its dealkylation products in lake and river waters by GC-FPD and GC-MS (gas chromatograph-mass spectrometer). Müller [29] used a tropolone-C_{18} silica cartridge and ethereal tropolone solution to extract organotin compounds from water and sediments, respectively. The extracted organotin compounds were ethylated and analyzed by using GC-FPD and GC-MS for confirmation. The technique was used for the analysis of tributyltin in samples of river and lake water, and sediment. The detection limits of less than 1 pg/l for water and 0.5 pg/l for sediment was obtained. An analytical method for fatty animal tissues like blubber, which interfere with accurate quantifications on gas chromatograms, was examined and concentrations of butyltin compounds was determined in the subcutaneous fat (blubber) of marine mammals [30]. A hydrolysis technique and a GC method were described for the determination of tributyltin and triphenyltin compounds in human hair and fish tissue by

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Nagase *et al.* [31]. The limit of determination of tributyltin and triphenyltin compounds in the sample were 5 and 10 ng/g, respectively, in their chloride forms. Kawata *et al.* [32] used a quartz-fibre filter and activated carbon-fibre filter for sampling method of organotin compounds in air. The organotin compounds were investigated for dibutyltin dichloride (DBT), tributyltin chloride (TBT), diphenyltin dichloride (DPT) and triphenyltin chloride (TPT). The minimum detectable concentrations were 0.2 ng/m^3 for DPT and TBT, 0.3 ng/m^3 for DPT and 0.4 ng/m^3 for TPT. This method was successfully applied to the determination of organotin compounds in indoor air.

With the recent developments in supercritical fluid extraction (SFE) technique conventional solvent extractions had been gradually replaced. In this technique, carbon dioxide was used at its critical state, which flow with low viscosity and penetrated the solid matrix. It dissolved and carried the analytes from the sample to a trap containing small amounts of solvent. It was simple and convenient, and eliminated the use of large volumes of solvents. Alzaga and Bayona [33] presented an analytical procedure using solid-phase extraction (SPE) with C_{18} extraction discs, in situ ethyl-Grignard derivatization and SFE with carbon dioxide and off-line capillary GC-FPD determination for tributyltin and its degradation products from seawater. The procedural minimization of sample handling and intermediate steps led to an enhancement of sensitivity and reproducibility and also analysis time reduction. The limit of detection of TBT (1L of seawater) was 6 ng/l. Furthermore, analysis time and solvent usage were reduced by 50 and 90%, respectively. Cai *et al.* [34] described a rapid, quantitative analytical procedure for the determination

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of butyl- and phenyltin compounds in sediment samples. This method employed an in situ Grignard hexylation of organotins with hexylmagnesium bromide on sediments followed by extraction with supercritical carbon dioxide and then analyzed by GC-FPD. No organic solvent extractions are involved and no clean-up steps were used prior to GC analysis. Dachs *et al.* [35] developed a SPE procedure for TBT determination in sediments and compared it with other procedures based on different desorption processes from the solid matrix (i.e. high-pressure liquid extraction, HPLE and atmospheric pressure liquid extraction, APE). The extraction efficiency of developed SPE method was about 30% higher than an APE procedure applied to the same samples.

Microwave-assisted digestion had gained wide acceptance as a rapid method for sample decomposition in inorganic analysis. Attacked at high temperatures (200-300 °C) with a mixture of concentrated acids in a pressurized vessel, which aims at complete dissolution (often associated with complete destruction of organic matter) was the most popular method. In contrast, a focused, low powered microwave field seemed to be more suitable to assure extraction of the organometallic species from the matrix without destroying the carbon-metal bonds. Laléra *et al.* [37] described the method of speciation analysis for organotin compounds in sediments by capillary GC-FPD after microwave-assisted acid leaching. The detection limit of the procedure was 10 ng/g for 0.5 g of sediment. The method was demonstrated that microwave-assisted leaching could be a cheap and useful method for the recovery of organometallic compounds in speciation analysis of complex matrices, e.g., sediments. The analysis

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time could be dramatically (20-100 times) decreased and yields from some species could be increased compared with conventional methods.

Atomic absorption spectrometry (AAS) could also be used as GC detection technique for the determination of organotin compounds. Dirkx et al. [38] used quartz furnace AAS detection technique (QF-AAS) for determination of tri- and dibutyltin in sediments. A detection limit of 2.5 ng/g (as Sn) can be achieved. The accuracy of the method had been tested through the analysis of certified materials with good agreement between the certified value and the results obtained with the proposed method.

Atomic absorption spectrometry, detection technique for GC, was a relatively unsensitive technique for the determination of tin due to the high atomization temperature needed and the possibility of the formation of refractory oxide. Plasma source providing higher atomization temperature were more advantageous. Helium atmospheric pressure microwave induced-plasma atomic emission spectrometry (MIP/AES) had recently gained popularity as a sensitive and selective detector for GC. £obinski *et al.* [4] demonstrated the potential of GC-MIP/AES for the speciation of organotin compounds in environmental samples. The method was developed for the determination of mono-, di-, tri-, and some tetraalkylated organotin compounds in water and sediments. The absolute detection limit was about 0.05 pg (as Sn). In a subsequent work, Szpunar- £obinska *et al.* [39] presented a comprehensive optimization study of the sorption of butyltin and phenyltin compounds using a C_{18} packed microcolumns and extraction discs, followed by in situ heterophase derivatization of ionic species using $NaBEt_4$. A semi-automated system for on-line

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preconcentration/derivatization of organotin compounds was developed and applied to the analysis of river water samples by capillary GC-MIP/AES. The detection limit of 0.1 ng/l could be obtained. The method eliminated the necessity for the storage of large volume of untreated water samples, which was not only cumbersome but might also result in losses and changes in speciation due to degradation processes. Suzuki *et al.* [40] used GC-MIP/AES for analysis of organotin compounds in biological samples. The analytical method for speciation of organotin compounds in environmental samples by using GC-MIP/AES was described by Tutschku *et al.* [41]. The method allowed to achieve absolute detection limits in the range of a few pg (as Sn). The signal intensity was influenced by several parameters of the MIP and GC system, such as reagent gases and the Sn emission lines used. An investigation of the parameters should depend on that the analytical response depended on the kind of tin compounds itself.

1.4 AIM AND SCOPE OF THE PROJECT

The aim of this project was to develop an analytical method for the determination of phenyltin compounds in environmental samples. The analytical method for the determination of phenyltin compounds combining alkylation with a Grignard reagent and gas-liquid chromatography with flame photometric detection (GC-FPD) was investigated in this study. Although the technique has been investigated by other authors [22,25-29], the optimization of each step in the procedure need to be studied for samples having different matrices and form.

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Many parameters were required in order to obtain the optimum conditions for determination of phenyltin species, such as He flow rate, H_2 flow rate, injector temperature and initial column temperature. The gas chromatographic data (i.e. detection limit and reproducibility) were presented for different kinds of GC columns. In the derivatizing step, the amount of Grignard reagent and the time required for such reaction were optimized. Propylmagnesium bromide was also used as an alternative reagent for derivatization reaction.

A suitable extraction technique for phenyltin species was developed. For determination of TPT, two alternative extraction techniques were used: liquid-liquid extraction (LLE) and solid-phase extraction (SPE) using silica column. SPE has an advantage over LLE, with less consumption of organic solvent and simpler preparation steps. Therefore, SPE was chosen for preconcentration technique. The preconcentration steps (i.e. load, wash and elution) were optimized to achieve high recovery. One part of this study concerned the selection of suitable concentrator column, the eluent and also the optimum eluent volume. The optimized procedure was applied to environmental water samples such as seawater and pond water.

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CHAPTER II

EXPERIMENTAL

2.1 INTRODUCTION

In this chapter, instrumentation, reagents, general method of preparation of solution and experimental procedure are presented.

The development of standard method for analysis was studied. Three types of columns (OV-17 packed column, HP-17 and HP-1 capillary column) were presented from the aspect of detection limit and calibration curve. The optimum conditions were selected from data with high sensitivity and reproducibility. Comparison between liquid-liquid extraction and solid phase extraction was performed for the determination of TPT. The development of preconcentration and sample clean-up step was studied for water samples containing differernt matrices (pond water and seawater) and applied to fish sample. The general details of procedure are presented.

2.2 INSTRUMENTATION

2.2.1 Gas Chromatography

A GC, model 5890 A (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a flame photometric detector was operated in the sulfur mode (filter 393 nm.). The detector output was recorded using an

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HP 3396 A integrator. The columns were OV-17 packed column 4 ft × 1/4 in. × 4 mm, 50% phenyl methyl silicone, HP-17 (cross-linked) 10 m × 0.53 mm × 2.0 μm, 50% phenyl and 50% methylpolysiloxane and HP-1 (crossed-linked Methyl Silicone Gum) 25 m × 0.2 mm × 0.3μm, 100% dimethylpolysiloxane.

2.2.2 Vacuum Manifold

Vacuum Manifold (BARNANT Co., illinois, U.S.A.) was used in the preconcentration step.

2.2.3 Centrifuge

A Centrifuge, Model EBA 12, Universal 16/16 R (Hettich, Germany) was used for sample preparation of fish sample.

2.2.4 Shaker

A Shaker, Model HS 250 (IKA, Germany) was used for agitating sample in the sample preparation.

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2.3 CHEMICALS AND REAGENTS

Triphenyltin chloride (TPT; >97% purity), ethylmagnesium chloride (25% in tetrahydrofuran), tropolone (2-hydroxycycloheptatrienone), ammonium chloride and silica gel (activated overnight at 120°c before use) were purchased from Fluka (Buchs, Switzerland). Diphenyltindichloride (DPT; 96% purity), monophenyltintrichloride (MPT; 98% purity) were from Aldrich (Wisconsin, U.S.A.). Propylmagnesium bromide (2 mol/l in tetrahydrofuran) was from Tokyo Kasei Kogyo (Tokyo, Japan). Sodium chloride and sodium sulphate anhydrous were from E.Merck (Darmstadt, Germany). Analytical grade diethyl ether, hexane and methanol were obtained from J.T. Baker (Phillipsburg, U.S.A.). Hydrochloric acid was from BDH (Poole, UK).

2.4 PREPARATION OF SOLUTION

2.4.1 Standard solution

Standard solution of phenyltin compounds were prepared by dissolving 0.0050 g of phenyltin compounds in 10 ml volumetric flask to give a concentration of 500 ppm of phenyltin compounds in hexane or methanol. These stock solutions were stored in dark glass bottles at a low temperature. Subsequent dilution was prepared freshly prior to use by diluting with hexane or methanol.

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2.4.2 Ammonium chloride solution

Ammonium chloride, NH_4Cl, was dissolved in distilled water to saturated concentration and stored in a plastic bottle at room temperature.

2.4.3 Sodium chloride solution

Sodium chloride, NaCl, was dissolved in distilled water to saturated concentration and stored in a plastic bottle at room temperature.

2.4.4 Etheral tropolone solution

Etheral tropolone solution was prepared freshly prior to use by dissolving 0.025 g of tropolone in ether and diluted to 25 ml in volumetric flask to give a concentration of 0.1% w/v. Subsequent dilution was prepared by using ether.

2.5 PROCEDURE

2.5.1 Development of standard method for analysis

2.5.1.1 Optimization of GC condition

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Standard solution of 500 ppm TPT (100 µl) in hexane was added into 1 ml volume of ether and then 2 ml of ethylmagnesium

chloride (25% in THF) was carefully added to derivatize the TPT. The mixture was allowed to stand at least 10 minutes and the excess of Grignard reagent was destroyed by adding about 10 ml of 2 M HCl dropwise. The ether layer containing the ethylated derivative was dried over anhydrous Na_2SO_4. The ether layer was evaporated until dry and made up to 5 ml with hexane to give a concentration of 10 ppm of ethylated derivative.

The working standard solution of 10 ppm was diluted with hexane to give a concentration of 1 ppm for further study.

2.5.1.2 Derivatization of phenyltin compounds

2.5.1.2.1 Effect of derivatization reaction time on signal of phenyltin compounds

Standard solution of 5 ppm MPT (5 µl) and 5 ppm DBT (30 µl) as an internal standard, in hexane were added into 1 ml volume of ether and 1.5 ml of ethylmagnesium chloride (25% in THF) was carefully added. The derivatization step was followed as Section 2.5.1.1 and the time required for completeness of derivatization reaction was varied from 5 to 30 minutes. The final solution containing 0.50 ppm of ethylated MPT was subjected to GC analysis. Ratio of peak height and peak area of MPT to dibutyltinchloride were measured.

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2.5.1.2.2 Effect of amount of Grignard reagent on derivatization for triphenyltin

Standard solution of 10 ppm TPT (100 µl) in hexane was added into 1 ml of ether . Ethylmagnesium chloride (25% in THF) was added with varying amount from 0.25 to 2 ml. The derivatization step was the same as mentioned in Section 2.5.1.1 and the residue was made up to 0.5 ml with hexane to give a final concentration of 2 ppm of ethylated TPT before GC analysis.

2.5.1.3 Calibration of phenyltin compounds by gas chromatography

Ethylated phenyltin compounds were prepared as described in Section 2.5.1.1 and the final solution containing 10 ppm of each derivative was used for calibration curve by appropriate dilution with hexane.

2.5.1.4 Use of alternative derivatizing agent

Propylated phenyltin compounds were prepared in the same as Section 2.5.1.1 but propylmagnesium bromide was used to be derivatizing agent. An excess of Grignard reagent was destroyed with 10 ml volume of saturated NH_4Cl solution. The final solution containing 10 ppm of each derivative was used for calibration curve by appropriate dilution with hexane.

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2.5.2 Effect of extraction volume ratio in liquid - liquid extraction for preconcentration

In a separatory funnel, standard solution of 25 ppm TPT (25 µl) in MeOH was added in distilled water with various volume from 25 to 200 ml. The TPT was extracted with diethyl ether by agitating for 10 minutes using a mechanical shaker and the extraction volume ratio was varied to be 1:5, 1:7 and 1:10 (v/v). The ether layer was dried with anhydrous Na_2SO_4 and concentrated to 1 ml. This solution was derivatized with ethylmagnesium chloride before introduction to GC, as mentioned in Section 2.5.1.1.

2.5.3 Effect of the amount of HCl in the sample extraction step

Different volume of distilled water (i.e. 25 to 200 ml) was employed for extraction. Standard solution of 25 ppm TPT (25 µl) in MeOH was spiked in the distilled water. The volume of concentrated HCl varying from 0 to 15 ml was added before extraction process. The solution was extracted with diethyl ether by agitation for 10 minutes using a mechanical shaker. The extraction volume ratio of 1:5 (v/ v) which was obtained from the study of the recovery of TPT by liquid-liquid extraction, described in Section 4.2.1, was used. After shaking, the upper phase was collected and dried over anhydrous Na_2SO_4. The ether layer was concentrated to 1 ml. The derivatizing step was then followed as mentioned in Section2.5.1.1.

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2.5.4 Effect of percent tropolone on extraction efficiency

The concentration of tropolone being used for the extraction were varied from 0 to 0.06% (w/v). Different water volumes (25 to 200 ml) which were spiked with standard solution of 25 ppm TPT (25 µl) in MeOH were used for performing preconcentration. The optimum extraction volume ratio (1:5 v/v) was used in the experiment. Extraction process and derivertizing step were followed as given in Section 2.5.3. The recoveries were obtained by comparing with the results when the same experiment was performed without preconcentration step that was equal to the amount of TPT, expected in the final solution.

2.5.5 Comparison between silica column and C_{18} column in preconcentration process

Standard solution of 15 ppm TPT (100 µl) in MeOH was added to 25 ml of distilled water. The solution was preconcentrated by passing the volume through silica gel column at 1 ml/min with the help of a vacuum manifold. Two types of columns were used : silica column and C_{18} column. The column was previously conditioned with 2 ml of hexane. The solution was passed through the prewashed column. The column was washed with 5.00 ml distilled water and allowed to dry for 10 minutes. Elution was carried out with various kinds of eluent with 5.00 ml eluent. The eluate was dried with anhydrous Na_2SO_4 and concentrated to 1 ml. The derivatization step was proceeded in the same manner as Section 2.5.1.1 but an excess of Grignard reagent was destroyed with 10 ml

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volume of saturated NH_4Cl solution. The residue was made up to 0.5 ml with hexane before GC analysis.

2.5.6 Study of breakthrough volume

Distilled water containing 100 ppb of TPT was preconcentrated by passing through the silica gel column at 1 ml/min with the help of a vacuum manifold. The silica gel column was previously conditioned with 2 ml of hexane. The distilled water containing TPT was passed through the prewashed column with the varying of distilled water from 5 to 200 ml, the column was washed with 0.50 ml distilled water and allowed to dry for 10 minutes. Elution was carried out with 3.00 ml diethyl ether. Ethylmagnesium chloride (25% in THF) 1.5 ml was carefully added to the eluate and the derivatization was proceeded as mentioned in Section 2.5.5.

2.5.7 Optimum elution volume in preconcentration step

Standard solution of 1 ppm TPT (50 µl) in MeOH was added in 50 and 100 ml of distilled water. The solution was preconcentrated by passing the volume through silica gel column at 1 ml/min with the help of a vacuum manifold. The silica gel column was previously conditioned with 2 ml of hexane. Distilled water containing TPT was passed through the prewashed column. The silica gel column was washed with 5.00 ml distilled water and allowed to dry for 10 minutes. Elution was carried out

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with diethyl ether that varied from 1 to 10 ml. The eluate was dried with anhydrous Na_2SO_4 and concentrated to 1 ml. The derivatization step was followed in the same manner as Section 2.5.5 but propylmagnesium bromide was used to be derivatizing agent. The results were compared with derivatized TPT without preconcentration step. The peak height was measured for recovery calculation.

2.5.8 Optimum wash volume in preconcentration step

Standard solution of 50 ppm TPT (30 µl) in MeOH was added in 50 and 100 ml of distilled water. The preconcentration and derivatization process were performed in the same manner as mentioned in Section 2.5.7 but the column was washed with distilled water with various volume from 1.00 to 15.00 ml. Elution was carried out with 3.00 ml diethyl ether (as obtained from Section 2.5.7). The recoveries were obtained in the same as Section 2.5.7.

2.5.9 Optimization of precontentration parameters for diphenyltin compound determination

Standard solution of 15 ppm DPT (50 µl) in MeOH was added in 25 ml of distilled water. The preconcentration process was performed as mentioned in Section 2.5.7 but in the elution step, tropolone in ether solution was selected for an eluent. The concentration of tropolone was varied from 0 to 0.08% (w/v). Elution was carried out with

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3.00 ml volume of eluent. The derivatization step was followed in the same manner as Section 2.5.7.

2.5.10 Optimum elution volume in preconcentration step for diphenyltin compound determination

Standard solution of 50 ppm DPT (30 µl) in MeOH was added in 25 ml of distilled water. The preconcentration process was performed as mentioned in Section 2.5.7 but elution was carried out with 0.06% (w/v) tropolone in ether solution (as obtained from Section 2.5.9). Elution volume was varied from 1.00 to 10.00 ml. The concentrated solution of DPT was derivatized before GC anlysis in the same manner as Section 2.5.7.

2.5.11 Modification of preconcentration step for diphenyltin compound determination in seawater

Standard solution of 50 ppm DPT (30 µl) in MeOH was added in 25 ml of seawater. The preconcentration process was performed as mentioned in Section 2.5.7 but the column was washed with distilled water with various volume from 1.00 to 20.00 ml. Elution was carried out with 3.00 ml of 0.06% tropolone in ether solution (as obtained from Section 2.5.9 and 2.5.10). The derivatization step was followed in the same manner as Section 2.5.7.

CHAPTER III

DEVELOPMENT OF STANDARD METHODS FOR ANALYSIS

3.1 INTRODUCTION

The abundant use of organotin compounds has led to their occurrence in the environment. As trace levels of organotin compounds are toxic to organisms and the toxicity of the various organotin compounds is widely different, sensitive and specific analysis is necessary for the ecotoxicological study of the compounds [42].

Sensitive determination method of organotin compounds by conventional gas chromatographic methods usually involved solvent extraction of samples, concentration, and, in most cases, derivatization to prepare stable volatile species for chromatographic separation; hydride formation [18,43] or an alkylation procedure [22,25-29] had usually been employed.

This work had been attempted to study the optimum conditions for phenyltin species that will be selected in order to give the high sensitivity and also reproducible data. These parameters are flow rate of carrier gas (He), fuel to H_2 ratio in FPD flame, injector temperature, initial column temperature and different types of GC column (packed column vs capillary column). For derivatizing step, the amount of Grignard reagent and the time required for such reaction will be optimized. Propylmagnesium bromide was also used as an alternative reagent for derivatization reaction.

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3.2 OPTIMIZATION OF GAS CHROMATOGRAPHIC CONDITIONS

3.2.1 Experimental

3.2.1.1 Instrumentation

GC model used as described in Section 2.2.1. The column was HP-17 capillary column (10 m × 0.53 mm × 2.0 μm), 50% phenyl and 50% methylpolysiloxane.

GC conditions using in this experiment

Column	:	HP-17 capillary column
Temperature	:	injector ; 250°C, detector ; 250°C
Oven	:	55°C(1 min), 40°C/min→ 240°C (15 min)
Air frow mode	:	H_2 ; 106.24 ml/min, air, 88.39 ml/min
Carrier gas	:	He 56.86 ml/min
Inlet type	:	Splitless
Split vent	:	260.9 ml/min
Column head pressure	:	11 psi

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3.2.1.2 Reagent and Procedures

TPT was used as standard to perform the optimization experiments. TPT was of analytical grade and used without further purification. All chemicals used were of analytical grade. Stock solutions (500 ppm) were prepared in AR grade hexane.

Stock solution was ethylated with ethylmagnesium chloride and prepared in hexane. Working solution ($EtPh_3Sn$) containing 10 ppm was diluted with hexane to 0.5 ppm and used for the study of optimun conditions.

Stock solutions of MPT and DPT were also prepared in the same way.

3.2.2 Results and discussion

Criteria used for selecting optimum conditions were high sensitivity and reproducible data. The parameters concerned were flow rate of carrier gas (He), fuel to H_2 ratio in FPD flame (obtained by varying flow rate of H_2), injector and initial column temperature in the temperature programmed. Although, the parameters that contained in temperature programmed were initial column temperature, rate of increasing temperature and final temperature, the parameter that had the greatest effect on reproducibility was the initial column temperature. The other parameters affected the resolution of the peak of the compounds. In this work, the chromatogram of phenyltin compounds which were obtained had

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enough resolution, therefore only the initial column temperature was varied during the method development.

Table 3.1, 3.2, 3.3 and 3.4 showed the results of varying flow rate of He, flow rate of H_2, injector and initial column temperatures, respectively. The results showed that best results (in term of sensitivity and reproducibility) were obtained when flow rate of He was 56.86 ml/min, flow rate of H_2 was 106.24 ml/min, injector temperature was 250°C and initial column temperature was in the range of 35°C-55°C when using HP-17 capillary column.

The optimum flow rate of H_2 was 106.24 ml/min, at a constant air flow rate of 88.39 ml/min. Therefore, the optimum hydrogen-to-air flow rate ratio was 1.2. This indicated that the detector response depended significantly on the hydrogen-to-air flow rate ratio. The photometric detection mechanism is attributed to light emission of the excited organotin species in the detector flame. The response was strongly dependent on the flame conditions, and the hydrogen-rich flame was found to enhance selectivity as well as the shape of the GC analysis [20].

When initial column temperature was varied, if the temperature was too high (65°C), the results were irreproducible because of solvent effect. The solvent used to dissolve the derivatized organotin was hexane. When passing inlet system it should be concentrated at the inlet and then transferred to the column for separation. If the temperature was higher than the boiling point of hexane, the capability of trapping and concentrating solute in this area was low, causing irreproducible results.

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The results of initial column temperature in the range of 35°C-55°C showed the high sensitivity and more reproducible data. To save analysis time, the initial column temperature of 55°C was chosen for the following experiments.

Table 3.1 Analytical data of triphenyltin at varying flow rate of He

He flow rate (ml/min)	Peak height mean[1]±S.D.[2]	%RSD[3]	Peak area mean[1]±S.D.[2]	%RSD[4]
28.99	19640 ± 1454	7.4	192144 ± 18109	9.4
43.56	22204 ± 3204	14.4	196850 ± 50426	25.6
56.86	23560 ± 320	1.3	224368 ± 3370	1.5
64.50	15563 ± 833	5.3	154712 ± 8331	5.3
71.15	18601 ± 472	2.6	182140 ± 3465	1.9
85.86	19709 ± 840	30.8	197551 ± 8212	49.4

[1]average in three runs; [2]standard deviation (n=3); [3] relative standard deviation of peak height (n=3); [4] relative standard deviation of peak area (n=3)

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Table 3.2 Analytical data of triphenyltin at varying flow rate of H_2

H_2 flow rate (ml/min)	Peak height mean[(1)]±S.D.[(2)]	%RSD[(3)]	Peak area mean[(1)]±S.D.[(2)]	%RSD[(4)]
106.24	19711 ± 259	1.3	181723 ± 2373	1.3
132.80	13624 ± 584	4.2	124969 ± 4884	3.9
146.08	13578 ± 808	5.9	115071 ± 3947	3.4

[(1)]average in three runs; [(2)]standard deviation (n=3); [(3)] relative standard deviation of peak height (n=3); [(4)] relative standard deviation of peak area (n=3)

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Table 3.3 Analytical data of triphenyltin at varying injector temperature

injector temperatue (^{0}C)	Peak height mean[1]±S.D.[2]	%RSD[3]	Peak area mean[1]±S.D.[2]	%RSD[4]
250	14666 ± 232	1.6	144869 ± 2651	1.8
280	12761 ± 368	2.9	115507 ± 7670	2.8
300	14720 ± 772	5.2	136734 ± 3387	5.3

[1]average in three runs; [2]standard deviation (n=3); [3] relative standard deviation of peak height (n=3); [4] relative standard deviation of peak area (n=3)

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Table 3.4 Analytical data of triphenyltin at varying initial column temperature

initial temperatue (^{0}C)	Peak height mean[(1)]±S.D.[(2)]	%RSD[(3)]	Peak area mean[(1)]±S.D.[(2)]	%RSD[(4)]
35	14919 ± 86	0.6	146842 ± 684	4.6
45	15117 ± 724	4.8	150533 ± 10379	6.8
55	15516 ± 501	3.2	157997 ± 10256	6.4
65	13236 ± 2083	15.8	125059 ± 20262	16.2

[(1)]average in three runs; [(2)]standard deviation (n=3); [(3)] relative standard deviation of peak height (n=3); [(4)] relative standard deviation of peak area (n=3)

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3.3 Derivatization of phenyltin compounds

3.3.1 Effect of derivatization reaction time on signal of phenyltin compounds

The effect of reaction time on the derivatization reaction of the organotin compounds was studied by Harino and Fukushima [25]. They proposed that the derivatization reaction time of phenyltin compounds was similar. In this work, MPT was chosen for the study of effect of derivatization reaction time. Standard solution of 5 ppm MPT (50 µl) and 5 ppm DBT (30 µl) as an internal standard, in hexane was ethylated by ethylmagnesium chloride. The time of derivatization reaction was varied from 5 to 30 minutes. The procedure and GC conditions were described in section 2.5.1.2.1. and 3.2.1.1, respectively. The results showed that at different reaction time no appreciable difference in peak height and peak area were obtained for monophenyltin. This could indicated that the derivatization reaction occured in less than 5 minutes after introducing Grignard reagent (ethylmagnesium chloride).

3.3.2 Effect of amount of Grignard reagent on derivatization for triphenyltin

Since only triphenyltin was stable in environmental samples, therefore the rest of the project would concern with triphenyltin analysis. The effect of amount of Grignard reagent on derivatization for triphenyltin was studied. Standard solution of 10 ppm TPT (100 µl) in hexane was ethylated by ethylmagnesium chloride and the amount of ethylmagnesium

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chloride was varied from 0.25 to 2 ml. The procedure and GC conditions were described in section 2.5.1.2.2. and 3.2.1.1, respectively. The results in Figure 3.1 showed that the signal increased with the amount of Grignard reagent. The signal reached a maximum at Grignard reagent of 1.5 ml. Therefore, the 1.5 ml volume will be utilized in the following experiments.

3.4 Calibration of phenyltin compounds by gas chromatography

The chromatograms of ethylated organotins (mono-, di-, and triphenyltin compounds) were shown in Figure; 3.2, 3.3 and 3.4. Under the condition employed, all peaks were well separated when using OV-17 packed column, HP-17 and HP-1 capillary column.

The calibration curves of the analysis performed on OV-17 packed column were shown in Figure 3.5 and the analytical data reported in Table 3.5. In capillary chromatographic mode, the analytical data performed on HP-17 column were reported on Figure 3.6 and Table 3.6. And the analytical result obtained on HP-1 capillary column were shown in Figure 3.7 and Table 3.7.

Results obtained from the three columns showed that the calibration curves were linear up to at least 2 ppm concentration of phenyltin compounds. Relative standard deviation, when using capillary columns, was lower than that of packed column. It might be because of the differences of sample introduction system in packed column and capillary

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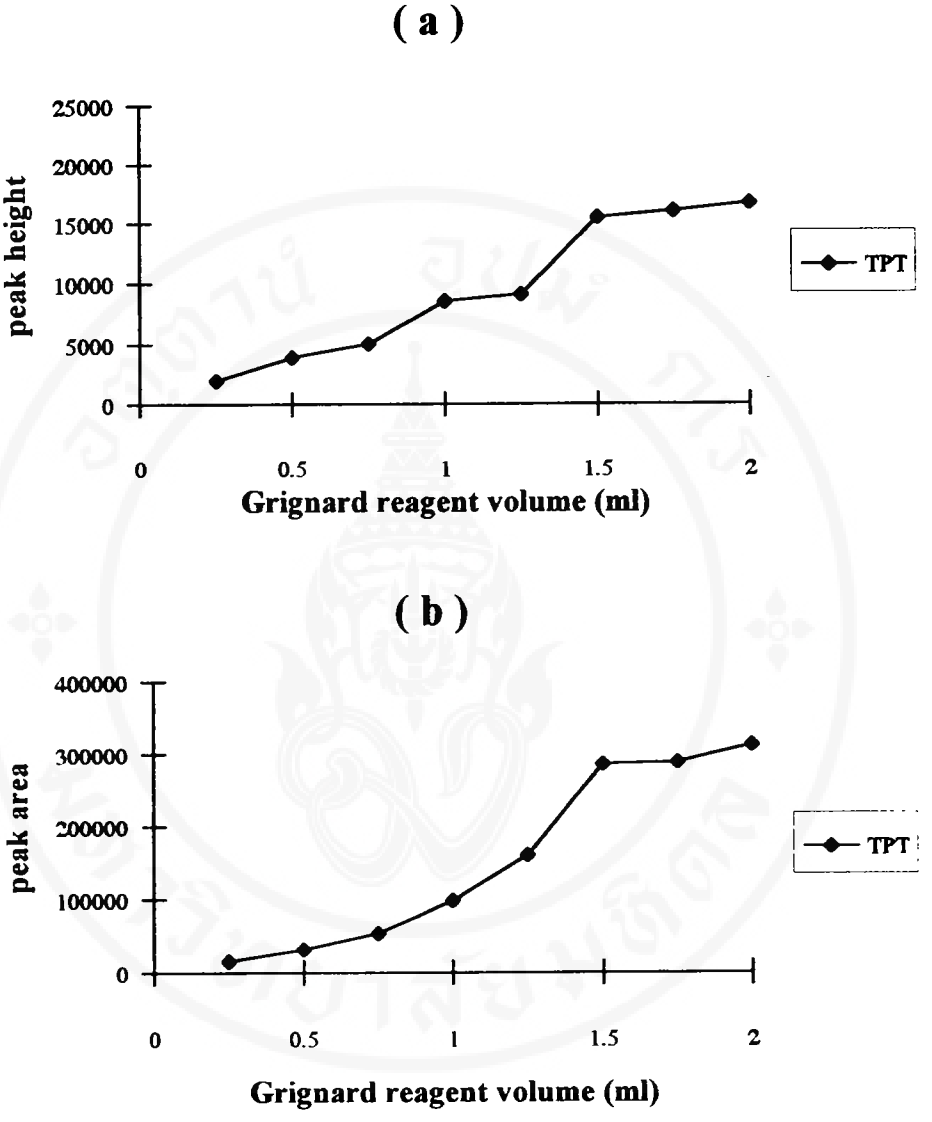

Figure 3.1 The effect of amount of Grignard reagent for TPT 2 ppm on a) peak height, b) peak area

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column. The superior character of inlet system in capillary mode (i.e. split or splitless) could be the reason for more reproducible data. The shape of splitless inlet had more space for the analyte vapor to concentrate before being transferred to the analytical column. Moreover, the lower temperature of initial temperature (i.e. 55°C) could prevent loss of analyte vapor. The detection limit of mono-, di- and triphenyltin compounds, obtained from HP-1 capillary column, were the poorest when compared with OV-17 packed column and HP-17 capillary column. Data of detection limit were summarized in Table 3.8. However, HP-1 capillary column was chosen for most of the work. This was because the HP-17 capillary column and OV-17 packed column degraded on prolonged use. They were not appropriate for further use.

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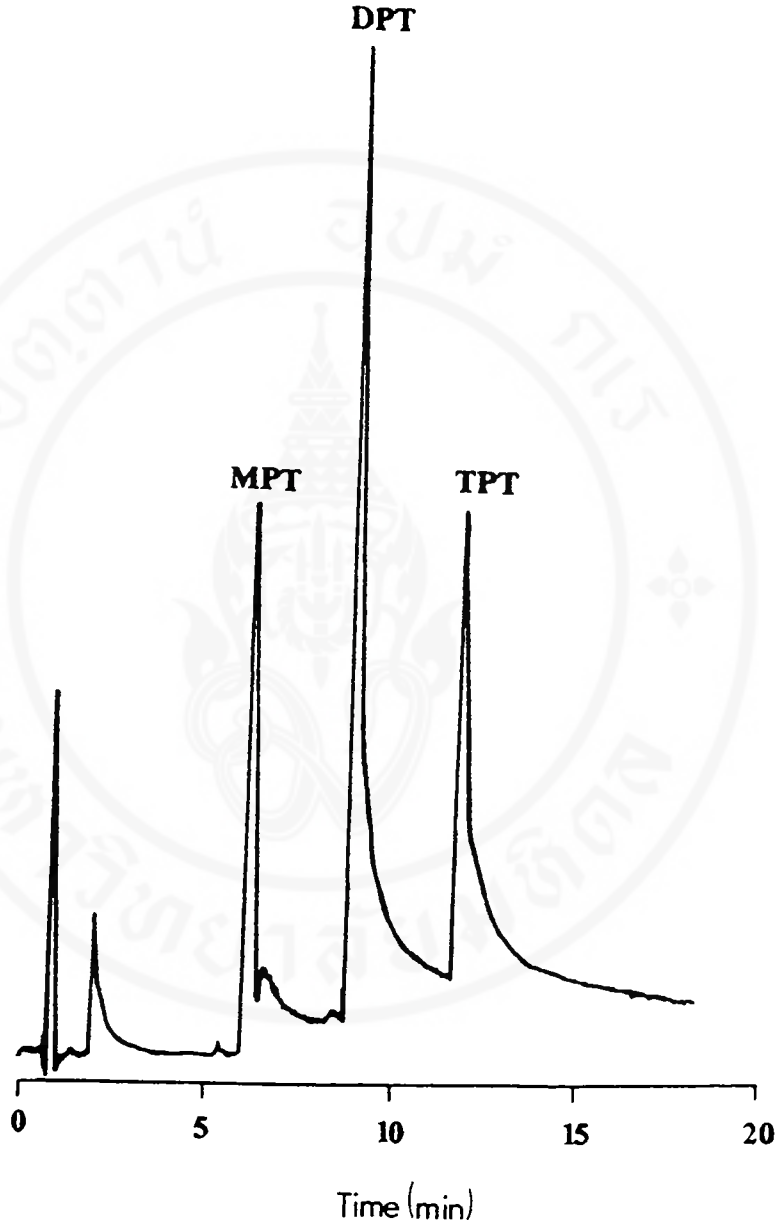

Figure 3.2 Gas chromatogram of ethylated derivative of phenyltin compounds using OV-17 packed column; MPT 1.5 ppm, DPT 4 ppm and TPT 3 ppm; GC conditions as described in Appendix I

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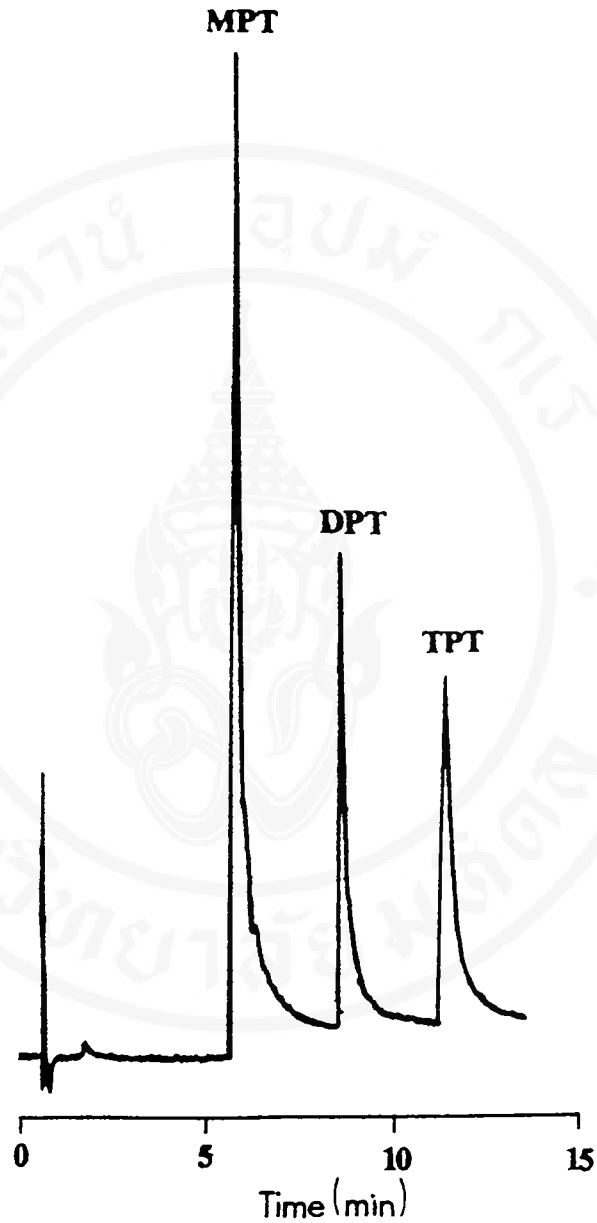

Figure 3.3 Gas chromatogram of ethylated derivative of phenyltin compounds using HP-17 capillary column; MPT 2.5 ppm, DPT 2 ppm and TPT 3 ppm; GC conditions as described in Appendix II

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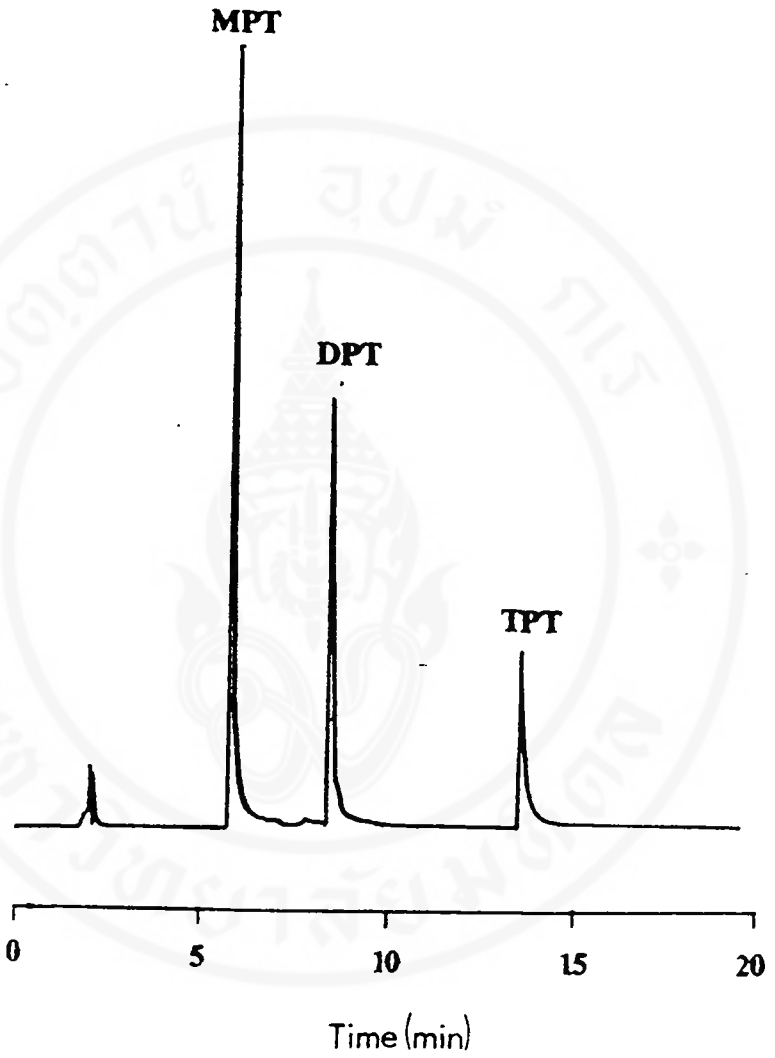

Figure 3.4 Gas chromatogram of ethylated derivative of phenyltin compounds using HP-1 capillary column; MPT 2 ppm, DPT 2 ppm and TPT 2 ppm; GC conditions as described in Appendix III

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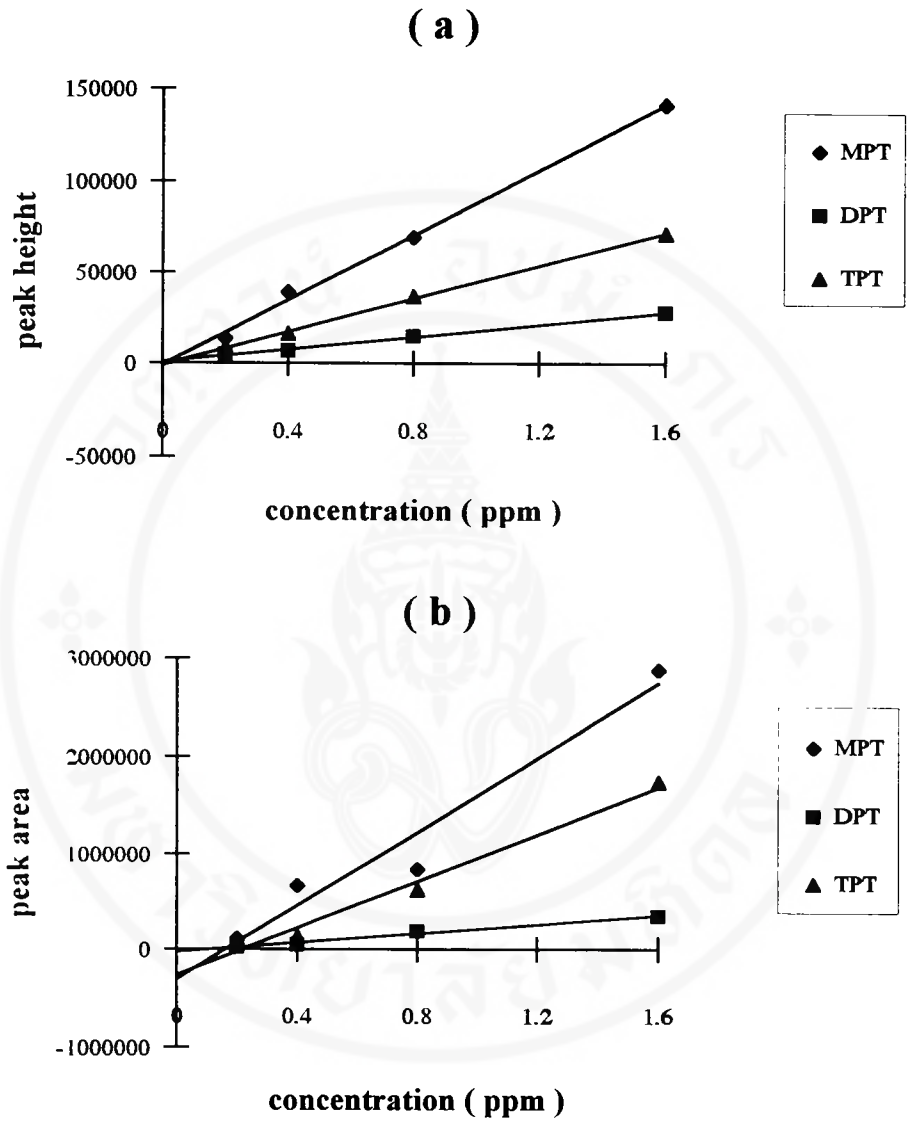

Figure 3.5 Calibration curves of phenyltin compounds obtained by plotting; a) peak height, b) peak area versus the concentration of phenyltin compounds using OV-17 packed column

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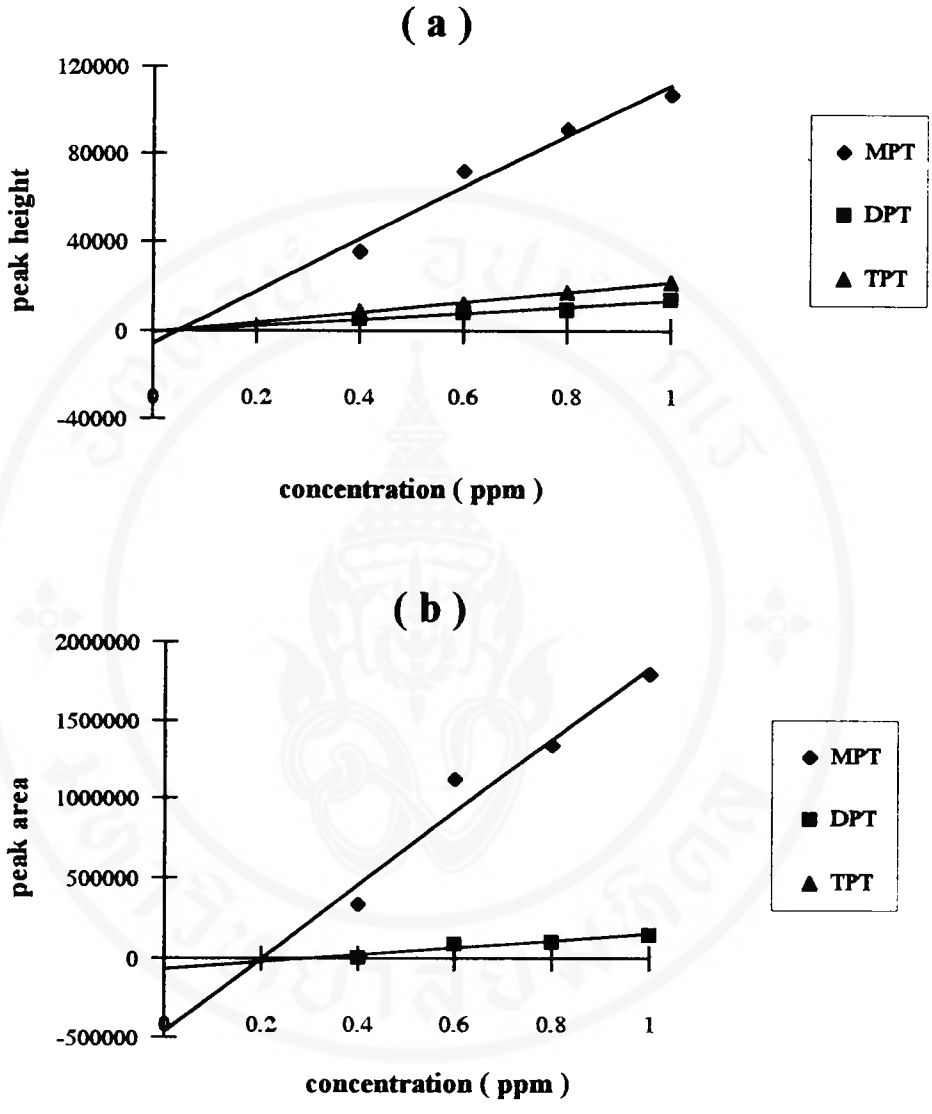

Figure 3.6 Calibration curves of phenyltin compounds obtained by plotting; a) peak height, b) peak area versus the concentration of phenyltin compounds using HP-17 capillary column

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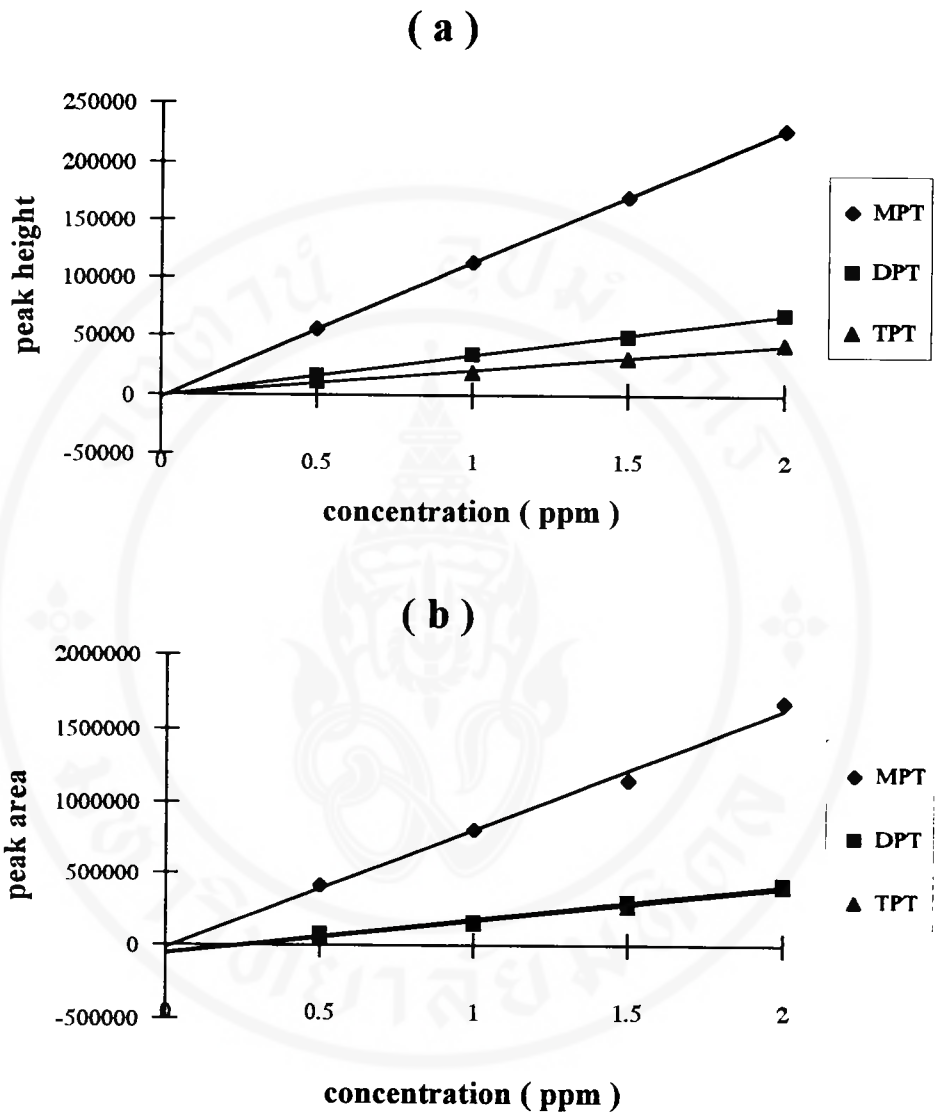

Figure 3.7 Calibration curves of phenyltin compounds obtained by plotting; a) peak height, b) peak area versus the concentration of phenyltin compounds using HP-1 capillary column

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Table 3.5 Analytical data obtained when using OV-17 packed column for phenyltin analysis

Phenyltin species	Detection limit[1] (ppb)	%RSD[2] Peak height	Regr.line corr.coeff of peak height	%RSD[2] Peak area	Regr.line corr.coeff of peak area
MPT	8.40	4.76	y = 8941x-1188.6 r = 0.9909	12.25	y = 2E+0.6x-316482 r = 0.8146
DPT	29.31	18.13	y = 16833x+91.154 r = 0.9985	23.40	y = 299873x-81929 r = 0.9898
TPT	26.15	12.60	y = 45524x-1039.5 r = 0.999	19.60	y = 1E+06x-266077 r = 0.9795

[1]S/N ratio = 2 ; [2] %R.S.D. at concentration 400 ppb (n = 3)

Chromatrographic conditions: column HP-1, He flow rate 20.69 ml/min, Air flow rate 88.39 ml/min, H_2 flow rate 106.24 ml/min, detector temp. 250 ^{0}C, injector temp. 250 ^{0}C, column temp. 55 ^{0}C(1min),40 ^{0}C/min → 240 ^{0}C(15 min).

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Table 3.6 Analytical data obtained when using HP-17 capillary column for phenyltin analysis

Phenyltin species	Detection limit[(1)] (ppb)	%RSD[(2)] Peak height	Regr.line corr.coeff of peak height	%RSD[(2)] Peak area	Regr.line corr.coeff of peak area
MPT	5.22	5.49	y = 116967x-5491.3 r = 0.9616	8.04	y = 2E+06x-464112 r = 0.8146
DPT	20	2.94	y = 13826x-386.1 r = 0.9487	4.69	y =152396x-9375.1 r = 0.963
TPT	20	1.65	y = 21633x+137 r = 0.9505	1.42	y = 218657x-4367.5 r = 0.9718

[(1)]S/N ratio = 2 ; [(2)] %R.S.D. at concentration 600 ppb (n = 3)

Chromatrographic conditions: column HP-1, He flow rate 1.42 ml/min, Air flow rate 88.39 ml/min, H_2 flow rate 106.24 ml/min, detector temp. 250 ^{0}C, injector temp. 250 ^{0}C, column temp. 55 ^{0}C(1min),40 ^{0}C/min → 240 ^{0}C(15 min).

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Table 3.7 Analytical data obtained when using HP-1 capillary column for phenyltin analysis

Phenyltin species	Detection limit[1] (ppb)	%RSD[2] Peak height	Regr.line corr.coeff of peak height	%RSD[2] Peak area	Regr.line corr.coeff of peak area
MPT	10.45	6.51	y = 115300x-2316.9 r = 0.9970	8.12	y =105030x-471.90 r = 0.9750
DPT	26.55	5.73	y = 34463x-1293 r = 0.9982	3.70	y =218031x-24839 r = 0.9817
TPT	40	1.41	y = 21216x-640.5 r = 0.9890	4.23	y = 229693x-63131 r = 0.9891

[1]S/N ratio = 2 ; [2] %R.S.D. at concentration 1 ppm (n = 3)

Chromatrographic conditions: column HP-1, He flow rate 1.42 ml/min, Air flow rate 88.39 ml/min, H_2 flow rate 106.24 ml/min, detector temp. 250 ^{0}C, injector temp. 250 ^{0}C, column temp. 55 ^{0}C(1min),40 ^{0}C/min → 240 ^{0}C(15 min).

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Table 3.8 Data of detection limit when using OV-17 packed column, HP-17 and HP-1 capillary column

Type of column	Stationary phase	Stationary phase polarity	Detection limit[1] (ppb)		
			MPT	DPT	TPT
OV-17	50%phenyl and 50%methylpolysiloxane	intermediate	8.40	29.31	26.15
HP-17	50%phenyl and 50%methylpolysiloxane	intermediate	5.22	20.00	20.00
HP-1	100% dimeyhylpolysiloxane	non polar	10.45	26.55	40.00

[1]S/N ratio = 2

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3.5 Use of alternative derivatizing agent

Ethylmagnesium chloride caused a problem of peak splitting of organotin compound which might be due to impurities of Grignard reagent. Therefore another derivatizing agent was chosen for analysis. In general, it was found that alkylmagnesium bromide was more reactive than alkylmagnesium chloride and therefore RMgBr reagent is prefered [21,24]. Furthermore, ethylation was not suitable for butyl- and phenyltin compounds as the derivatives were fairly volatile and could be lost during routine concentration procedures involving removal of solvents [24]. To overcome the volatility problem, propylmagnesium bromide was used for derivatizing agent. The n-propyl derivatives were prepared for analysis of phenyltin compounds in this work. The n-propyl derivatives were sufficiently non- volatile to prevent the loss during solvent removal, yet sufficiently volatile for use in analyse by GC. Figure 3.8 showed the gas chromatogram obtained with mono-, di- and triphenyl propyltin at concentration of 3 ppm each. The chromatogram showed a good separation of the three organotin compounds and sensitive detection. Moreover, there was a system peak that occured before the phenyltin compounds peaks but it did not disturbed the phenyltin compounds analysis. The calibration curves of n-propyl derivatives were shown in Figure 3.9 and the chromatographic results reported in Table 3.9. It was found that the calibration curves were linear up to at least 1.5 ppm concentration of phenyltin compounds.

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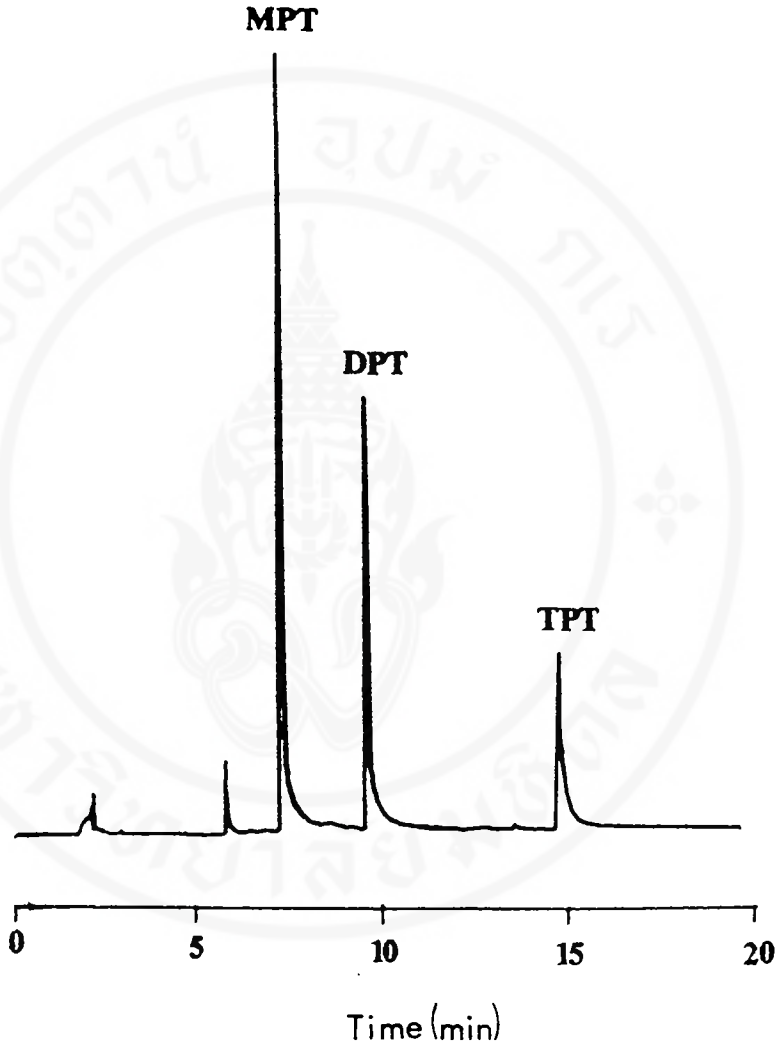

Figure 3.8 Gas chromatogram of n-propyl derivative of phenyltin compounds using HP-1 capillary column; MPT 2 ppm, DPT 2 ppm and TPT 2 ppm; GC conditions as described in Appendix III

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Table 3.9 Analytical data obtained when using propylmagnesium bromide as derivatizing agent

Phenyltin species	Detection limit[(1)] (ppb)	%RSD[(2)] Peak height	Regr.line corr.coeff of peak height	%RSD[(2)] Peak area	Regr.line corr.coeff of peak area
MPT	13.97	2.08	y = 52380x-2347 r = 0.9832	13.60	y =272958x+26581 r = 0.8605
DPT	35.56	4.95	y = 19437x-198.5 r = 0.9724	5.21	y =196274x-17499 r = 0.9625
TPT	48	3.42	y = 11709x+825.8 r = 0.9867	7.07	y = 217063x-57720 r = 0.7735

[(1)]S/N ratio = 2 ; [(2)] %R.S.D. at concentration 1.2 ppm (n = 3)

Chromatrographic conditions: column HP-1, He flow rate 1.42 ml/min, Air flow rate 88.39 ml/min, H_2 flow rate 106.24 ml/min. detector temp. 250 ^{0}C, injector temp. 250 ^{0}C, column temp. 55 ^{0}C(1min),40 ^{0}C/min → 240 ^{0}C(15 min).

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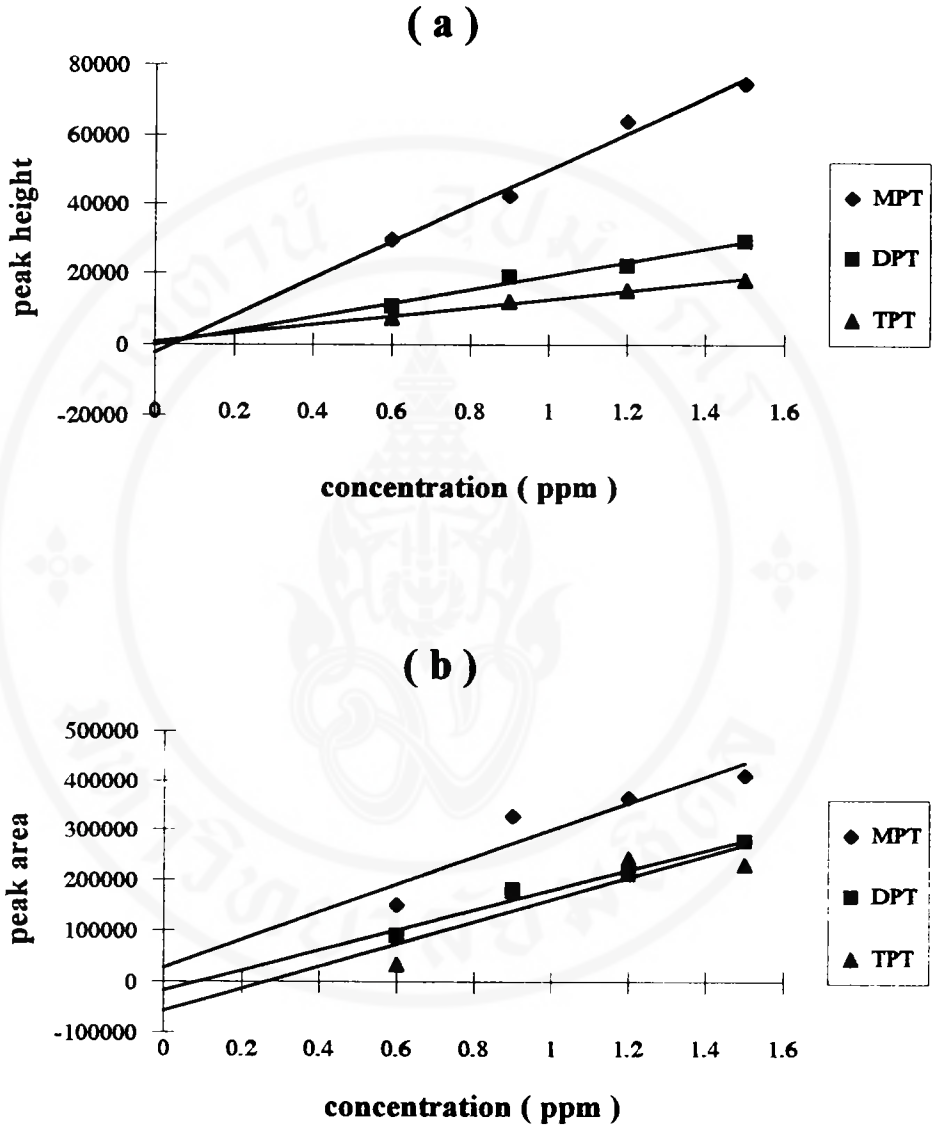

Figure 3.9 Calibration curves of phenyltin compounds obtained by plotting; a) peak height, b) peak area versus the concentration of phenyltin compounds using HP-1 capillary column and propylmagnesium bromide as derivatizing agent

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3.6 CONCLUSION

Gas chromatographic method was developed for phenyltin compounds. The GC conditions such as flow rate of carrier gas (He), fuel to H_2 ratio in FPD flame, injector and initial column temperature were optimized in order to obtain reproducible results as well as high sensitivity. The data when using packed and capillary column were demonstrated and HP-1 capillary column was chosen for analysis. In derivatizing step, the optimized volume of Grignard reagent was found to be 1.5 ml and no appreciable effect from different reaction time was observed. Propylmagnesium bromide was also tried to see if there was any advantages over ethylmagnesium chloride. The results showed similar performance.

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CHAPTER IV

COMPARISON BETWEEN LIQUID-LIQUID EXTRACTION AND SOLID-PHASE EXTRACTION

4.1 INTRODUCTION

The growing awareness of the pollution of the aquatic environment by organotin compounds in recent years has resulted in a steadily increasing number of reports concerning the analytical chemistry of these contaminants. The greatest hazard to biological life is associated with the use of tributyl- (TBT) and triphenyltin (TPT) compounds as biocides, especially in antifouling paints. The most affected are waters with intense ship activity. Once released, organotin can undergo a variety of physico-chemical processes such as biodegradation, biomethylation, sorption on the particulate matter or vaporization [40].

The toxicity of organotin compounds is species dependent so a viable analytical method should allow the determination of the target compounds without an interference from other organotin species. At present, the speciation analysis of organotin is usually performed by coupled techniques based on a combination of a chromatographic separation technique with a sensitive and element selective detection method. Gas chromatography enjoys a wider popularity despite more complex sample preparation procedures often required because of insufficient volatility of the organotin compounds. Generally, almost every

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procedure for speciation analysis consists of five successive steps:- (1) separation of the analytes from the sample matrices, (2) formation of volatile derivatives, (3) preconcentration, (4) clean-up and (5) determination.

Recent rapid advances in analytical instrumentation enable the instrumental analysis of many samples. However, despite these advances it is still often necessary to use separation and preconcentration method prior to the quantitative determination. Preconcentration step is intended to reduce the limit of detection, both by removal of interferences and increase of the concentration of the species of interest. Among the preconcentration techniques, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used methods for sample emrichment.

LLE is a major sample preparation tecnique for the determination of organotin [21, 26, 27] but due to the drawbacks of the technique it is begining to be replaced by SPE for routine applications with high througput. LLE tends to be time consuming and laborious. It is also more expensive as large volumes of solvent may be required and many of these solvents are potential health hazards. Furthermore, a major problem of LLE is the formation of emulsions which are extremely difficult to break and often cannot be separated by either centrifugation or ultrasonication. More recently, SPE has been an attractive approach to the preconcentration of traces of analytes. Its advantages over LLE include a higher enrichment factor, lower risk of contamination and the ease of application to field sampling and in on-line manifolds. In this field SPE

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has been reported as a suitable technique for the preconcentration of organotin compounds [19, 29, 33].

As mentioned in the aims of the studies, this work was attempted to develop a suitable extraction technique for phenyltin analysis. For determination of TPT, two alternative extraction techniques were used : liquid-liquid extraction and solid-phase extraction (silica column). In LLE, many parameters were selected for study such as the extraction volume ratio, the effect of HCl and %tropolone to the recovery of TPT. For SPE, the optimization of preconcentration steps were studied. SPE ensured good results and was more suitable in the field, avoiding high volumes of organic solvent and transport of fragile glassware. From the advantages of SPE, it was chosen for preconcentration technique and applied for environmental water samples and fish sample analysis which were shown in details in the next chapter.

4.2 LIQUID-LIQUID EXTRACTION

4.2.1 Effect of extraction volume ratio in liquid-liquid extraction for preconcentration

Standard solution of 25 ppm TPT (25 µl) in MeOH was added in distilled water with various volume from 25 to 200 ml and the optimum ratio of extraction volume was studied for LLE by varying in the ratio of 1:5, 1:7 and 1:10 (v/v). The recovery was obtained by comparing with standard TPT solution that was equal to the amount of TPT, expected in the final solution. Table 4.1 showed that the recoveries of TPT obtained

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when performing preconcentration by liquid-liquid extraction. It was seen that the large volume of distilled water (i.e. 150 and 200 ml) gave higher recovery than small volume and large extraction volume ratio gave the better recovery. Therefore, the optimum extraction volume ratio at 1:5 (v/v) was chosen for further study.

In Table 4.1, it was noticed that with the same extraction volume ratio, the % recovery of TPT still not the same with various distilled water volume, like it should be. It was indicated that not only the sample volume but also the concentration of TPT should be awared. Because TPT had to dissolve in restricted volume of ether, the unequal solubility of TPT was occured. At 1:5 (v/v) of extraction volume ratio, when used small volume of water (i.e. 25 ml) which corresponded to the TPT concentration in distilled water of 25 ppb, the low recovery was obtained. It showed that the factors concerned with LLE process should be come from both extraction volume ratio as well as concentration of TPT in water. This meant that the limitation of LLE technique came from the fact that it should provide enough volume of extractant to dissolve the interested compounds otherwise the technique would become less effective. Unlike SPE technique, the eluent volume required for dissolving the analyte from the column was less amount.

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Table 4.1 Percent recovery of TPT obtained when performing preconcentration by liquid-liquid extraction

TPT solution*		extraction volume ratio		
volume (ml)	concentration (ppb)	1:5	1:7	1:10
25	25.00	71.12	69.38	4.55
50	12.50	88.84	78.22	7.87
150	4.17	90.67	86.98	56.40
200	3.13	93.08	88.09	59.15

*all contain 0.625 μg of TPT

4.2.2 Effect of the amount of HCl added in the sample extraction step

Extraction of organotin compounds based on acidification with hydrochloric (HCl), hydrobromic (HBr) and acetic acid (HAc) of the sample to release alkyltin and phenyltin compounds from the suspended particulate matter and to convert them into the respective halogenides which were then extracted into a variety of organic solvents, therefore effect of the addition of HCl in the sample extraction step was studied. Equal amounts of standard TPT solution were spiked in different volume of distilled water (i.e. 25 to 200 ml). The concentrated HCl was added with various volume (0 to 15 ml) and the optimum extraction

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volume ratio (1:5 v/v) obtained from Section 4.2.1 was used. The recoveries were obtained in the same manner as in the previous section. The results were shown in Table 4.2 and it was found that the addition of HCl improved the recoveries to be greater than 90%, except for the experiment using 25 ml of distilled water. The addition of concentrated HCl 15 ml to 25 ml of distilled water gave low recovery because the concentration of HCl (37.5% v/v) was too high for effectiveness of TPT extraction.

Table 4.2 Percent recovery of TPT obtained from the addition of concentrated HCl in the sample extraction step

volume of TPT solution* (ml)	HCl added			
	0 ml	5 ml	10 ml	15 ml
25	71.12	72.04	69.43	4.27
50	88.84	97.14	96.25	95.53
150	90.67	100.90	99.22	96.04
200	93.08	103.04	98.15	97.26

*all contain 0.625 μg of TPT

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4.2.3 Effect of percent tropolone on extraction efficiency

To avoid the problems arising from the extractant of more polar organotin compounds, the effect of % tropolone added to the extractant was tested. This reagent was frequently recommended for organotin species, and was used in previous studies. The concentrations of tropolone in ether solution which were used for the extractant were varied from 0 to 0.06% (w/v). Different water volumes were spiked with TPT and extractant with the optimum extraction volume ratio (1:5 v/v) obtained from Section 4.2.1. The effect of % tropolone, added to the extractant to recovery of TPT was shown in Table 4.3. It was found that the recoveries of extraction were good and satisfactory for large volume of water when using tropolone in ether solution as extractant.

Table 4.3 Percent recovery of TPT showing the effect of % tropolone on extraction efficiency

volume of TPT solution* (ml)	% tropolone			
	0 %	0.02 %	0.04 %	0.06 %
25	71.12	74.67	73.31	72.35
50	88.84	91.82	97.46	97.15
150	90.67	97.27	106.83	103.05
200	93.08	100.90	108.65	104.98

*all contain 0.625 μg of TPT

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4.3 SOLID-PHASE EXTRACTION

Solid-phase extraction (SPE) provides samples that are in solution, free of interfering matrix components, and concentrated enough for detection. Preconcentration steps for SPE consisted of the followings such as, conditioning the column, loading the sample, washing the column and elution of interested compounds. Usually the first step of process was conditioning of column with appropriate solvents which depend on the column packing and the application. The column was conditioned in order to open up the hydrocarbon chains and hence increase the surface area available for interaction with the analyte and to remove residues from the packing material that might cause the interference in the analysis. The next step is adding or loading where the sample is transfered and retained on the column. For washing step a suitable solvent or water is passed through the column to remove selectively compounds from the sample matrix which might interfere with analysis. The last step is elution that elute sample with a suitable solvent and collect eluate for further analysis. Each step is important for the success of preconcentration process.

4.3.1 Comparison between silica column and C_{18} column in preconcentration process

Two type of columns were selected for preconcentration step. They are silica column and C_{18} column. Samples which were comprised of distilled water and spiked standard TPT solution were passed through the prewashed column. After allowing air passed through the column for

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10 minutes, the concentrated solute being trapped on the precolumn was eluted with different kinds of solvent. The volume of eluent was fixed at 5.00 ml and the results obtained in Table 4.4 showed the effect of different kinds of eluent for each types of packing materials. It was noticed that the best result was obtained when using silica column and ether for elution. The recovery greater than 90% was obtained which might be because of the comparable polarity between TPT and silica as well as the polarity of ether. The similar polarity between the eluent and TPT made them eluted in a suitable time. Therefore, at this stage the preconcentration step for determination of triphenyltin compound was performed by silica column and ether as a preconcentrator column and eluent, respectively.

Table 4.4 Percent recovery of TPT obtained from using three kinds of eluent in C_{18} and Silica column*

eluent	SPE column	
	C_{18}	silica
hexane	0.00	57.97
methanol	58.07	74.95
ether	31.53	92.13

*TPT solution of 60 ppb 25 ml, 5.00 ml eluent were used.

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4.3.2 Study of breakthrough volume

For loading step the sample volume was passed through the conditioned-silica column. To increase the sensitivity and lower the detection limit it was necessary to extract larger volume of sample. Therefore, the potential breakthrough volume of the silica column was determined. Various volume of distilled water containing standard solution of TPT at 100 ppb level was loaded on to the conditioned-silica column. The column was washed with 0.50 ml distilled water and allowed to dry for 10 minutes. TPT was eluted from the column with 3.00 ml ether. The results were shown in Table 4.5 and Figure 4.1. It could be seen that there was no measurable breakthrough of the analyte from the silica column utilized in the experiment for at least up to the sample volume of 200 ml. Therefore, the load sample volume of 200 ml did not lead to overloading of column. This volume (200 ml) was considered to be large enough to concentrate the sample of interest to reach the detectable level. Therefore, further experiment was not continued to obtain a breakthrough volume.

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Table 4.5 Analytical data obtained from the study of breakthrough volume

loaded water volume * (ml)	peak height	regression line correlation coeff.
5	8222	
10	17971	
20	35519	$Y = 1227X + 12664$
30	56882	$R^2 = 0.9899$
50	81212	
100	150471	
150	193281	
200	250927	

* 100 ppb TPT was used

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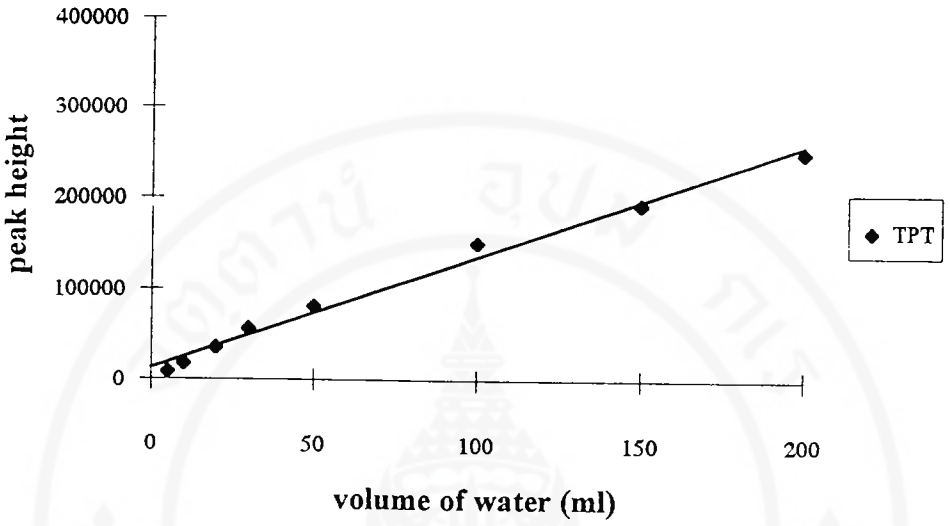

Figure 4.1 Calibration curve from the study of breakthrough volume

4.3.3 Optimum elution volume in preconcentration step

As commonly, the preconcentration steps consisted of "load", "wash" and "elute" step. It is importance to optimize each step to give the best results. To judge and select the best condition it was necessary to compare the preconcentration step with direct injection mode. The condition will be accepted when the result from direct injection and preconcentration was similar or 100% recovery. In elution step the volume of eluent was optimized for use in further study. Standard solution of TPT was spiked in various volumes of distilled water and the sample volume was loaded on to a conditioned- silica column mounted on a vacumn manifold. After the column was dried for 10 minutes, the ether

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volume was varied for eluting TPT from the column. The results were obtained by employing the eluent as shown in Table 4.6. It could be seen that no difference in recoveries was observed with various water volumes and the optimum elution volume was 3.00 ml for elution of TPT compound. Furthermore, the polarity of eluent was increased by adding THF in ether solution for complete elution of TPT compound. By varying the concentration of THF in ether solution in the range of 5-10 % (v/v) and also with the various volume of eluent, no difference in the recoveries was observed even when up to 10.00 ml was added. Therefore, it was decided that THF should not be added as a modifier in the elution step.

Table 4.6 Percent recovery of TPT from spiked distilled water using varying ether volume at 50 and 100 ml loading volume

elution volume of ether (ml)	loading volume	
	50 ml*	100 ml*
0.50	60.82	62.95
1.00	70.00	81.77
1.50	75.34	84.55
3.00	96.64	98.92
5.00	94.13	96.75
8.00	95.16	97.22
10.00	96.02	95.57

*contain 0.05 μg of TPT

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4.3.4 Optimum wash volume in preconcentration step

The purpose of wash step with water or suitable eluent was that it could help to remove any interferences which were retained on the column after loading sample. Therefore, the water volume should be optimized to give best results. Different volume of distilled water was spiked with standard TPT solution and passed through the conditioned-silica column. Washing step with various volume of distilled water was carried out and the results were compared. The optimum ether volume from Section 4.3.3 was used for elution step Table 4.7 presented the results that the optimum water volume for washing the column was in the range of 5.00-15.00 ml.

Table 4.7 Percent recovery of TPT obtained with varying wash volumes at 50 and 100 ml loading volume*

wash volume (ml)	loading volume	
	50 ml	100 ml
1.00	79.34	86.98
3.00	90.47	91.56
5.00	101.14	105.94
10.00	97.92	96.72
15.00	99.33	97.13

*contain 1.5 μg of TPT and ether volume 3.00 ml was used as eluent.

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4.3.5 Optimization of preconcentration parameters for diphenyltin compound determination

This section intended to find suitable eluents for the elution step. No difference in the recoveries was observed with various volumes of distilled water (see Section 4.3.3), therefore the water volume was fixed at 25 ml for the following experiments. The volume of eluent was obtained from Section 4.3.3 and used for this work. Two kinds of eluent were used; they are ether and solution containing tropolone in ether. It was found that ether was not suitable as it had weaker strength for eluting DPT compound from the column even when 10.00 ml of ether was used. Therefore, the concentration of % tropolone in ether solution was varied. 25 ml of standard solution of DPT at 60.0 ppb level was loaded to the conditioned-silica column to perform preconcentration process. After leaving the column dry for 10 minutes, solution containing tropolone in ether was used as eluent and was passed through the column using the same volume as obtained from Section 4.3.3. Table 4.8 showed the results obtained by varying the concentration of tropolone in ether solution. It was found that the recovery was much improved when using tropolone in ether solution as the eluting solution (i.e. from 0.00 to 72.86%). Tropolone was acting as a complexing agent that could be bound with DPT and made them less polar. The lowering in polarity of DPT made them elute easily by ether which led to higher recoveries. The results showed that the optimum concentration of tropolone in ether solution was 0.06% (w/v) that was chosen for further study.

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Table 4.8 Effect of concentration of tropolone in eluent on recovery of DPT*

% tropolone	% Recovery
0	0.00
0.02	72.86
0.04	89.98
0.06	99.97
0.08	97.07

*TPT solution of 30 ppb 25 ml and eluent volume of 3.00 ml were used

4.3.6 Optimum elution volume in preconcentration step for diphenyltin compound determination

Standard solution of 50 ppm DPT (30 μl) in MeOH was spiked in 25 ml volume of distilled water and the sample volume was loaded on to a conditioned - silica column mounted on a vacumn manifold. The column was washed with 5.00 ml distilled water . After the column was allowed to dry for 10 minutes, the elution volume was varied for eluting DPT from the column with 0.06% (w/v) tropolone in ether solution. The results were obtained by employing the eluent as shown in Table 4.9. It was obvious that the recovery was good and satisfactory when using 3.00 ml of eluent volume.

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Table 4.9 Effect of eluent volume on recovery of DPT

eluent volume (ml)	%Recovery
1.00	57.04
3.00	102.95
5.00	96.15
8.00	98.36
10.00	99.91

*0.06% (w/v) tropolone in ether was used as eluent

4.3.7 Modification of preconcentration step for diphenyltin compound determination in seawater

The purpose of washing step with water was to remove any interferences such as co-anions in seawater. Therefore, the water volume for washing step was optimized. DPT was chosen for this study and the result would be applied for DPT and TPT determination in environmental water samples. Standard solution of 50 ppm DPT (30 µl) in MeOH was spiked in 25 ml of seawater and passed though the conditioned- silica column. The distilled water volumes were varied in washing step. The concentration of tropolone in ether solution and volume of eluent were 0.06% (w/v) and 3.00 ml, respectively. The results were shown in Table 4.10. It was found that the optimum wash volume was 15.00 ml.

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Table 4.10 Effect of wash volume on recovery of DPT in seawater*

wash volume (ml)	%Recovery
1.00	52.80
5.00	83.95
10.00	90.74
15.00	101.40
20.00	98.90

*DPT solution of 60 ppb 25 ml and 0.06%(w/v) tropolone in ether 3.00 ml was used as eluent.

4.4 CONCLUSION

The results indicated that the use of solid phase extraction (SPE) could avoid the possible complication associated with a comparable liquid-liquid extraction (LLE). LLE tends to be time consuming and laborious. It also required large amounts of solvents and these solvents were potential health hazards. The volume of eluting solvent in this SPE procedure was 3.00 ml. Which was much less when compared with LLE procedure. Therefore, the SPE was chosen for preconcentration step for diphenyltin and triphenyltin compounds determination in environmental water samples. Moreover, SPE acts as a clean-up treatment to remove some interferences from fish sample.

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CHAPTER V

APPLICATION TO REAL SAMPLES ANALYSIS

5.1 INTRODUCTION

Solid phase extraction (SPE) has general advantages over liquid-liquid extraction (LLE) method, i.e. solvent reduction, higher preconcentration factor and ease of application in the field (see chapter IV). Development of analytical technique used for determination of DPT and TPT in environmental samples was studied. This chapter emphasized on the development of preconcentration and sample clean-up step for water and fish samples. The optimum conditions of SPE method obtained from the study in chapter IV, were applied for real sample analysis. The success of this technique was achieved by exploring the recovery of preconcentration of analyte in environmental water samples.

5.2 EXPERIMENTAL

5.2.1 Instrumentation

GC model used as described in Section 2.2.1 and the column was HP-1 (cross-linked Methyl Silicone Gum) 25m × 0.2mm × 0.3 μm, 100% dimethylpolysiloxane.

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GC conditions using in this experiment

Temperature	:	injector ; 250°C, detector ; 250°C
Oven	:	55°C (1 min), 40°C/min→ 240°C (15 min)
Air flow mode	:	H_2 ; 106.24 ml/min, air ; 88.39 ml/min
Carrier gas	:	He 1.38 ml/min
Inlet type	:	Splitless
Split vent	:	260.90 ml/min
Column head pressure	:	25 psi

5.2.2 Reagents and procedures

5.2.2.1 Determination of DPT in water samples

The environmental water samples were filtered through paper filter, Whatman no.6 (Whatman, Kent, U.S.A) and were then filtered through 0.45 μm membrane filter, Millipore Type HA (Millipore, MA, U.S.A.) prior to spiking with DPT.

Water samples from pond water and seawater were spiked with standard solution of DPT 50 ppm and were diluted to 25 ml volumetric flask to obtained the final concentration of 30, 60 and 90 ppb. The solution was preconcentrated by passing through silica gel column at 1 ml/min. The silica gel used for packing the column was

activated at 120°C overnight prior to use. The column was previously conditioned with 2 ml of hexane. Water samples containing DPT was passed through the prewashed column. The column was washed with 15.00 ml distilled water (which was the optimum volume obtained from Section 4.3.7). The air was passed through the column for 10 minutes and the elution was carried out by 3.00 ml of 0.06% (w/v) tropolone in ether solution (which was the optimum volume obtained from Section 4.3.6). Propylmagnesium bromide (2 mol/l in THF) 1.5 ml was carefully added to the eluate and the mixture was allowed to stand at least for 10 minutes. The excess of Grignard reagent was destroyed by adding 10 ml of saturated NH_4Cl solution. The ether layer, containing the ethylated derivatived, was dried with anhydrous Na_2SO_4 and evaporated until dry. The residue was made up to 0.5 ml with hexane for GC analysis. DPT in distilled water was also prepared in the same manner. The peak height was measured for the recovery experiment.

5.2.2.2 Determination of TPT in water samples

Water samples were filtered as mentioned in the previous section. Standard TPT solution 50 ppm was spiked in water samples which were diluted to 25 ml in the volumetric flask to obtained the final concentration of 30, 60 and 90 ppb. The solution was preconcentrated by passing through a silica gel column at 1 ml/min. After conditioning the column with 2 ml of hexane, water samples containing TPT was passed through the prewashed column. The column was washed with 15.00 ml of distilled water (which was the optimum volume obtained

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from Section 4.3.7). The air was passed through the column for 10 minutes and the elution was carried out by 3.00 ml of diethyl ether (which was the optimum volume obtained from Section 4.3.3). The eluate was dried over anhydrous Na_2SO_4 and concentrated to 1 ml. The concentrated solution of TPT was derivatized with propylmagnesium bromide (2 mol/l in THF) before GC analysis in the same manner as Section 5.2.2.1.

5.2.2.3 Development of method for fish sample analysis

A fish sample weighing 5.0 g (wet wt.) was macerated in 2 ml of saturated sodium chloride solution. Add 1.5 g of NaCl and 1 ml of HBr (48%). When the evolution of gas ceased, The slumy was extracted with two portions (10 ml each) of diethyl ether. After being shaked vigorously and centrifuged, the ether layer was combined and dried with anhydrous Na_2SO_4. The combined ether layer were reduced to 2 ml then the derivatization step was proceeded in the same manner as described before. The ether layer containing the ethylated derivatives, was dried with anhydrous Na_2SO_4 and was applied to a silica gel column. Silica gel 0.3446 g was packed in a column at a height of 1 cm. Silica column was prewashed with 2 ml of hexane. The ether layer was passed through the prewashed column. The column was washed with 5.00 ml of distilled water and then allowed to dry for 10 minutes. The ethylated derivatives were eluted with 3.00 ml of diethyl ether. The effluent was evaporated until dry and made up to 0.5 ml with hexane for GC analysis.

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5.3 RESULTS AND DISCUSSION

5.3.1 Development of preconcentration step in water samples analysis

The whole process of SPE in combination with GC and flame photometric detection was developed for pond water and seawater. Samples were usually filtered before analysis to eliminate suspended matter and interfering matrix. Standard solution (at ppb level) was spiked in samples. Four replicates of each sample were analysed using the proposed method. The recoveries of water samples spiked with DPT and TPT were shown in Table 5.1 and 5.2, respectively.

It was found that no difference in peak shape of DPT and TPT was observed between water samples and distilled water. Furthermore, there was no interfering peak in the chromatogram from water samples. Table 5.1 and 5.2 showed that greater than 90% recoveries could be obtained. The relative standard deviation was acceptable for this technique. The determination of monophenyltin compound was also studied. Four kinds of eluent were used: diethyl ether, tropolone in ether solution, hexane and MeOH. It was found that these eluents were not suitable as they were not strong enough to elute MPT from the silica column. The other reason was that MPT might not be stable during preconcentration process therefore it could not be trapped on pre-column. At this stage, development of preconcentration step in water analysis for the determination of monophenyltin compound has not yet been achieved.

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Table 5.1 Percent recoveries of DPT from spiked water samples

Water samples	Concentration (ppb)	%Recovery[1]	%RSD[2]
Seawater	30	98.31 ± 1.82	2.62
	60	97.50 ± 7.52	7.72
	90	105.33 ± 8.39	3.93
Pond water	30	97.43 ± 6.17	6.08
	60	103.50 ± 4.60	4.71
	90	101.50 ± 5.60	5.93

[1]Recovery (mean ± S.D. for n=4), [2]Relative standard deviation

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Table 5.2 Percent recoveries of TPT from spiked water samples

Water samples	Concentration (ppb)	%Recovery[(1)]	%RSD[(2)]
Seawater	30	96.90 ± 3.11	3.55
	60	95.38 ± 5.19	6.71
	90	101.75 ± 5.28	5.94
Pond water	30	97.55 ± 6.11	5.13
	60	101.23 ± 1.77	1.75
	90	98.02 ± 6.15	6.39

[(1)]Recovery (mean ± S.D. for n=4), [(2)]Relative standard deviation

5.3.2 Development of method for fish sample analysis

The experiments were performed by using fish sample spiked with standard TPT solution. The sample was then extracted and derivatized. Before injecting into GC, the sample was loaded on the silica column to clean-up. Three kinds of eluent were used: ether, ether: THF (90:10) and tropolone in ether solution. It was found that these eluents were not suitable for elution of TPT in fish sample analysis as low

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recoveries were obtained, even when 10.00 ml of eluents were used for elution. The low recoveries might come from the effect of matrices (i.e. fat or protein in fish) or from the nature of absorption onto the clean-up column. Effect of TPT absorption on column was therefore studied. Two types of column were used: silica and florisil column for clean-up. Ether was employed as the eluent. It was found that the peak of triphenyltin compound was observable in the chromatogram for both columns when analysing standard TPT solution. It might be concluded that triphenyltin compound could be absorbed on the column and then eluted normally. When TPT was spiked in fish sample, the peak of TPT was observable in the chromatogram but with low recovery. Therefore at this stage, it could be explained that the low recovery was due to matrix effect from fish sample, not from the permanent absorption of TPT on the clean-up column. It was important to note here that the phenyltin compounds should be derivatized before loading onto the clean-up column in fish sample analysis. Unlike water samples, the preconcentration as well as clean-up step was able to perform before derivatization with Grignard reagent. The possible assumption for low recovery of TPT from fish sample which can be made here was that, firstly the phenyltin compounds could not be trapped efficiently on the pre-column due to the interfering substance in tissue such as fat and protein, or secondly the phenyltin compounds might be trapped on the pre-column quantitatively but the eluent used could not elute them from the pre-column efficiently. To prove this, further research work was required. Other eluents such as hexane, ethylacetate, toluene or suitable mixed solvents could be tried in order to enhance elution efficiency. The efficiency of sample clean-up technique to

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remove fat, lipid, protein or other interfering substances that prevented phenyltin from absorbing onto the column was necessary to improve the analysis.

5.4 CONCLUSION

SPE was chosen for preconcentration technique in environmental samples analysis. By using SPE as an extraction technique, the analysis of trace level of phenyltin compounds (at ppb level) could be performed. Not only concentrating the analyte, it could be used for clean-up the sample. I "Wash step" in preconcentration process was proven as the effective way to remove the interferences from sample. In addition, sort of eluent and the volume used were optimized carefully to obtain the high recoveries (up to 95%) and good reproducible results. Nevertheless the fish sample could not be analysed successfully, the preliminary study of this clean-up step might be helpful in developing the technique of SPE to clean the tissue samples in the future.

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CHAPTER VI

CONCLUSION

Phenyltin compounds were analysed after derivatization using a Grignard reagent by gas chromatography with flame photometric detection (GC-FPD). The study was aimed for the optimum conditions for phenyltin species when using either capillary column or packed column. The column providing higher sensitivity and reproducible data was selected for further investigation. As a matter of fact, the forms of mono-, di- and trisubstituted organotin compounds usually presented in the environmental hydroxides and sulfides[44]. In the process of extraction they would be converted into the respective chlorides, which are suited for extraction into an organic solvent. It could be assumed that these compounds were polar and stable due to their high boiling points. To convert them to the form possessing enough volatility and suitable for GC analysis, the precolumn derivatizing reaction was required. Grignard reagent was one of the most often used for derivatization of organotin compounds due to its convenience and shorter time of reaction. Moreover, the product obtained from derivatization reaction would become less polar and could be adsorbed properly on the common GC column which contained nonpolar phase (i.e. HP-1), or weakly polar phase (i.e. HP-17). Using packed column and capillary column for separation of phenyltin compounds, it was found that they were well separated with the capillary column (i.e. HP-17 and HP-1) giving more reproducible data. However, HP-1 capillary

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column (25m×0.2mm×0.3μm, 100% dimethylpolysiloxane) was chosen for most of the work owing to the fact that it was commonly found in an ordinary laboratory.

Another concern of this study was also came from derivatization reaction. In the beginning of this work, ethylmagnesium chloride was selected for this purpose, It was found that ethylmagnesium chloride caused a problem of peak splitting of organotin compounds. To overcome the problem, propylmagnesium bromide was also tried in the same column that gave peak splitting . Although this system also gave a system peak in the chromatogram, it did not interfere the peak of interest of the analysis and importantly, the peak splitting was disappeared. However, it was found that the reproducible obtained when using two kinds of Grignard reagent was similar. The number of carbon in alkyl chain of ethyl- and propyl- might not give the distinct volatility that made an improvement of reproducibility. Further research works were required to obtain the optimum condition for this derivatizing reaction and to eliminate an interfering peak in the system. In order to identify the unknown product, a more sophisticated instrument such as GC-MS was necessary.

This work also included the comparative study between liquid-liquid extraction (LLE) and solid-phase extraction (SPE). For LLE, ether was used as extractant with the optimum ratio of 1:5 (v/v). The addition of HCl and tropolone improved the recovery of TPT when the large sample volume was used. Silica column and ether as eluent were chosen for TPT analysis in SPE. Silica column was previously conditioned with 2.00 ml of hexane and the sample volume was passed through the prewashed column.

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After washing off the interferences with 5.00 ml of distilled water, triphenyltin was eluted with 3.00 ml of ether. For diphenyltin analysis, it was eluted with 3.00 ml of 0.06% (w/v) tropolone in ether solution after washing off the interferences with 15.00 ml of distilled water. The SPE was found to give more advantages over LLE, therefore it was applied to the determination of diphenyltin and triphenyltin in pond water and seawater which allowed the determination at the ppb levels with satisfactory recovery.

This work could be considered as a preliminary study for further application to more complicate samples such as soils, sediments and fish which were the main source of trace elements that entered the food chain and the main receptor of contaminants from antropogenic activities. As the toxicity of organotin is strongly species dependent, a viable analytical method for monitoring these contaminants should be species selectives, allowing for the determination of the target compounds (e.g., triphenyltin and tributyltin) without interference from other organotin species. The reliable analytical method will enable the study of fate and distribution of organotin compounds in the environment. This would lead to adequate informations for concerned agencies or personnels in issuing regulations or planning of counter measures to protect our environment.

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APPENDIX I

Chromatographic conditions for the study of calibration of phenyltin compounds

Column	:	OV-17 packed column
Temperature	:	injector ; 250°C, detector ; 250°C
Oven	:	55°C(1 min), 40°C/min→ 240°C (15 min)
Air flow mode	:	H_2 ; 106.24 ml/min, air, 88.39 ml/min
Carrier gas	:	He 20.69 ml/min
Column head pressure	:	24 psi

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APPENDIX II

Chromatographic conditions for the study of standard method development

Column	:	HP-17 capillary column
Temperature	:	injector ; 250°C, detector ; 250°C
Oven	:	55°C(1 min), 40°C/min→ 240°C (15 min)
Air flow mode	:	H_2 ; 106.24 ml/min, air, 88.39 ml/min
Carrier gas	:	He 56.86 ml/min
Inlet type	:	Splitless
Split vent	:	260.9 ml/min
Column head pressure	:	11 psi

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APPENDIX III

Chromatographic conditions for the study of standard method development when using propylmagnesium bromide as derivatizing agent and the comparative study between LLE and SPE method

Column	:	HP-1 capillary column
Temperature	:	injector ; 250°C, detector ; 250°C
Oven	:	55°C(1 min), 40°C/min→ 240°C (15 min)
Air flow mode	:	H_2 ; 106.24 ml/min, air, 88.39 ml/min
Carrier gas	:	He 1.38 ml/min
Inlet type	:	Splitless
Split vent	:	260.9 ml/min
Column head pressure	:	25 psi

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