DEVELOPMENT OF ANALYTICAL METHODS FOR SPECIATION OF ORGANOTIN COMPOUNDS IN ENVIRONMENTAL SAMPLES USING GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Mitja Vahčič

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Evaluation Board:

Prof. dr. Milena Horvat, Institute Jožef Stefan, Chairman, Jamova 39, SI-1000 Ljubljana,

Assoc. Prof. dr. Radmila Milačič, Institute Jožef Stefan, Member, Jamova 39, SI-1000 Ljubljana,

Prof. dr. Lucija Zupančič-Kralj, Member, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, SI-1000 Ljubljana

MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
JOŽEF STEFAN INTERNATIONAL POSTGRADUATE SCHOOL

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RAZVOJ ANALIZNIH METOD ZA SPECIACIJO ORGANOKOSITROVIH SPOJIN V OKOLJSKIH VZORCIH Z UPORABO PLINSKE KROMATOGRAFIJE IN MASNE SPEKTROMETRIJE

Doktorska disertacija

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Ljubljana, Slovenia, April 2011

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Abstract

Organotin compounds (OTCs) are the organometallic compounds with the most numerous applications. Today, it is more than 50 years of their intensive development and use. While they are highly useful chemicals they are also extremely toxic to a wide array of aquatic and terrestrial living organisms at very low concentrations. The quantification and identification of OTCs present in various environmental and biological samples is performed by speciation analysis.

In the present doctoral dissertation analytical methods for speciation of 12 OTCs (methyl-, butyl-, phenyl- and octyl-tins) in different aqueous environmental samples were developed.

A rapid analytical method for the simultaneous determination of 12 OTCs in salty or fresh water sample comprises of *in situ* derivatisation (by using $NaBEt_4$) of OTC in salty or fresh water sample matrix adjusted to pH 6 with Tris-citrate buffer, extraction of ethylated OTCs into hexane, separation of OTCs in organic phase on 15 m GC column and subsequent quantitative determination of separated OTCs by ICP-MS. To optimise the pH of ethylation, phosphate, carbonate and Tris-citrate buffer were investigated alternatively to commonly applied sodium acetate – acetic acid buffer. The ethylation yields in Tris-citrate buffer were found to be better for TBT, MOcT and DOcT in comparison to commonly used acetate buffer. Iso-octane and hexane were examined as organic phase for extraction of ethylated OTCs. The advantage of hexane was in its ability for quantitative determination of TMeT. GC column of 15 m in length was used for separation of studied OTCs under the optimised separation conditions and its performances compared to 30 m column. The analytical method developed enables sensitive simultaneous determination of 12 different OTCs and appreciably shortened analysis time in larger series of water samples. LOD's obtained for the developed method ranged from 0.05 – 0.06 ng Sn L^{-1} for methyl-, 0.11 - 0.45 ng Sn L^{-1} for butyl-, 0.11 – 0.16 ng Sn L^{-1} for phenyl-, and 0.07 – 0.10 ng Sn L^{-1} for octyl-tins. By applying the developed analytical method, marine water samples from the Northern Adriatic Sea containing mainly butyl- and methyl-tin species were analysed to confirm the proposed method's applicability.

OTCs represent an important group of pollutants also in landfill leachates. For their accurate detection and quantification a new analytical procedure was developed. By using this procedure 12 OTCs were simultaneously determined. The applicability of methanol as co-extraction reagent and Tris-citrate buffer for adjustment of pH for derivatisation of OTCs in sample matrix was first carefully investigated. The use of $NaBEt_4$ and $NaBPr_4$ as derivatisation reagents for liquid-liquid extraction into hexane was then critically evaluated. 15 m GC column was used for rapid separation of OTCs. The developed analytical procedure was sensitive (LODs for OTCs investigated in general better than 2 ng Sn L^{-1}) with good repeatability of measurement (RSDs mostly better than 3 %) and was successfully applied in the analysis of OTCs in landfill leachates using standard addition calibration method. Due to its simplicity and reliability it is appropriate to be used in routine laboratories for monitoring of OTCs in landfill leachates.

The newly developed analytical methods for speciation of OTCs in marine, fresh and landfill leachate samples will be used in investigations of the distribution, transformations and life cycle of OTCs in environmental ecosystems as well as serve as a tool for routine laboratory analyses.

Povzetek

Organokositrove spojine (OKS) spadajo med organokovinske spojine, ki imajo največje število aplikacij. Danes mineva že več kot 50 let od začetka njihovega intenzivnega razvoja in uporabe. Poleg tega, da so zelo uporabne kemikalije so, v že zelo nizkih koncentracijah, strupene za široko skupino vodnih in kopenskih živih organizmov. Identifikacija in kvantifikacija prisotnosti OKS v različnih okoljskih in bioloških vzorcih se izvaja s pomočjo speciacijske analize.

V predloženi doktorski disertaciji je predstavljen razvoj analiznih metod za speciacijo 12 OKS (metil-, butil-, fenil- in oktil-kositrovih spojin) v različnih vodnih okoljskih vzorcih.

Hitra analizna metoda za hkratno določitev 12 OKS v slanih in sladko vodnih vzorcih se sestoji iz *in situ* derivatizacije (z uporabo $NaBEt_4$) OKS, uravnavo slano ali sladko vodne matrice vzorca na pH 6 s Tris-citratnim pufrom, ekstrakcije etiliranih OKS v heksan, ločbe OKS v organski fazi na 15 m koloni za plinsko kromatografijo (GC) in kvantitativne določitve ločenih OKS z ICP-MS. Pri optimizaciji etilacijskega pH smo proučili fosfatni, karbonatni in Tris-citratni pufer, kot alternativo splošno uporabljanemu acetatnemu pufru. Uporaba Tris-citratnega pufra je dala boljše izkoristke pri etilaciji TBT, MOcT in DOcT spojin, v primerjavi s splošno uporabljanim acetatnim pufrom. Za ekstrakcijo etiliranih OKS v organsko fazo smo proučili uporabo heksana in izooktana. Prednost uporabe heksana je bila v zmožnosti kvantitativne določitve TMeT. Pri separaciji OKS z GC smo primerjali uporabo 15 m in 30 m GC kolone pod optimalnimi pogoji kromatografske ločbe. Razvita analizna metoda omogoča občutljivo in hkratno določitev 12 različnih OKS in je znatno skrajšala analizni čas za večje serije vodnih vzorcev. Meje detekcije razvite analizne metode so od 0.05 – 0.06 ng Sn L^{-1} za metil-, 0.11 - 0.45 ng Sn L^{-1} za butil-, 0.11 – 0.16 ng Sn L^{-1} za fenil-, in 0.07 – 0.10 ng Sn L^{-1} za oktil-kositrove spojine. Razvito analizno metod smo uporabili za analizo OKS v vzorcih morske vode iz slovenskega dela Jadranskega morja. Vzorci so večinoma vsebovali metil- in butil-kositrove spojine.

OKS spojine predstavljajo tudi pomembno skupino onesnažil v izcednih vodah iz deponij odpadkov. Za njihovo zanesljivo določitev smo razvili nov analizni postopek. Z uporabo tega postopka je mogoča hkratna določitev 12 OKS. Najprej smo proučili uporabnost metanola, kot ko-ekstrakcijskega reagenta, in Tris-citratnega pufra, za uravnavo derivatizacijskega pH OKS, v matrici vzorcev. Nato smo kritično primerjali uporabo derivatizacijskih reagentov $NaBEt_4$ in $NaBPr_4$ za tekočinsko-tekočinsko ekstrakcijo derivatiziranih OKS v heksan. Za hitro kromatografsko ločbo smo uporabili 15 m GC kolono. Razviti analizni postopek je občutljiv (LOD-ji za proučevane OKS so v splošnem boljši od 2 ng Sn L^{-1}) z dobro ponovljivostjo meritev (RSD je večinoma boljša kot 3 %). Postopek smo nato uspešno uporabili za analizo OKS v izcednih vodah iz odlagališča komunalnih odpadkov. Pri kvantifikaciji smo uporabili metodo standardnega dodatka. Zaradi enostavnosti in zanesljivosti je metoda lahko uporabna za rutinske nadzorne meritve OKS v izcednih vodah iz odlagališč odpadkov v analiznih laboratorijih.

Novo razviti analizni metodi za speciacijo OKS v morskih, sladkovodnih in izcednih vodah iz odlagališč odpadkov sta lahko uporabljeni za proučevanje porazdelitve, pretvorb in življenjskih ciklov OKS v okoljskih ekosistemih. Služita lahko tudi kot orodje za rutinske laboratorijske analize.

Abbreviations

AAS = atomic absorption spectrometry
AC = alternating current
AES = atomic emission spectrometry
AED = atomic emission detection
CE = capillary electrophoresis
CRM = certified reference material
DBT = dibutyltin
DMeT = dimethyltin
DOcT = dioctyltin
DPhT = diphenyltin
EM = electron multiplier
ETQC = Environmental Quality Target Concentration
FPD = flame photometric detector
GC = gas chromatography
HCl = hydrochloride
ICP-MS = inductively coupled plasma mass spectrometry
ID = isotope dilution
ID-MS = isotope dilution mass spectrometry
ID-ICP-MS = isotope dilution inductively coupled plasma mass spectrometry
IMO = International Maritime Organization
IUPAC = International Union of Pure and Applied Chemistry
LLE = liquid-liquid extraction
LPME = liquid-phase micro extraction
LOD = limit of detection
LOQ = limit of quantification
MBT = monobutyltin
MMeT = monomethyltin
MOcT = monooctyltin
MPhT = monophenyltin
MPEC = Maritime Environmental Protection Committee
MS = mass spectrometry
OTC = organotin compound
Q = quadruple
PFPD = pulsed flame photometric detector
PVC = polyvinyl chloride
RSD = relative standard deviation
SBSE = stir bar sorptive extraction
SFE = supercritical fluid extraction
Sn = tin
SPD = suspended particulate matter
SPE = solid phase extraction
SPME = solid-phase microextraction
TBT = tributyltin

TEtT = triethyltin
TMeT = trimethyltin
TOcT = trioctyltin
TOF = time of flight
TPhT = triphenyltin
UV = ultra violet
WHO = World Health Organization

1 Introduction

1.1 General introduction

Tin (Sn) is metal with atomic number of 50 and atomic mass of 118.69. It is the 24th most abundant element in earth's crust with average concentration of 2.2 mg kg^{-1} and has 10 stable isotopes (Rossenberg, 2005).

It has been known to mankind since the Bronze Age. For several thousands of years Sn and Sn alloys were used for production of consumer and military goods. To this day, Sn remains important element for a wide range of industrial applications. Sn does not occur as a free metal in nature and must be extracted from a base compound, usually cassitterite (tinstone). Approximately 200.000 tons of Sn is produced each year worldwide (Rossenberg, 2005).

Sn occurs in the valence states of 2 and 4, where divalent state is always positive. The valence state 4 however has amphoteric properties and appears as +4 and -4, depending on the reaction partner. Sn has also two allotropic modifications α- and β-form. The normally occurring modification of Sn is the β-form (white tin) which is transformed into α-form (grey tin) below 13.2 °C. Impurities normally present in Sn influence the transformation from white to gray tin. Sn is inert to water and air and even to oxygen at normal temperatures. Only at higher temperatures Sn reacts with oxygen to form oxides. Sn reacts with chlorine, bromine and iodine to form respective stannic halides at room temperatures and with fluorine at elevated temperatures. Sn also reacts vigorously at elevated temperatures with sulphur, selenium and tellurium. Due to its amphoteric nature Sn reacts with both strong acids and strong bases. Metallic Sn is attacked by hydrogen halides and is readily dissolved by hot alkaline solutions to form alkaline stannite and hydrogen (Lazarini and Brenčič, 1984; Rossenberg, 2005).

Major interest on Sn today is in its organic chemistry. First OTC was prepared by Sir Edward Frankland in 1849 (diethyltin-diiodide). However for 100 years OTCs remained laboratory curiosity without any known practical application. This changed in 1940s, when plastics industry discovered the stabilizing effect of certain OTCs for polyvinyl chloride (PVC) (Hoch, 2001). Under the influence of heat and light PVC progressively losses hydrogen chloride (HCl) leading to a system of conjugated double bonds with resulting colour formation and loss of physical properties. The addition of OTCs, traps released HCl, functions as antioxidant and thus significantly reduces degradation of PVC (Batt, 2006). The discovery of biocidal properties of OTCs in the mid-1950s led to their important industrial applications such as the use of OTCs as toxic ingredients in timber preservatives, fungicides, miticides, molluscides, rodent repellents, wood preservatives and antifouling paints. As a result of their widespread usability more than 800 OTCs is known today and the production of OTCs went from 50 t per year to more than 40000 t annually today (Hoch, 2001; Mercier et al., 1994). In developed countries (US, EU, Japan) 76 % of all OTCs are used as stabilizers for PVC, 10 % as antifouling biocides, 8 % as agricultural biocides and 5 % as catalysts in production of silicones and polyurethanes. In less industrialized countries the pattern of use is more biased towards agricultural applications.

The widespread use of OTCs has consequently led to their entrance into various ecosystems. Due to their high toxicity, long half-life and bioaccumulation potential, to both terrestrial and aquatic life, they pose serious environmental and health problems for present and future. In order to better understand the OTCs transportation, distribution and fate in the environment it is necessary to know the chemical species in which OTCs are present in the environment. Because of low levels of OTCs in the environmental samples and highly variable matrices, speciation of OTCs can still be beset with difficulties especially in more complex samples (Hoch, 2001; Rossenberg et al., 2005).

Speciation analysis is an area of analytical chemistry occupied with separation, quantification and identification of chemical species of a given element within a sample. The most common approach to speciation analysis is the use of hyphenated analytical techniques, where an efficient separation

technique is coupled to an on-line or off-line element specific atomic detector. In the case of OTCs, gas chromatography (GC) is the most widely preferred and used species separation technique although it is not the only option. For simultaneous detection of various Sn species many different detectors are used such as atomic absorption (AAS), atomic emission (AES), mass spectrometry (MS) or inductively coupled plasma mass spectrometry (ICP-MS), and flame (FPD) or pulsed flame (PFPD) photometric detection.

1.1.1 Chemical and physical properties of OTCs

OTCs are characterized by the presence of covalent carbon-tin bond(s) and have the following general formula:

$R_xSn(L)_{(4-x)}$

where R is an organic alkyl or aryl group (methyl, butyl, phenyl, …) and L is an organic or inorganic ligand (for example chloride, oxide, hydroxide or some other functional group). The Sn-C bonds are stable in the presence of water, atmospheric O_2 and temperature to up to 200 ºC (Hoch, 2001). This implies that thermal decomposition is not significant route of decomposition in nature. Compared to the energy of carbon-tin bonds, the association energy with the anionic ligand (L) is low, and has a tendency to dissociate both in use and in the environment. The physical and chemical properties of organotin compounds vary significantly, depending upon the number and nature of the R groups in particular, but also upon the type of ligand (L) (Dobson et al., 2006).

OTCs are strong reducing agents which mean that tin-carbon bond can be readily cleaved by strong acids, halogens and other electrophilic agents.

Divalent organotin compounds also exist but they are insignificant because they have no practical use (Hoch, 2001).

1.1.1.1 Solubility of OTCs

Because of hydrocarbon substituents, OTCs are hydrophobic. The extent of hydrophobicity depends on the degree of substitution of the central atom with alkyl or aryl groups and their length. The water solubility of most OTCs is low and dependent on pH, ionic strength and temperature. However, hydrolysis of the reactive ligand and/or ligand exchange in the environment leads to the formation of species that are more soluble. The environmental behavior of OTCs is also strongly influenced by their partition coefficients (Dobson et al., 2006). Depending on environmental conditions OTCs may exist in waters as neutral ion pairs, complexes or cations. The cationic forms of OTCs are stable below their respective acid constant (pKa). The vapor pressures of most OTCs are low and the amount of volatized OTCs depends on actual conditions in the environment. Volatility can also be reduced at pH values below respective pKa values of OTCs, because the major fraction of molecules exists as ions.

1.1.1.2 Sorption of OTCs

Adsorption of OTC species depends on the size and number of their alkyl and aryl groups and the physicochemical properties of sorbent material and its surroundings (Donard et al., 1993). Because soil and sediments may serve as traps for OTCs the adsorption behavior of organotin species is important in determination of the transport processes as well as their bioavailability. Dissolved species are more likely to be expelled to the sea or be accumulated in the food chain by bioaccumulation (Hoch, 2001).

Adsorption of OTCs in soils, sediments and particulate matter may be caused by:

- Electrostatic interaction between the positive charge on the OTCs and the negative charges on the sorbent material (Sun et al., 1996)
- Complexation of the OTCs cation by negatively charged ligands provided by organic matter (Arnold et al., 1998)
- Hydrophobic interaction (Randall et al., 1986)

Sorption of OTCs to organic phase is significantly higher than sorption to mineral phases, including clay mineral as well as Si-, Al- and Fe-hydroxides (Arnold et al., 1998; Weidenhaupt, 1997). Huang and Matzner, showed that mono substituted species exhibit strong and almost irreversible adsorption to soils rich in organic matter, which points to the relevance of electrostatic interactions for the adsorption of OTCs in soils. The adsorption of di- and tri- substituted OTCs to soils rich in organic matter is also almost irreversible suggesting strong OTCs complexation to soils with organic matter in addition to electrostatic interactions. Sorption and desorption studies indicate that the amount of organic material and the substitution level of OTCs plays crucial role to the mechanism and strength of adsorption. The strong adsorption of OTCs to organic matter in soils means that the compounds are largely retained and are only slowly leached to subsurface waters.

In aquatic environments adsorption of OTCs to sediments is largely influenced by pH, salinity, types of exchangeable cations present, mineralogical and chemical composition of adsorption material, the of amount particulates present and the amount of dissolved organic matter (M. Hoch, 2001; Weidenhaupt, 1997). It was found that the maximum adsorption of OTCs in sediments is between pH from 6 to 7 which is close to the pKa values of most OTCs present in sediment and is mostly a consequence of electrostatic interactions. At pH ≥ 8 the driving force for adsorption is the hydrophobic character of OTCs (M. Hoch et al., 2002). High salinity inhibits the adsorption of OTCs to sediments because of the competition of other cations to sorbents anions and possible coagulation of sorbents (O.F.X. Donard & Weber, 1985; Randall & Weber, 1986).

OTCs sorption to sediments is fast and reversible process involving primarily particulate matter constituents which contains fulvic and humic substances rich in carboxylic and phenolic groups. Sorption occurs by reversible formation of chemical bonds between Sn atom and Lewis bases (carboxyl and phenol groups). Thus the mobility, bioavailability and degradation of OTCs in marine sediments and consequently marine ecosystems are strongly influenced by organic matter (Oliveira et al., 2010; Pinochet et al., 2009).

1.1.1.3 Degradation of OTCs

OTCs are in general colorless and odorless compounds and fairly stable towards water and air. Light, atmospheric oxygen, strong acids, electrophilic agents and certain microorganisms degrade OTCs relatively fast, by splitting hydrocarbon groups off, which after time leaves only nontoxic Sn behind. Degradation of OTCs can be defined as progressive loss of organic groups from Sn cation (Hoch et al., 2001).

$$R_4Sn \rightarrow R_3Sn^+ \rightarrow R_2Sn^{2+} \rightarrow RSn^{3+}$$

The sequential removal of organic groups from OTC species reduces their toxicity and can be caused by various processes. These are:

- Photolysis

Photolysis by UV radiation is the fastest degradation mechanism. It occurs when OTCs are exposed to direct sunlight. The mean bond dissociation energy of Sn – C bond is in the range of 190 – 220 kJ mol^{-1}. UV radiation of wavelength of 290 nm corresponds to energy of approximately 300 kJ mol^{-1}. When OTCs are exposed to and the absorption of light takes place, Sn-C bond cleavage can occur (Blunden and Chapman, 1986). However, when considering this degradation mechanism it has to be kept in mind that UV

radiation is strongly absorbed by water, sediment and soils where most OTCs can be found. Thus, photolytic degradation is not the most important degradation route in greater depths of water, sediments and soils but is predominant in air. Among the different OTC species, TPhT and TCyT species are the most rapidly decomposed by UV irradiation (Barnes et al., 1973).

- Chemical cleavage

The Sn-C bond can be attacked by both nucleophile and electrophile reagents. For example mineral acids, carboxylic acid and alkali metals are agents that are able to heterolytically cleave Sn-C bond (Blunden and Chapman, 1986).

- Biological cleavage

Some microorganisms and fungi in soils and some microalgae in aquatic environments have the ability to metabolise and degrade OTCs. Biological degradation was first observed by Barnes et al. when phenyltin acetate was stepwise decomposed to di-, mono-phenyltin and inorganic tin in soil, but in sterile soil this process was not observed. It was found that degradation was due to the ability of some bacteria like *Pseudomonas aeruginosa, Pseudomonas putida C*, and *Alcaligenes faecalis* to degrade OTCs under certain conditions. Some microalgae species like *Skeletonem costatum* are also capable of degrading TBT (Tsang et al., 1999). Up to date, only a limited number of microorganisms that are capable of OTCs degradation have been identified. Biological activity and ability of microorganisms to degrade OTCs is restricted by toxic concentrations of OTCs and other limiting conditions concerning their life, like light, temperature or the availability of nutrients (Hoch et al., 2001).

Half-lives of OTCs in nature are hard to be determined and reported half-lives are often related to laboratory experimental conditions and thus are not directly comparable with natural conditions. The breakdown depends on numerous factors and circumstances (e.g. intensity of sunlight, presence of microorganisms…)(Hoch et al., 2001).

1.1.1.4 Bio methylation of OTCs

Majority of OTCs found in nature are of anthropogenic origin, but some methyltin and trans methyltin species can be formed in nature by bio-methylation processes. Biomethylation may occur under the action of biotic methylating agents such as bacteria, algae and seaweeds or under abiotic methylating agents (Vella, 2001; Omae, 2006). The principal naturally occurring methylating agents are methylcobalmin (the methyl coenzyme of vitamin B_{12}), S-adenosylmethionine (methyl-group donor, active methionine), methyl iodide (CH_3I) and N-methyltetrahydrofolate (Omae, 2006; Rossenberg, 2005). However, the main methylating agent for tin compounds is believed to be methylcobalmin (CH_3CoB_{12}). Methylcobalmin appears to be the sole natural carbanion (CH_3^-) donor and is able to convert inorganic Sn (IV) and organic Sn compounds in the presence of Fe (III) and Co (III) to several methyltin species (Craig and Rapsomanikis, 1985). Regardless of the methylating agent, only one methyl group is transferred to the metal(loid) at one time, although further methyl groups may be transferred to the same receiving atom. Reactivity of organotin intermediates towards further methylation seems to decrease as the number of organic groups increases.

Methylated butyltin species have been found in harbour sediments and surface waters. These substances are far more volatile than polar butyltin species (Amouroux, 2000; Cooney, 1988).

Biomethylations are believed to represent one path of degradation of OTCs through microbial activities in the sediment. The degradation of OTCs proceeds by two methylation reactions with a sulphate-reducing and debutylation with nitrate-reducing microbial activity (Omae, 2006).

Another important reaction is the trans-methylation of methyltin compounds with other metals (Pb, Hg, As). This process is of great ecological relevance because some methylated metals have higher toxicity to organisms than the inorganic metals (e.g. OTCs, methylmercury, …) (Hoch et al., 2001).

1.1.2 Toxicity of Sn

Sn in its inorganic form is generally considered as being non-toxic. The toxicological pattern of organic tin compounds is very complex. Biological effects of OTCs depend on both the nature and the number of the organic moieties bound to the Sn cation. In general the tri-substituted species demonstrate the highest level of toxicity and biocidic activity, followed by di-substituted, mono-substituted and tetra-substituted organotin species. The nature of the anion (L) attached to the organotin species $R_xSn(L)_{(4-x)}$ has little or no effect on toxicological and biocidic properties, except in cases when L is itself toxic. In this case the biological activity of a given OTC may be enhanced (Hoch et al., 2001).

Among different tri-substituted OTCs there are considerable variations in toxicity which depend on the nature and the length of the side chain of the alkyl groups. The increase in the chain length produces sharp drop in the biocidic activity and the long-chain species, such as octyltin derivates which are essentially non-toxic to all organisms. Thus, octyltin compounds can be used as stabilizers in PVC and food packaging materials. The most toxic OTC to mammals is triethyltin acetate (Et_3SnOAc). In contrast to mammals butyl- and phenyl- tin species exhibit the highest toxicity towards sensitive organisms. Chronic and acute poisoning of aquatic organisms such as algae, zooplankton, mollusks and fish larvae has been demonstrated to occur even at low ng L^{-1} concentrations of butyl- and phenyl-tins. Lethal concentration are in the range of 0.04 – 16 µg L^{-1} for short term exposure, depending on the aquatic species (WHO, 1990). Because of these properties butyl- and phenyl- tin species have been extensively used as antifouling agents in boat paints since the 1960s till 2008 when they were banned from commercial use as antifouling agents. In addition to tri-substituted OTCs species less toxic di-substituted and mono-substituted derivates also have to be considered. They are significantly more soluble in water than their tri-substituted analogues which may lead to potentially higher concentrations in aquatic environments.

1.1.2.1 Effects on microorganisms

Toxicity of OTCs towards microorganisms generally decreases in the order R_3SnL > R_2SnL_2 > $RSnL_3$, with R_4Sn compounds being of low toxicity. Tributyl-, tripropyl- and triphenyltins are the most toxic OTCs, because of their ready association with cell membranes which is an important prerequisite for their toxicity (J. J. Cooney, 1995; Gadd, 2000). It was proposed that toxicity also correlates with the total molecular surface area of the compound. In this case butyl-, phenyl- and pentyl-tins are the most toxic while methyltins the least (Gadd, 2000).

The inhibition of microbial processes by the most widely used trisubstituted OTCs has been recorded for all major groups of aerobic and anaerobic microorganisms. The main interaction is occurring at cellular membranes and chloroplasts or the mitochondria in eukaryotes. Toxicity of OTCs to microorganisms depends mainly on environmental factors and the species itself. It was also demonstrated that bacterial chemo taxis can be affected by concentrations below those which prevent growth, meaning that subtoxic concentration levels may still affect the microbial populations (Han, 1995).

1.1.2.2 Effect on aquatic organisms

Toxic potentials of the most widely used OTCs to aquatic organisms are well documented. Research undertaken since 1970s has shown that butyl- and phenyl- tins are

very toxic to a large number of aquatic organisms. Several studies have shown that tributyltin (TBT) and triphenyltin (TPhT) can cause changes in the endocrine system of marine organisms at concentrations as low as 1 ng L^{-1} by disturbing the proper function of mitochondria. These changes include the development of male sexual characteristics in female gastropods, known as imposex, which can lead to sterilization and death of affected organisms. Other responses of marine organisms usually involve reduced growth rates and photosynthesis, as well as cell death depending on the type and quantity of the organotin compound (Rosenberg, 2005).

Historically, the decline of marine molluscs in coastal areas due to imposex has been of particular concern historically. The embryonic and larval stages of marine invertebrates are less tolerant to toxicants than adults and thus have been used to assess the biological quality of marine water and sediments. While OTCs are in general toxic to all aquatic organisms, juveniles in early stages of life show extreme sensitivity. Exposure to OTCs causes growth impairment, shell deformations and greatly increases mortality. It is not yet clear whether this is due to TBT-induced alterations in the uptake and elimination kinetics or differences in the tissue concentration (Meador and Rice, 2001). The shell thickening and deformations caused by TBT in oysters was found to be due to the appearance of chambers in the oyster shell and interlamellar gel formation in these cavities. TBT is known to inhibit oxidative phosphorylation and it has been suggested that this forms the basis of its action on the shell. The effect on calcification derives from inadequate calcium addition to the organic matrix (a process dependent on ATP) and incorrect deposition of this matrix. The observed shell abnormalities had been attributed to sediment contaminated with high concentrations of butyl- and phenyl- tins along busy shipping lanes, harbours and marinas. It was also observed that toxicity levels among different aquatic species vary enormously. This difference in sensitivity to TBT was attributed to the species specific capacity to metabolize TBT. Furthermore it was observed that the increases in TBT concentration increase the proportion of females which was attributed gender related differences in sensitivity to toxic effects of OTCs present (Rosenberg, 2005). Differences in species sensitivity to OTCs were also observed between freshwater and saltwater species. Leung et al. (2007) reported that saltwater species are more susceptible to TBT than their freshwater counterparts. Higher toxicity of OTCs in saltwater may be due to “salting out effect”. The solubility and thus chemical activity of lipophilic compounds can differ between fresh and salt waters because of strong ionic interactions, resulting in reduced solubility in salt waters. At levels below saturation, the effective concentration of the substance is higher, leading to an increased activity and greater bioavailability in saltwater conditions (Wheeler et al., 2002). Among important environmental parameters which affect the toxicity of OTCs is temperature. It was observed that by raising temperature by 10 ºC OTCs toxicity increases significantly. This phenomenon could be attributed to the increase in the metabolic rate at higher temperatures and a consequent faster depletion of energy reserves resulting in an increase of susceptibility to TBT. The effect of temperature was observed to be greater than the effect of salinity. The combined effect of both salinity and temperature was suggested in the case where mortality increases with temperature but decreases with salinity (Kwok and Leung, 2005).

1.1.2.3 Effects on humans

Because of widespread use of OTCs, human exposure is inevitable. Yet, relatively little is known about harmful effects of OTCs to humans. Most of the data comes from poisoning or occupational exposure to OTCs. The most predominant pathways of human exposure are the consumption of either contaminated drinking water or beverages or the consumption of marine foods (Antizar-Ladislao, 2008; Azenha et al. 2004; Forsyth et al., 1997; Lo et al., 2003). In spite of the evidence that such sources expose humans to OTCs, limited data on deposition of OTCs in humans is available. Human risk assessment has mainly been based on immunological studies in experimental animals, estimated human intake of marine food sources, poisonings and occupational exposure.

Since the main source of OTCs in human exposure is the ingestion of marine fishery products, which may contain high levels of butyltins, most of the toxicological studies have focused on them. After the ingestion, TBT undergoes dealkylation by cytochrome P450 systems, (Ohhira et al., 2006) producing metabolites that are generally less toxic than parent compounds. It was found that

permeability pattern of OTCs in gastrointestinal tract correlates to the general *in vivo* toxicity pattern (trialkyltin > dialkyltin >> monoalkyltin) but was different from accumulation pattern (DBT > TBT > MBT). Thus, it was suggested that the toxicological potential of OTCs species depends on membrane permeability. This hypothesis was supported by observation that the butyltin species mainly deposit in liver with DBT as predominant species (Appel, 2004).

Toxic effects of TBT to mammalian cells include apoptosis or necrosis and influence fundamental process such as mitochondrial respiration, ion channels, steroid genesis, receptor activation and gene transcription (Cooke, 2006; Nakanishi, 2007), while DBT mainly acts as partial inhibitor of human aromatase activity. Butlytins also affect natural killer cells lymphocytes in human blood, which are primary immune defence against tumour and virally infected cells (Kannan, 1999). Animal experiments have suggested that the spectrum of potential adverse chronic systemic effects of OTCs in humans may include immunosuppressive, endocrinopathic, neurotoxic, metabolic and enzymatic activity as well as potential ocular, dermal, cardiovascular, upper respiratory, pulmonary, gastrointestinal, blood dyscrasias, reproductive/teratogenic/developmental, liver, kidney, bioaccumulative and possibly carcinogenic activity. Symptoms of dialkyl- and trialkyltin poisoning in humans include general malaise, nausea, gastric pain, dryness of the mouth, vomiting, headache, visual disturbance, shortness of breath and electroencephalographic anomalies (Hoch et al., 2001).

1.1.3 Occurrence and distribution of OTCs in the environment

Due to the extensive use in numerous areas of human activity, large amounts of OTCs have entered the environment. Significant concentrations of OTCs and their degradation products have been detected in various ecosystems, with the highest concentrations in all compartments of aquatic environment. Amounts of OTCs in the atmosphere have been found to be negligible (Rossenberg et al., 2005).

1.1.3.1 OTCs in water, sediment and biota

Since the discovery of biocidal properties of OTCs in the mid 1950's until 2008 OTCs have been extensively used as effective antifouling agents in many applications, mainly as biocides in protective coatings for shipping vessels. As a consequence significant amounts of OTCs have entered aquatic environment.

The main sources of marine pollution were antifouling paints, which are mainly self-polishing copolymers that release butyltins continuously. Harbour areas, shipyards and high shipping lanes were specially affected by butyltin contamination. Flakes of anti-fouling paints from removal of old coatings ended in harbours and shipyards sediments. Now they serve as reservoirs of OTCs that can cause locally high contamination (Oliveira and Santelli, 2010). For other compounds such as phenyltins, impute via their use as pesticides in agriculture is more important. Further imputes to the environment result from runoff waters contaminated with OTCs by industrial effluents, large scale use of PVC, leachates from landfills, municipal wastewater and sewage sludge.

The distribution of OTCs present in the aquatic environment is influenced by different factors such as the species population density of aquatic organisms, dissolved and suspended organic material, pH, salinity, temperature and solubility in water (Weidenhaupt et al., 1997). The cation forms of OTC species are stable at pH values below their respective acid constants (pKa). At normal pH of seawater (pH 8), most of the OTC species are predominantly present as hydroxides and carbonates. Recent studies report that according to the species present in sea water (ionic compounds: TBT^{+}, DBT^{2+}, MBT^{3+}; and neutral compounds: TBTOH, DBTOH, MBTOH, $TBTNO_3$, TBTCl, etc,…) they may be retained on the surface of particulate matter by cation exchange and Coulomb forces. For example at pH between 4 and 7, DBT adsorbs to sediment more effectively than TBT. At pH < 7, the cationic OTCs are dominant species in aqueous solution and there is electrostatic attraction between the positively charged organotin molecule and the negatively charged surface of clay minerals. At pH 8 the affinity for these compounds in the sediment is inverted to TBT > DBT, corresponding to the hydrophobicity of the compounds (Bianchi et al., 2006; M. Hoch et al., 2003).

Half-lives of OTCs in the aquatic environment vary and depend on the type of OTC, its lipophilic character and the amount of dissolved or suspended organic and particulate matter. For example, half-life of TBT in sea water is usually 6 h, because of TBT degradation.

Concentration of OTCs in sediments can be up to three orders of magnitude higher than in water column. This is a consequence of low aqueous solubility and low mobility of OTCs in water. OTCs are also easily adsorbed onto suspended particulate matter (SPD). The sedimentation of SPD results in significant accumulation of OTCs in sediments.

Sources of OTCs in sediments are a consequence of shipping activities, untreated discharges from various industries, municipal landfills and waste water treatment plant. Because sediments are often anoxic environments half-lives of OTCs are considerably longer than those in water column and are measured in the range of years instead of days or weeks. As a consequence OTCs can accumulate in sediments and present persistent ecotoxicological risk (Hoch, 2001). Not all OTCs in sediments are of anthropogenic origin. Some are presumably formed by biomethylation. Methylated Sn species are less toxic to aquatic life than the equivalent butyltin species. The toxicities of mixed butylmethyltin species are still unknown (Hoch, 2001).

Contaminated sediments presents long term problem because they remain a source of OTC species in water. Once in water, they become available to filter- or sediment-feeding organisms even long after the source of contamination has been removed. Another risk for aquatic systems is the possible contamination from resuspended sediments. Re-suspension of particles and remobilisation of the pollutants can be caused by dredging, swirling or desorption. The ability of marine sediments to accumulate OTCs varies geographically and geologically, according to the physicochemical characteristics of the sediments (e.g. particle size and organic carbon content) (Carvalho et al., 2009; Rainbow, 1995).

1.1.3.2 OTCs in soil

Origin of OTCs in soil can be attributed to the spreading of contaminated sewage sludge as a fertilizer over the fields and the use of phenyltins as a contact fungicide for treatment of variety of crops including potatoes, sugar beets, peanuts and rice.

OTCs are readily adsorbed to soils, not easily leached and have considerable half-lives. Mobility of OTCs in soils can be affected by the sorption onto the soil solid phase and the rate of their degradation is depended on its physical and chemical properties, such as polarity, pH, organic matter content, mineralogical composition, redox potential and the presence of microorganisms. Barnes et al. showed that 1 year after application of phenyltin acetate (fungicide) to the soil, residues of the TPhT remained in the top layer of soil (0-10 cm) and less than 4 % of the applied TPhT leached from the soil.

Research also showed that the persistence of OTCs in soil is as follows: TPhT < DPhT < TBT < MPhT < DBT < MBT. The stability of persistent OTCs was attributed to the nature of organic group present in the OTCs and was inversely proportional to the degree of substitution of a given OTC (Barnes et al., 2010; Heroult et al., 2008).

1.1.3.3 OTCs in municipal landfills

The most predominant route of municipal waste disposal today is landfilling. Municipal solid wastes contain great diversity of chemical compounds and elements which are being released and/or produced during the decomposition of waste material, either by microbiological or chemical pathways (Mersiowsky et al., 2001; Pinel-Raffaitin et al., 2008). The release of chemical substances and compounds in municipal waste sites occurs through two main pathways, leachates and biogases. (Pinel-Raffaitin et al., 2008).

Organometallic compounds are an important group of pollutants which are present in both landfill leachates and biogases. The main sources of OTCs in landfill leachates are wastes, which contain OTCs (PVC, silicones, polyurethanes…). Partly their presence is also a consequence of microbiological transformations. Inorganic Sn and OTCs can be mobilised and released to the environment by percolation of water through the waste layers. OTCs can also be released in the gaseous phase, which contains volatile Sn species. Landfill conditions enhance the chemical and biological transformation of Sn. Inorganic Sn and OTCs present in the waste can be modified by hydration, methylation, ethylation, dealkylation or trans alkylation reactions. In this way, new Sn species both in liquid and gaseous phases are generated (Amouroux, 2000). Transformation of species

can enhance the mobility and toxicity of OTCs in landfill leachates and represent danger to the environment (Pinel-Raffaitin et al., 2008).

1.1.4 Speciation of OTCs

Chemical elements exist in nature in various chemical species. Their toxicity, environmental mobility and bioaccumulation potential vary significantly between different species of the same element. Definition of speciation analysis in analytical chemistry was given by IUPAC and is defined as analytical activities of identifying and/or measuring the quantities of one or more individual chemical species of element in a sample. Chemical specie is defined as specific form of an element as to its isotopic composition, electronic or oxidation state, and/or complex or molecular structure (Templeton et al., 2000).

A variety of analytical techniques have been developed for the chemical speciation of OTCs. In this time, great progress has been made in development of analytical methodologies and instrumentation for speciation analysis.

For successful speciation of OTCs in water, sediment, soil or biota several critical steps are involved. These steps usually include: sampling, sample storage, extraction of OTCs (transfer of analytes of interest from complex matrix to a simpler solution), preconcentration, "clean up" (removal of impurities co-extracted together with the compounds of interest that may interfere with the results), derivatisation (transformation of the analytes into a measurable volatile compound), and the use of an appropriate analytical technique for the identification, quantification and interpretation of the results (Oliveira et al., 2010).

1.1.4.1 Sampling and storing

For chemical speciation of OTCs, extreme caution in sampling is necessary since the environmental concentrations of OTCs are generally very low (on the order of ng L^{-1} in water samples and ng g^{-1} in sediment or biological samples) and there is spatial and temporal variability of OTCs concentrations. Therefore, the choice of sample locations and sampling period should be taken into account in both biotic and abiotic samples (Quevauviller et al., 1992).

Seasonal variations in the concentrations of OTCs in water between hot and cold seasons often occur due to the increase of anthropogenic sources during summer (tourism, increased boating activity in summer and spring) and degradation of OTCs (Gómez-Ariza et al., 2000; Quevauviller and Donard, 1991; Stang, 1992). For sediments, only undisturbed surficial layers must be sampled (Venkatesan et al., 1998). Higher concentrations of OTCs can be found in the water-sediment interface due to the degradation of TBT and higher hydrophilicity of its main degradation product DBT (Quevauviller and Donard, 1991; Venkatesan et al., 1998). Resuspension of sediments can also lead to resolubilisation of sorbed OTCs and consequently to increased concentrations of OTCs in water. Sediment location also plays important role. Sediments from locations near maritime activity (shipyards, wharves, harbours) usually have higher content of OTCs. Concentration of OTCs in is related to their higher bioavailability, which is higher during spring and summer (Aguerre et al., 2001).

Storage is especially important for biological, sediment and landfill leachate samples, because of the risk of physical and chemical changes that may affect the concentration of OTCs (Aguerre et al., 2001; Gomez-Ariza, 1997; Muñoz et al., 2004; Serra and Nogueira, 2005). Experimental data shows that water samples are stable when they are acidified to pH 2 and stored in the dark at +4 °C. For storage of biota, sediments, soils and biologically active samples (landfill leachate, sewage sludge), it is recommended that they are stored at -20 °C in the dark to ensure their long term stability and the balance between chemical forms (Gómez-Ariza et al., 2000).

1.1.4.2 Sample preparation

Prior to speciation analysis is sample preparation. It may consist of several steps. Steps

required depend mainly on the physico-chemical properties of the sample, matrix complexity and the analytical method used. Initially in sample preparation, the sample undergoes a series of physical and chemical processes, which ensure that the elements or compounds can be identified and/or adequately quantified. Because this stage involves direct sample handling (e.g. crushing, extraction, filtration, dilution, concentration, etc…), contamination can easily occur. Therefore, care should be taken to avoid loss or alteration of analytes.

1.1.4.3 Extraction

In the extraction of OTCs two conflicting issues have to be balanced. First is obtaining an adequate recovery and second preventing losses especially destruction of the OTCs. The extraction should be performed in such a way that the analyte is separated from the sample matrix without losses, contamination, change of speciation and with the minimum of interferences (Quevauviller, 2000).

Extraction of OTCs from aqueous matrices depends on the complexity of liquid matrix (sea water, landfill leachate…). In order to extract OTCs successfully, various binding interactions, such as ion-dipole, dipole-dipole, Van der Waals forces, hydrophobic partitioning or complexation with organic particles and matter must be overcome. Interaction of tri-substituted OTCs with matrix is mainly a consequence of hydrophobic forces while interaction of mono-substituted compounds is caused mainly by ionic forces (Ipolyi et al., 2006; Smedes et al., 2000). Binding interactions influence the efficiency of the extraction methods.

Different extraction techniques have been developed to isolate and concentrate the OTCs from various matrices. Among them, the most used techniques are liquid-liquid extraction (LLE) (Milivojevič et al., 2002; Milivojevič et al., 2008; Montignyet al., 1998), Soxhlet extraction (Dietz et al., 2007), solid phase extraction (SPE) (Abalos et al., 1997; Heroult et al., 2008; Montigny et al., 1998; Nemanic et al., 2007; Nemanič, 2007; Scancar et al., 2007; Zuliani et al., 2010), supercritical fluid extraction (SFE) (Cai et al., 1994), solid-phase microextraction (SPME) (Aguerre et al., 2001; Alzaga et al., 1993; Arthur et al., 1990, Zuliani et al., 2006; Zuliani et al., 2008), stir bar sorptive extraction (SBSE) (Baltussen, 1999; Prieto et al., 2008; Vercauteren et al., 2001) and liquid-phase microextraction (LPME) (Liu, 1996).

LLE is one of the most widespread techniques for the extraction of OTCs from aqueous samples. Its advantages are high preconcentration factors and simplicity of use. Disadvantages of LLE are, high economic and environmental costs due to relatively large volume of solvents used and poor time efficiency when only a few samples are processed.

To achieve better extraction efficiencies different extraction techniques have been used. Most commonly used extraction techniques for LLE are mechanical shaking and microwave-assisted extraction. Mechanical shaking is efficient, simple and non-expensive procedure, which increases contact surface area between solvent phases and in turn increases the rate of mass transfer between organic and inorganic phase. Its main disadvantage is that it is time consuming for samples with complex matrices.

Low-power microwave-assisted LLE is mainly used when complex liquid matrix is present (landfill leachate or sewage sludge). It is used to digest organic matter making complete solubilisation possible in a few minutes while keeping the integrity of OTCs (Rodríguez et al., 1997). Some degradation of PhT has been observed when using this technique. Because leaching process takes place in open glass container the exposition time is limited by the boiling point of the mixture (Camel, 2000).

Solvents with low to medium polarity such as hexane, isooctane, pentane, toluene or dichloromethane are usually employed in LLE. Medium polarity solvents are advisable for extraction of OTC species, but they also increase the number of substances that are co-extracted and impair derivatisation reaction (Gómez-Riza et al., 2001; Smedes et al., 2000). One of the most important factors within the extraction conditions is the pH. The mildly acidic conditions reduce the strength of mineral binding, break bonds between the matrix and the target OTCs and provide positively charged ionic species (Ipolyi et al., 2006). However, too acidic or basic conditions lead to significant degradation of OTCs.

1.1.4.4 Derivatisation

OTCs present in the environment are mostly found in their polar forms, which are not sufficiently volatile and/or thermally liable for gas chromatographic separation (GC). Before GC analysis they need to be derivatised. The most common derivatisation reactions are alkylation with Grignard reagents (Abalos et al., 1997; Milivojevič et al., 2002), hydride generation with sodium tetrahydroborate ($NaBH_4$) (Abalos et al., 1997) and *in situ* ethylation or propylation with sodium tetraethylborate or sodium tetrapropylborate ($NaBEt_4$ or $NaBPr_4$) (Abalos et al., 1997; Heroult et al., 2008; Milivojevič et al., 2008; Montigny et al., 1998; Nemanic et al., 2007a; Nemanič et al., 2007b; Scancar et al., 2007; Tang and Wang, 2007; Zuliani et al., 2010; Zuliani et al., 2006; Zuliani et al., 2008). The use of $NaBPr_4$ as a derivatisation agent enables determination of ethylated Sn compounds.

1.1.4.4.1 Derivatisation by Grignard reactions

Chemical derivatisation by Grignard alkylation depends on the reaction of OTCs with a Grignard reagent to convert the ionic OTCs into their corresponding non-polar tetrasubstituted compounds:

$$R_nSn_{(4-n)} + R'MgX \text{ (in organic solvent)} \rightarrow RnSnR'_{(4-n)}$$

n = 1, 2, 3; R, R' = organic groups; X = anion.
The procedure for the determination of OTCs with Grignard alkylation involves acidification of samples, extraction with organic solvent, derivatisation, clean-up, preconcentration and analysis. Extraction with organic solvent is necessary because reaction with Grignard reagent has to be carried out in aprotic solvents such as benzene, toluene, diethylether or hexane.

A number of Grignard reagents (methyl, ethyl, propyl, butyl, pentyl and hexyl –magnesium chloride/bromides) have been used to convert the ionic OTCs in environmental samples into their volatile forms. Ethylation and pentylation are usually employed as they allow the determination of methyl, propyl, butyl and phenyltin species, which are the most important in environmental concern.

The main disadvantages of Grignard derivatisation are the violent nature of Grignard reagent, long analysis time, formation of mono-alkylated disulfides when sulfur is present, the need for anhydrous conditions and the use of apolar solvents.

1.1.4.4.2 Hydride derivatisation

The reaction of hydrides was originally utilized for the generation of trace amounts of stannate (SnH_4) from aqueous solutions of Sn and its subsequent determination by atomic absorption spectrometry (Oliveira et al., 2010). The derivatisation to hydrides, using $NaBH_4$ is applied to volatilise the butyltins for purge and trap analysis (Smedes et al., 2000). In general, the reduction is usually performed at a pH that is a few units below the pK_a of the species of interest (Attar, 1998):

$$R_nSn_{aq}^{(4-n)} + NaBH_4/H^+ \rightarrow R_nSnH_{(4-n)} + H_2$$

n = 1, 2, 3; R = organic group.
This method of derivatisation gives high yields and good separation from sample matrix in simple aqueous solutions. For more complex matrices (sediment, biota, and landfill

leachate) serious interferences might occur due to the presence of humic substances and high metal concentrations. Hydridization reagents are unstable and should be prepared immediate before use (Morabito, 2000; Abalos et al., 1997). The instability and volatility of butyltin hydrides and poor reproducibility of hydridization of phenyltins can lead to losses of compounds and consequently to an underestimation of contamination (Cassi et al., 2002).

1.1.4.4.3 Reaction with sodium tetraehtylborate

In contrast to Grignard reagent, the $NaBEt_4$ is sufficiently stable in water to be used for direct *in situ* derivatisation of organometallic species. Etyhlation can take place in aqueous or methanolic solutions. With $NaBEt_4$ simultaneous ethylation and extraction into organic phase (iso-octane or hexane) can be performed at pH between 4 and 6 in buffered solution (Bowles et al., 2007; Smedes et al., 2000). At pH less than 4, the $NaBEt_4$ decomposes faster, as it reacts with H^+ ions, and at higher pH OTCs form hydroxides, which hinders reaction efficiency (Smedes et al., 2000).

Derivatisation with $NaBEt_4$ reduces the number of steps required to perform derivatisation and extraction into organic phase. It is robust and sensitive. However, it is also subjected to interferences when applied to samples with complex matrices (Bowles et al., 2007). The derivatisation yields of OTCs may decrease due to the consumption of $NaBEt_4$ by matrix components (S, Pb, Hg,…). Thus, it is necessary to ensure the excess of $NaBEt_4$. $NaBEt_4$ is also extremely air-sensitive and must be handled with care to keep its chemical integrity.

1.1.4.5 Quality control

In order to obtain accurate analytical results and to correct for recovery losses during the mishandling of the sample, inefficiency of the extraction procedure, incomplete derivatisation, losses through volatilisation, etc., it is common to use certified reference materials (CRM), isotope dilution or spiking to assess recoveries of the analytes (Abalos et al., 1997).

Whenever CRMs are not available spiking is the most commonly applied procedure for recovery assessment. Spiking includes the addition of known amounts of analytes to the sample, equilibration time to allow the incorporation of the spike into the sample matrix and processing and analysis of the spiked sample. The main risk is that the behaviour of added analytes may not be the same as that of the natural and the recovery obtained may be an overestimation.

Since there are no commercially available CRMs for determination of OTCs in water matrices of any kind, it is necessary to prepare standard solutions of OTCs. Standard solutions of 1000 mg Sn L^{-1} are usually prepared from pure OTCs salts which typically have purity of 98 % or more. These standard solutions are then used for preparation of appropriate working standard solutions and those for spiking of the samples and for the preparation of calibration curves.

1.1.4.6 Sources of measurement uncertainty

Sources of measurement uncertainty are important part of quality control and method validation. In complex procedures, such as analysis OTCs in complex matrices, it is important to identify which parts of the overall procedure contribute the most to the method uncertainty. The most important sources of measurement uncertainty in OTCs analysis in complex matrices are extraction and derivatisation steps, which depend heavily on matrix complexity, and chromatographic separation. These sources of uncertainty are the hardest to control. Contribution from other sources (e.g. pH, volume of sample, concentration of standards, pipette accuracy, time of derivatisation,…) are minor in comparison, mainly because they can be easily controlled. It is important, that the biggest contributors to measurement uncertainty are studied and if possible minimized through optimization of the analytical procedure.

1.1.4.7 Traceability of measurements

Methods for determination of OTCs and organometallic compounds in general are based on complex method procedures (extraction, derivatisation, separation and detection), which means traceability of measurements is hard to achieve. In the case of organometallic compounds determinations in environmental matrices, primary methods, which assure traceability, do not exist since there are no means at present to prove that extraction or chemical reactions (e.g. derivatisation) yield a 100 % recovery. The best approximation in achieving traceability for organometallic compounds in environmental samples in general is the use of isotope dilution coupled to mass spectrometry (ID-MS) or to inductively coupled plasma mass spectrometry (ID-ICP-MS). It is argued that the final ID-based results are actually traceable to the "true" value of organometallic compounds in extracts of the analysed environmental samples but not necessarily to the true value in the sample. In addition, stable isotopes are not commercially available for all OTCs of interest (Quevauviller, 2004).

Proficiency testing and interlaboratory studies are another way which, in principle, enables laboratories to establish "external" references for evaluating the performance of their methods. One or more reference materials are usually distributed by a central organisation to several laboratories for the determination of given substances. Comparing laboratory results (based on different methods) allows the detection of possible sources of errors linked to a specific procedure or related to the way a method was applied by a given laboratory. Measured values obtained in the framework of interlaboratory studies or proficiency testing (using different techniques) may be considered as an anchorage point representing the analytical state-of-the-art; this offers laboratories a means to achieve comparability (i.e. traceability) of their results to a recognised reference, which in this case is a consensus value (generally the mean of laboratory means). However this reference does not enable traceability to the true value of the substance in the medium, but it represents a very useful method for achieving comparability of environmental measurements using an external quality control scheme (Quevauviller, 2004).

In the case of OTCs in fresh and marine water matrices there have been a few interlaboratory studies to establish comparability (i.e. traceability) of results and to establish reference material viability. However in the case of OTCs in landfill leachates matrices, there have been no intelaboratory studies or proficiency testing. Thus the best approximation to achieve traceability of measurement in landfill leachates or in general for OTCs analysis is the use of stable isotopes.

1.1.4.8 Instrumental analysis

Determination of total element concentration cannot usually provide the required information about mobility, bioavailability and the impact of elements on ecological systems or biological organisms (Milačič, 2005). In case of Sn, its organic forms are far more toxic than inorganic ones. In order to estimate the overall environmental and biological toxicity of a sample it is necessary to know the exact concentration of each OTC species present (Oliveira et al., 2010). Most of the analytical methods developed to quantify OTCs require hyphenated analytical techniques, which are the on-line combination of a separation technique with a detection technique with a specific detector suitable for identification and quantification of a specific molecule or element. Alternatively, separations can also be performed off-line, with species being separated and determined independently. Although a variety of separation techniques are used for OTC species separation, GC is by far the most common (Oliveira et al., 2010).

1.1.4.8.1 Separation

GC is the most widely used technique for separation of OTCs. It has superior resolution and sensitivity, great variety of coupled detectors and the ability to simultaneously separate many different OTC species (methyl-, ethyl-, butyl-, phenyl-, cyclohexyl- and

octyltin compounds) (Abalos et al., 1997). Another advantage of GC is the possibility of using different internal standards and surrogates, which allow the steps of the analytical procedure to be traced (Morabito, 1995). Extracts containing derivatised OTCs are injected onto GC column by standard systems, such as split-splitless, temperature or pressure programed injection. The use of autosampler is essential to obtain reproducible injections and constant equilibration times that decrease the variation in retention times (Morabito, 1995).

For separation of OTCs, common nonpolar or semi polar capillary chromatographic columns with stationary phases such as methyl-polysiloxsane or 5% diphenyl-dimethyl-polysiloxane are used. Typical column has a length of 15 to 30 m with inner diameter of 0.25 mm and film thickness of 0.1 to 0.3 μm. Helium is used as carrier gas, when mass spectrometry is used for detection of Sn. For adequate separation of OTCs oven temperature program must be optimized prior to analysis. This ensures constant retention times and subsequently accurate analysis (Smedes et al., 2000).

1.1.4.8.2 Detection

The combination of GC with ICP-MS provides a powerful and sensitive technique for on-line elemental speciation of OTCs, because of its simplicity, robustness and high reliability and reproducibility (Nelms, 2005). After chromatographic separation, several detectors may be used for detection of OTCs. In the early years of OTCs speciation GC was coupled to elemental specific detectors such as atomic absorption spectrometers (AAS) (Cal et al., 1993) and atomic emission spectrometers (AED) (Aguerre et al., 2001). Limits of detection were latter improved by using flame photometric detector (FPD), pulsed flame photometric detector (PFPD) and mass spectrometry (MS).

Lately the progress in the field of speciation has been achieved by the use of inductively coupled plasma mass spectrometry (ICP-MS) as a very sensitive detector. ICP-MS is arguably the most versatile trace elemental analysis technique available today. Since its first commercial introduction in 1983 ICP-MS has become applicable in various fields of research such as environmental, biological, food and agriculture, semiconductor, clinical and pharmaceutical, geological, nuclear, forensic and petrochemical (Agilent Technologies, 2005; Nelms, 2005). Its advantages are high sensitivity, rapid multi-elemental analysis, wide dynamic range, good precision and accuracy, better detection limits for the majority of elements and the ability for semi-quantitative analysis and isotope dilution (ID) quantification, which is a unique asset and enables both highly accurate and precise speciation measurements (Aguerre et al., 2001). Further, ICP-MS is a robust detector that can be coupled to various separation techniques such as liquid and gas chromatography and capillary electrophoresis (CE). However, ICP-MS has some drawbacks such as destructive character of the technique, costly maintenance and the need for skilled analysts. The main components of a typical ICP-MS instrument are sample introduction system, plasma, interface, ion focusing, collision/reaction cell, mass analyser and detector.

Sample introduction system is one of the most important components of the entire ICP-MS system. The principal purpose of the sample introduction system is conversion of a liquid sample into an aerosol and transport of the smaller droplets efficiently into the plasma. However, when GC is coupled to the ICP-MS, sample is transported into plasma in gaseous form via special transfer line. The transfer line interface connects the two instruments via a passivated, heated Sulfinert®tube between the GC column and the tip of the ICP injector using a special torch with a heated injector to maintain constant high temperature and inertness. Introduction of gaseous sample into the plasma increases the sensitivity and lowers the detection limit of ICP-MS detector.

Upon entering the plasma, sample atomisation and ionization occur (Thomas, 2004). The positively charged ions that are produced in the plasma are extracted into the vacuum system, via a pair of interface cones. The cones, sample and skimmer cone, are essentially metal plates with central orifices through which the ions pass. Electrostatic lens, located within the intermediate stage of the vacuum system, focuses the ions in a compact ion beam and separates positively charged ions from photons and neutral species. After the main ion lenses, also in the intermediate stage of the vacuum system, the collision/reaction cell (CRC) system is located. In the final analyser stage, the low pressure allows effective transmission of the ions through a mass analyser to the detector. Three different types of

mass analysers have been used with ICP-MS, these are quadrupole (Q), magnetic sector or double focusing and time of flight (TOF). By far the most common mass analyser used in ICP-MS is the Q. Due to its ease of use, rapid scan across the mass range (2 to 260 amu), good linearity and relatively accessible price, Q represents approximately 95 % of all ICP-MS used today (Agilent Technologies, 2005; Thomas, 2004). The Q is a sequential mass filter, which separates ions based on their mass to charge ration (m/z). It consists of four cylindrical hyperbolic metallic rods of the same length and diameter. One set of rods is at positive electrical potential and the other at a negative potential. By varying the alternating current (AC) and direct current (DC) voltages, but keeping the ratio between them constant, different masses can be selectively allowed to pass through the filter (Thomas, 2004). Each ion exiting the mass analyser is detected by an electron multiplier (EM). EM is largely responsible for the characteristics of very high sensitivity, wide linear dynamic range and low random background, for which the technique is well known. The detector electronics count and store the total signal for each mass (m/z), creating a mass spectrum. The spectrum that is produced provides a simple and accurate qualitative representation of the sample. The magnitude of each peak is directly proportional to the concentration of and element in a sample (Agilent Technologies, 2005).

1.1.5 Legislation

The first use of the organotin-based antifouling boat bottom paints began in the early 1960s. In 1974, oyster growers first reported the occurrence of abnormal shell growth in *Crassostrea gigas* (the Pacific oyster) along the east coast of England. However, it was not until the mid-1980s that researchers in France and the UK began to suggest that the use of TBT in antifouling paints was adversely impacting a number of marine species other than fouling organisms (Champ, 2000).

In 1982 France was the first country to regulate the use of antifouling paints containing OTCs and was followed by the UK in 1985. The use of TBT-containing paints was restricted for vessels less than 25 m in length. Based on the lethal concentrations for a few commercially important molluscs UK set an Environmental Quality Target Concentration (ETQC) for TBT at 20 ng l^{-1} in 1986. Regarding the high toxicity of the agent the value was reduced by a factor of 10 only a year later to achieve environmental protection. In the following years Canada, Australia, Japan, Sweden, Austria and Switzerland banned the use of TBT-containing antifouling paints (Hoch et al., 2001; Takahashi et al., 1999).

In 1999 the international maritime organization's (IMO) Maritime Environmental Protection Committee (MPEC) proposed global prohibition on the application of organotin-based antifouling paints on ships by 2008. In addition the MPEC also proposed that the IMO promotes the use of environmentally safe antifouling technologies to replace TBT (Champ, 2000).

In Europe, the current Water Framework Directive is the major Community instrument for the control of point and diffuse discharges of dangerous substances. Water Framework Directive (2000/60/EC) and Pollutant Emission Register (2000/479/EC) define 11 priority hazardous substances, including TBT and DBT compounds.

In spite of the banning or regulation of usage of TBT in EU and other developed countries, contamination continues in the aquatic environment because not all countries abide by IMO prohibition. Reasons for that is that the TBT-containing antifouling paints have very high performance, up to ten years of life and are very cost effective. Alternatives on the other hand do not provide high confidence in antifouling activity and still cause environmental problems (Omae, 2006).

Phenyltin compounds which are also toxic to various non-target organisms are still used in agriculture. The World Health Organization (WHO) pronounced TPhT as "safe agriculture chemicals" considering that their concentrations on treated plants decrease rapidly due to photo degradation. WHO also stated that residues of OTCs in foods, vegetables and fruits could partly or completely be removed by washing, peeling and cooking before consumption. However recent studies show that cooking is not effective way to eliminate OTCs from food (Takahashi et al., 1999).

2 Aims and Hypothesis

Extensive use of OTCs based biocides, land filling of OTCs containing products, such as plastics, together with their wide industrial use and commercial applications made OTCs one of the most ubiquitous pollutants in terrestrial and aquatic ecosystems today. Since the discovery of harmful effects of OTCs a few decades ago, worldwide studies have shown their detrimental effects to aquatic ecosystems. While numerous reports on the contamination of marine environment with OTCs can be found in relevant literature, much less is known on their presence in terrestrial environment.

For the determination of the lowest toxic concentrations, half-lives, environmental mobility and transformations, bioaccumulation potential, biological transformations and effects of different OTCs on living organisms and the environment, speciation analysis is required. Sensitive, selective and robust analytical methods must be applied for speciation of OTCs in order to simultaneously detect and quantify all OTCs species of concern present in various samples. Advances in instrumentation and the introduction of hyphenated analytical techniques, such as GC-ICP-MS, have enabled the development of such methods.

Speciation analysis of OTCs in aqueous environmental samples depends on the complexity of sample matrix and typically consists of the following steps: pre-extraction, liquid – liquid extraction with simultaneous derivatisation and extraction into organic phase, clean-up of the organic phase, and separation and detection by suitable hyphenated analytical technique. Each step is critical and can contribute to poor analytical and method performance.

The aim of the presented PhD research was to develop and evaluate new sensitive analytical methods for reliable simultaneous determination of 12 OTCs (methyl-, ethyl-, butyl-, phenyl- and octyltin compounds) in different environmental aqueous samples (fresh water, sea water and landfill leachates). For this purpose, liquid-liquid extraction of OTCs with their *in situ* derivatisation and extraction into organic phase was applied before GC separation and ICP-MS detection. An extraction method for efficient extraction of methyl-, ethyl-, butyl-, phenyl- and octyltin compounds, present at trace levels, from fresh water, seawater and municipal landfill leachate samples was first optimised. Ethylation or propylation, which allows the determination of ethyltin compounds, was used for derivatisation of extracted OTCs. Derivatised OTCs were simultaneously extracted into iso-octane or hexane. Individual species in organic phase were determined by GC-ICP-MS. Identification of the individual OTC specie was performed on the basis of its respective retention times determined by the analysis of standard solutions of investigated compounds.

Investigations in the presented doctoral dissertation enabled the development of reliable analytical methods for the sensitive determination of OTCs in environmental water samples and landfill leachate samples with highly complex matrix.

3 Materials and Methods

3.1 Apparatus

The determination of OTCs was carried out on an Agilent 6890 gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, USA) equipped with Agilent 6890 Series Autosampler Injector that was coupled to Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan) via heated transfer line and fitted with 30 m × 0.25 mm or 15 m × 0.25 mm DB-5MS capillary column (film thickness 0.25 μm) coated with 5 % phenyl-methylpolysiloxane (Agilent J&W Scientific, Palo Alto, CA, USA). Control and operation of the coupled system was achieved by using Agilent ChemStation Software.
For the separation of OTCs on 15 m column the following GC temperature program was applied: at the start the column temperature was held at 50 °C for 0.8 min, then raised to 200 °C at heating rate of 20 °C min^{-1} and held there for 2 min, then raised to 220 °C at heating rate of 40 °C min^{-1} and held there for 0.5 min and, in final step, raised to 280 °C at heating rate of 50 °C min^{-1} and held at this temperature for 2 minutes. The temperature program of GC separation on 30 m column was as follows: at the start the column temperature was held at 60 °C for one minute, then raised to 180 °C at heating rate of 18 °C min^{-1}, then raised to 280 °C at heating rate of 40 °C min^{-1} and held at final temperature for 6.5 minutes. In both separations, inlet temperature was held at 240 °C and transfer – line at 280 °C, helium at flow rate of 1 mL min^{-1} was used as carrier gas, injection mode was split-less and injection volume 2μL.
ICP-MS operated under conditions listed in Table 1.

Table 1: *Operating parameters for ICP-MS and GC.*

ICP-MS	
Parameter	Unit
RF power	1000 W
Sample Depth	8.0 mm
Carrier Gas	0.69 L min^{-1}
Optional Gas (O2)	5.5 % (v/v in carrier gas)
Integration time per isotope	0.1 s
Isotopes measured	^{118}Sn and ^{120}Sn
Tune gas	100 ppm Xe in Ar
Total acquisition time	778 s
GC	
Injection volume	2 μL
Mode	Splitless
Gas	He
Inlet temperature	240 ºC
Column flow	1 mL min^{-1}
Transfer line temperature	280 ºC

Mechanical shaking during the extraction procedure was performed on orbital shaker Vibromix 40 (Tehtnica, Železniki, Slovenia).

3.2 Reagents and Materials

All reagents used were of analytical-reagent grade. Milli-Q water (18.2 MΩ) (Milipore, Bedford, MA, USA) was used for the preparation of all aqueous solutions. Monomethyltin trichloride ($MMTCl_3$, 98 %), dimethyltin dichloride ($DMTCl_2$, 95 %), and trimethyltin chloride (TMTCl, 99 %), were purchased from Acros Organics, (New Jersey, NY, USA). Monobutyltin trichloride ($MBTCl_3$, 95 %), tributyltin chloride (TBTCl, 96 %), monophenyltin trichloride ($MPhTCl_3$, 98 %), diphenyltin dichloride ($DPhTCl_2$, 96 %), triphenyltin chloride (TPhTCl, 97 %) and triethylbromide (TEtBr, 97%) were purchased from Aldrich (Milwaukee, WI, USA). Dibutyltin dichloride ($DBTCl_2$, 98 %), tetrabutyltin (TeBuT), that was included in the speciation procedure as a standard which does not need alkylation, and tripropyltin chloride (TPrTCl, 98 %) were obtained from Merck (Darmstadt, Germany). Monooctyltin trichloride ($MOcTCl_3$, 99 %) and dioctyltin dichloride ($DOcTCl_2$, 99 %) were purchased from LGC Promochem (Wesel, Germany) and trioctyltin chloride (TOcTCl, > 90 %) from Fluka (Buchs, Switzerland).

OTCs standard stock solutions containing 1000 mg (expressed as Sn) L^{-1} were prepared in methanol. Stored in the dark at 4 ºC, they were stable for 6 months. Working OTCs standard solutions were prepared daily.

Iso-octane, hydrochloric acid (HCl), sodium chloride (NaCl), 25% water solution of ammonia, Tris (hydroxymethyl)aminomethane (Tris), hydrogen peroxide 30 % (H_2O_2) and citric acid monohydrate were obtained from Merck (Darmstadt, Germany). Acetic acid, nitric acid and anhydrous sodium acetate were purchased from Carlo Erba (Milan, Italy), hexane and methanol from J.T. Baker (Deventer, Holland), sodium tetraethyl borate ($NaBEt_4$, 98 %) from Strem Chemicals (Newburyport, MA, USA), potassium di-hydrogen phosphate and di-potassium hydrogen phosphate from Riedel-de Haen (Seeize, Germany), sodium hydrogen carbonate and di-sodium carbonate from Kemika (Zagreb, Croatia) and sodium tetrapropyl borate ($NaBPr_4$, 99 %) from ABCR (Karlsruhe, Germany). Aqueous solutions of sodium tetraethyl borate ($NaBEt_4$) (2 % (w/v)) and sodium tetrapropyl borate (($NaBPr_4$) (2 % (w/v)) were prepared just before derivatisation. All buffers were prepared daily.

3.3 Cleaning procedures

To avoid contamination all glassware were rinsed three times with tap water, soaked in 20 % nitric acid for 48 hours, rinsed three times with tap water, three times with deionised water and heated at 400 °C for at least 4 hours.

3.4 Sampling procedures

Marine and river water samples were collected with 5L Niskin Water sampler bottles, then transferred in 2.5 L dark glass bottles, acidified with nitric acid to pH 2.0 and transported using iceboxes. Until analysis they were stored in refrigerator at 4 ºC.
Landfill leachate samples were collected from each collector basin (landfill Barje) using sample collector which was thoroughly washed beforehand. Samples were then transferred into 2.5 L dark glass bottles and immediately transported using iceboxes. Then leachates were frozen at -20 ºC until analysis. Before sample collection all glass bottles were washed with 20% nitric acid and rinsed three times with Milli-Q water.

3.5 Analytical procedures

3.5.1 Determination of OTCs in marine and river waters

Developed liquid – liquid extraction procedure was used prior to the determination of OTCs in different water samples by GC-ICP-MS. 300 mL aliquot of water sample (deionised water, salt water containing 3.8 % NaCl, sea water from the Northern Adriatic Sea) was transferred into 500 mL dark glass reactor vessel along with 100 mL of selected 0.2 M buffer solution. Phosphate, carbonate and Tris-citrate buffers were used to optimise the pH of derivatisation of OTCs in fresh or salty water samples. Their applicability was critically evaluated and compared to acetate buffer. Phosphate buffer was prepared from potassium di-hydrogen phosphate and di-potassium hydrogen phosphate salt in appropriate ratios to match pH range from 4.4 to 9.0, carbonate buffer from di-sodium carbonate and sodium hydrogen carbonate salts to match pH range from 4.0 – 10.0 (pH was adjusted with HCl), and Tris-citrate buffer from Tris and citric acid salts to match pH range from 3.0 to 10.0 (pH was adjusted with citric acid or ammonia). Sodium acetate – acetic acid buffer was prepared at pH of 4.8. All samples were spiked with internal standard solution TPrT, TeBuT and methyl-, butyl-, phenyl- and octyl-tin standard solutions in concentration range from 0.06 to 33.3 ng Sn L^{-1}. To spiked samples 0.5 mL of 2 % (m/V) $NaBEt_4$ for derivatisation and 1 mL of iso-octane or hexane as an extraction agent for ethylated OTC species were added. Samples were mechanically shaken for 45 min and after that organic phase for analysis collected into 2 mL dark vials using Pasteur pipette. Blank samples were spiked only with internal standard (TPrT) and determined after applying the same analytical procedure as for samples. All the analyses were made in triplicate.

3.5.2 Determination of OTCs in landfill leachates

A liquid – liquid extraction procedure was used prior to the determination of OTCs in landfill leachate samples by GC-ICP-MS. Briefly, 200 mL aliquots of landfill leachate samples were transferred into 500 mL dark glass reactor vessels along with methanol that was added in concentration 5 % relative to sample volume. Mixtures were mechanically shaken for 2 hours. After shaking 190 mL of 0.2 mol L^{-1} Tris-citrate buffer was added to each sample, so that the total volume (sample, methanol plus buffer) was 400 mL. Tris-citrate buffer was prepared from Tris and citric acid to match pH 6 for derivatisation. pH was adjusted with citric acid or ammonia. Addition of TPrT internal standard solution (20 ng Sn) followed. For the standard addition calibration method mixtures of methyl-, butyl-, phenyl- and octyl-tins were added to sample aliquots in concentrations ranging from 0.2 to 100 ng Sn. For derivatisation 2 mL of 2 % (m/V) $NaBEt_4$ were added to the sample extracts, followed by the addition of 2 mL of hexane, as an extraction agent for ethylated OTC species. Samples were then mechanically shaken for 16 hours. After that organic phase was collected into 15 mL dark glass vials using Pasteur pipette. Organic phase contained dispersed emulsion. To separate the emulsion from the organic phase, 1 mL of 25 % KOH in methanol was added, shaken for 5 min and centrifuged for 20 min at 4200 x *g*. Clear organic phase was transferred into a dark glass vials for GC-ICP-MS analysis. For the determination of ethyltins, TeBuT (20 ng Sn) was used as internal standard solution and 2 % (m/V) of $NaBPr_4$ (2 mL) as derivatisation agent. To control the purity of reagents used, the same analytical procedure was applied to Milli-Q water. All the analyses were made in triplicate.

3.5.3 Determination of total Sn concentrations in landfill leachates

5 mL of samples were added into Teflon beakers. Then 2 mL of nitric acid and 2 mL of hydrogen peroxide (s.p.) were added and samples subjected to closed vessel microwave digestion at maximal power of 1200 W: ramp to temperature 20 min, 180 ºC, pressure 10 bar, hold 20 min, cooling 20 min. Clear solutions were quantitatively transferred into 20 mL glass volumetric flasks and filed to mark with Milli-Q water. The same procedure, with exception that no sample was added, was applied to determine blank. The total concentrations of Sn in the digested samples were determined by ICP-MS. Analyses were made in triplicate.

4 Results and Discussion

4.1 Optimisation of the analytical procedure for determination of OTCs in water samples

In order to develop optimal analytical procedure for the determination of OTCs in water samples, several parameters were critically evaluated.

4.1.1 Optimization of derivatisation of OTCs in water samples

For "*in situ*" derivatisation of ionic OTCs in water samples by $NaBEt_4$, pH that was adjusted to around 5 with sodium acetate-acetic acid buffer was frequently chosen as optimal. In these conditions, the yield of ethylation of OTCs depends on the degree of substitution and the nature of the alkyl groups linked to the tin atom. However, as was found reported in the literature, optimal pH can be quite higher than this value, due to the sample matrix that can retard ethylation rate, and pH effect on stability of $NaBEt_4$, which decomposed more rapidly at lower pH (M.Prat et al., 2002). Therefore, in the present study, optimisation of pH for "*in situ*" ethylation of ionic methyl-, butyl-, phenyl- and octyl-tin compounds was first performed in Milli Q water at pH ranging from 4 to 10. For this purpose, phosphate, carbonate, and Tris-citrate buffers were chosen as an alternative to commonly used acetate buffer. Ethylated OTCs were extracted into iso-octane and determined by GC-ICP-MS as described previously in section 3.5.1. For separation 15 m GC column was used. Experimental results of this preliminary study demonstrated that for all buffers investigated optimal pH of ethylation lied between pH 5 and 7.5 (data not shown). This pH range was then studied more in details. The OTCs signals normalised to TPrT that correspond to ethylation yields of OTCs in Milli Q water samples spiked with methyl-, buthyl- phenyl- and octyl-tin compounds (33.3 ng Sn L^{-1}) in pH range from 5 to 7.5 are shown in Figures 1-4. It was experimentally proven that normalisation to TeBuT gave similar results.

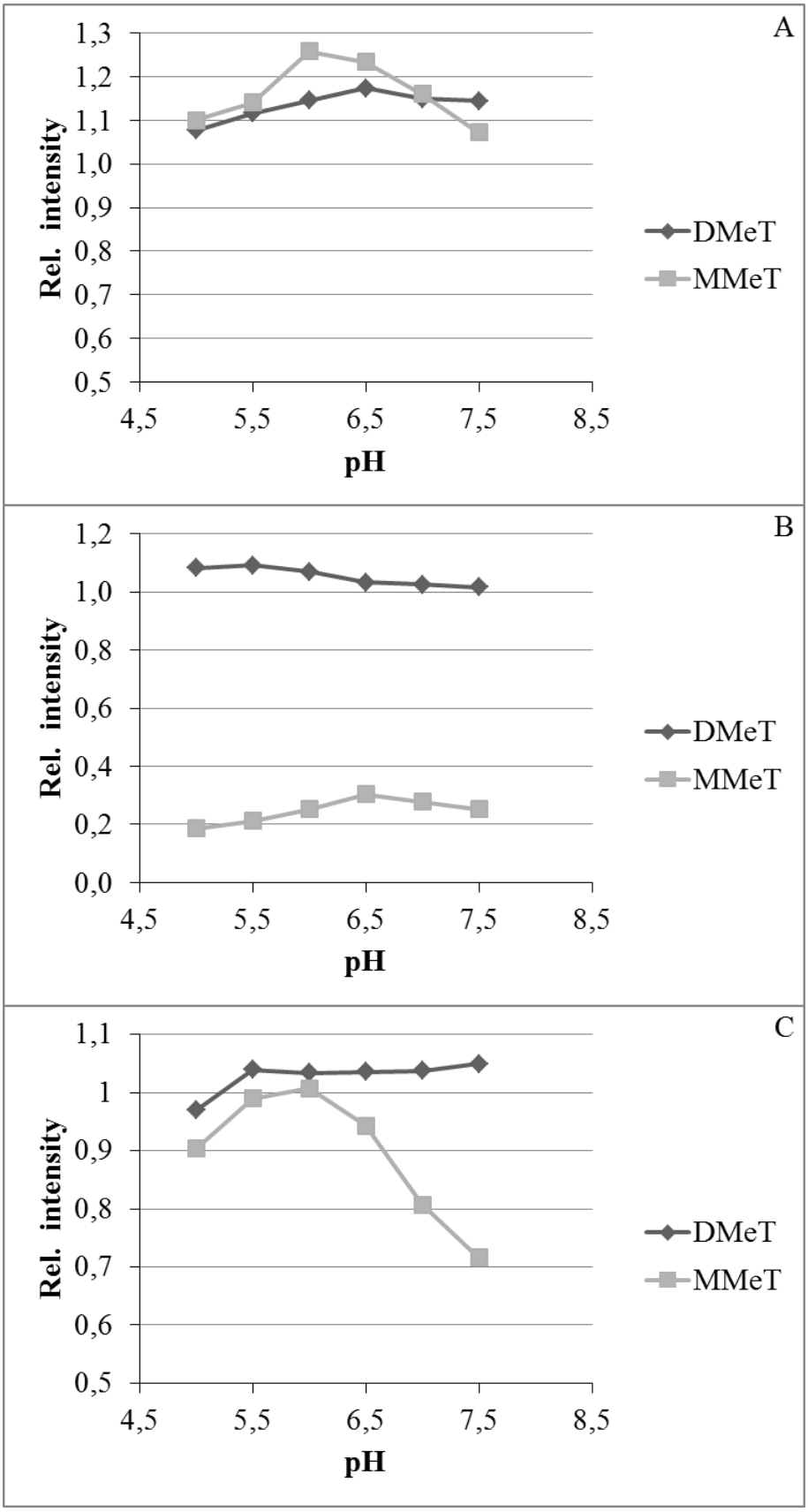

Figure 1: *Effect of pH on the relative signals of spiked methyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.*

Results of optimisation of pH for ethylation of methyl-tin compounds (Figure 1) show that when carbonate buffer was used (Figure 1A), ethylation of monomethyl-tin (MMeT) and dimethyl-tin (DMeT) was optimal at around pH 6.0. Similarly, maximum ethylation yield for MMeT and DMeT was observed at around pH 6.0 when phosphate (Figure 1B) or Tris-citrate (Figure 1C) buffer was used for pH adjustment. Results for optimisation of ethylation pH are not shown for trimethyl-tin (TMeT) since TMeT was not separated quantitatively in iso-octane which was used as organic phase for extraction of ethylated OTCs.

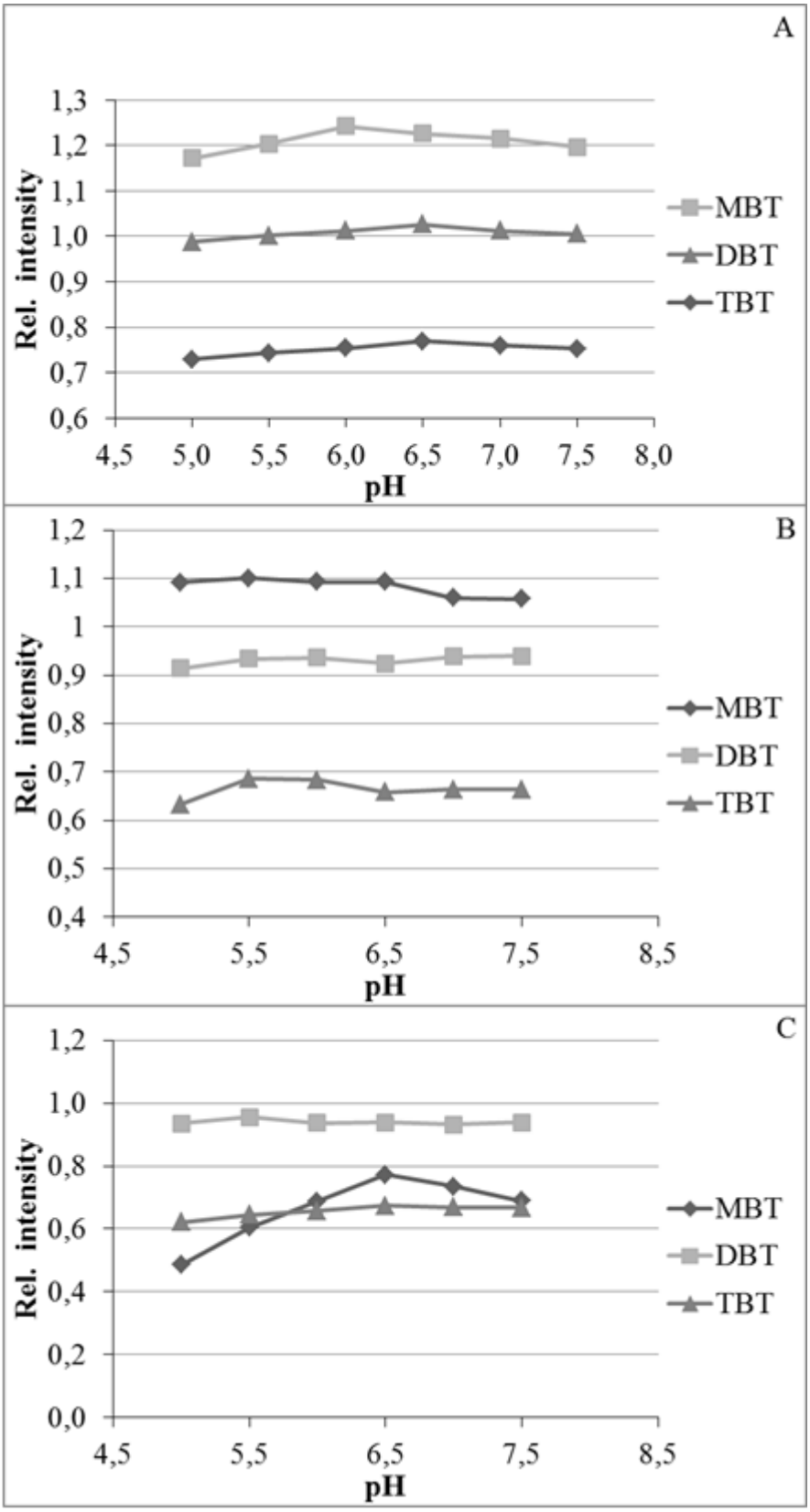

Figure 2: *Effect of pH on the relative signals of standard solution of butyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.*

In Figure 2 ethylation yields (as analytical signal intensities) for monobutyl-tin (MBT), dibutyl-tin (DBT) and tributyl-tin TBT are presented. When carbonate buffer was used (Figure 2A), maximum ethylation yield for all buty-tin compounds lied between pH 6.0 and 7.0. It is also evident that the ethylation of ionic butyl-tin compounds was almost constant over the whole investigated pH range. Similarly, more or less constant ethylation yield over the whole pH range investigated was obtained with the phosphate (Figure 2B) and Tris-citrate (Figure 2C) buffers.

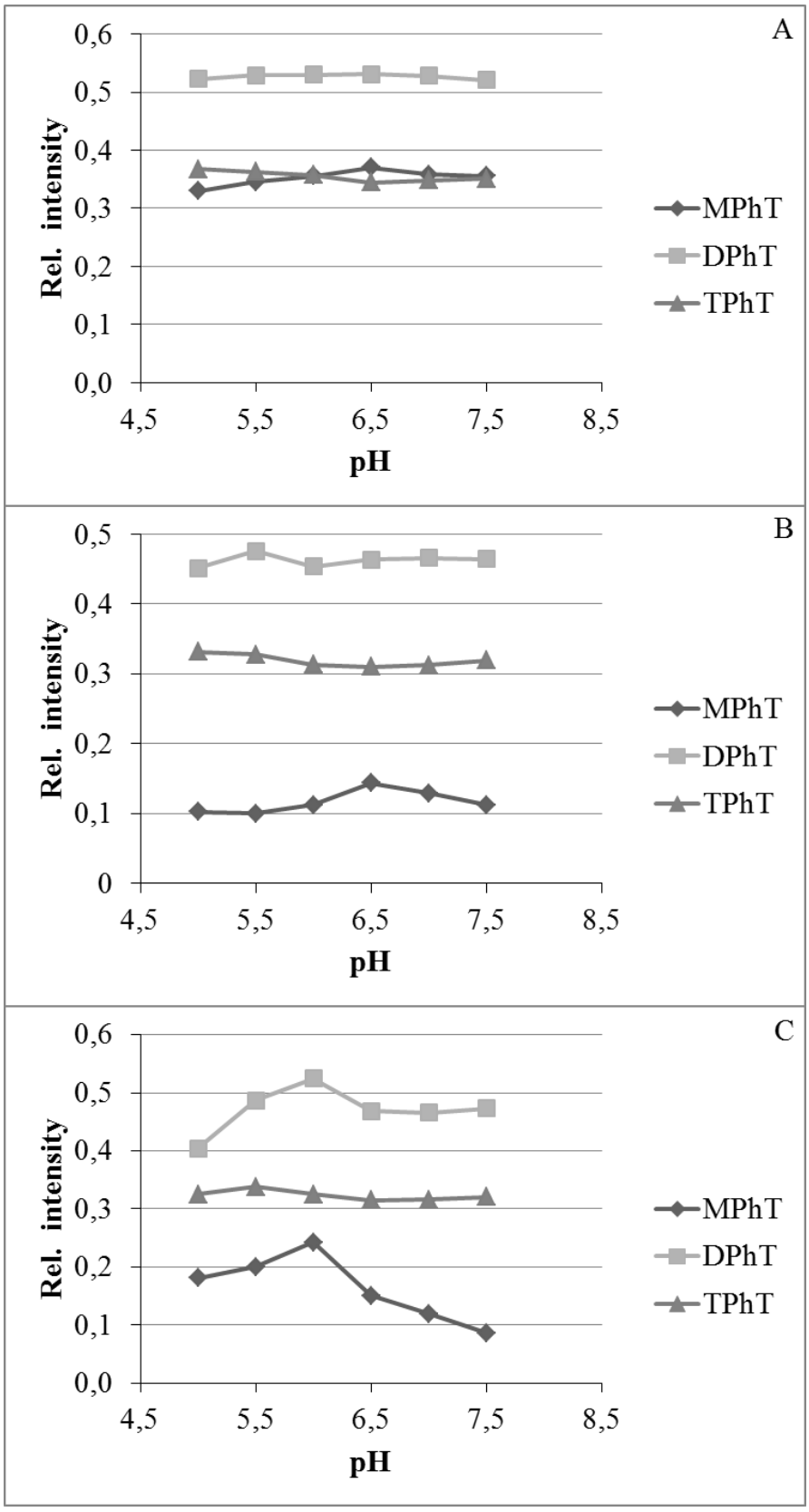

Figure 3: *Effect of pH on the relative signals of standard solution of phenyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.*

Figure 3 presents ethylation yields of monophenyl-tin (MPhT), diphenyl-tin, (DPhT) and triphenyl-tin TPhT. As it can be seen from Figure 3A, when carbonate buffer was used for pH adjustment at pH below 7, ethylation of phenyl-tin compounds didn't depend significantly on pH while at higher pH ethylation yield for phenyl-tin started to decline. Applying phosphate buffer (Figure 3B), the ethylation of MPhT and TPhT was constant in pH range between pH 5.0 and 8.0, and for DPhT optimal between pH 5.0 and 6.0. In Tris-citrate buffer (Figure 3C) maximum ethylation yield for MPhT and DPhT was observed at pH 6.0, while for TPhT it remained relatively constant over the whole pH range investigated.

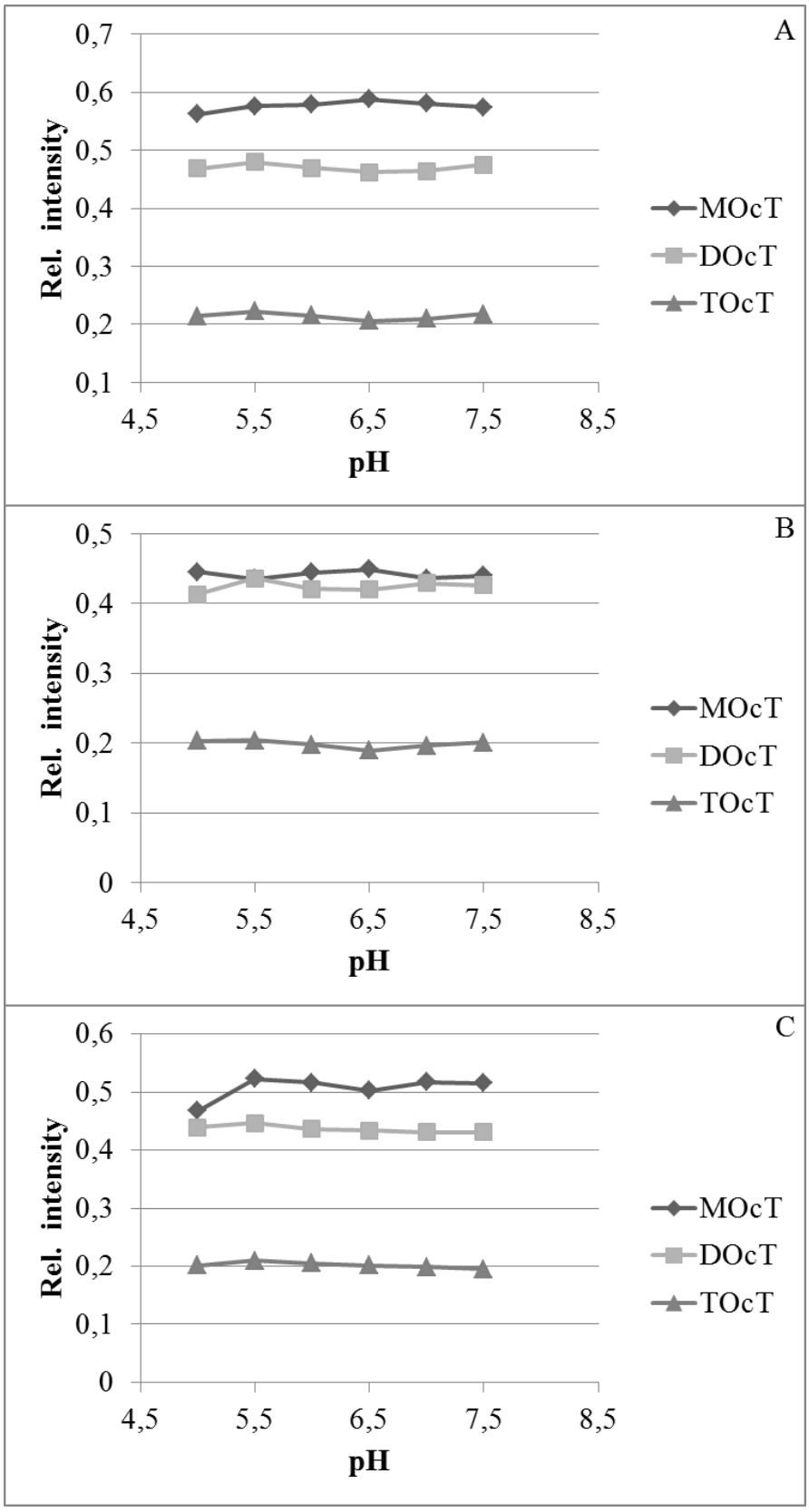

Figure 4: *Effect of pH on the relative signals of standard solution of octyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.*

Results of optimisation of pH for ethylation of octyl-tin compounds are presented in Figure 4. In carbonate buffer (Figure 4A) ethylation of all tested octyl-tin compounds was optimal between pH 6.0 and 7.0. Similar were results when phosphate (Figure 4B) or Tris-citrate (Figure 4C) buffer was applied.

In general, experimental results has shown that the effect of pH on the ethylation of OTCs tested was the most pronounced for methy-tin compounds and progressively decreased with the size of the alkyl- (butyl- and octyl-tin compounds) or aryl- (phenyl-tin compounds) functional groups. It should be also pointed out that above described studies were carried out in Milli-Q water due to the fact that in salty water (artificial salt water with 3.8 % NaCl or sea water) at pH higher than 6, as expected, visible precipitates in phosphate and carbonate buffers, and bubbles in carbonate buffer at pH lower than 4 have started to form. Precipitates or bubbles were not observed when Tris-citrate buffer was applied. Among buffers studied Tris-citrate buffer was found to be the most appropriate for adjustment of pH in different water samples. This buffer can be used for pH adjustment for ethylation of OTCs in fresh and salty water samples in wide pH range. Experimental results also demonstrated that for particular OTC the optimal pH of ethylation slightly varies, but overall optimal ethylation yields for all OTCs can be achieved at pH 6.0.

4.1.2 Performances of GC columns with different length, applicability of iso-octane and hexane, and comparison of Tris-citrate and acetate buffer

Survey of the relevant published literature indicates that GC columns of 30 m in length were mostly applied for the separation of derivatised OTCs in various environmental samples. In Figure 5 signal intensities of spiked OTCs (33.3 ng Sn L^{-1}) in water samples, extracted into iso-octane or hexane, separated on 15 m or 30 m GC columns, using Tris-citrate or acetate buffer, are shown.

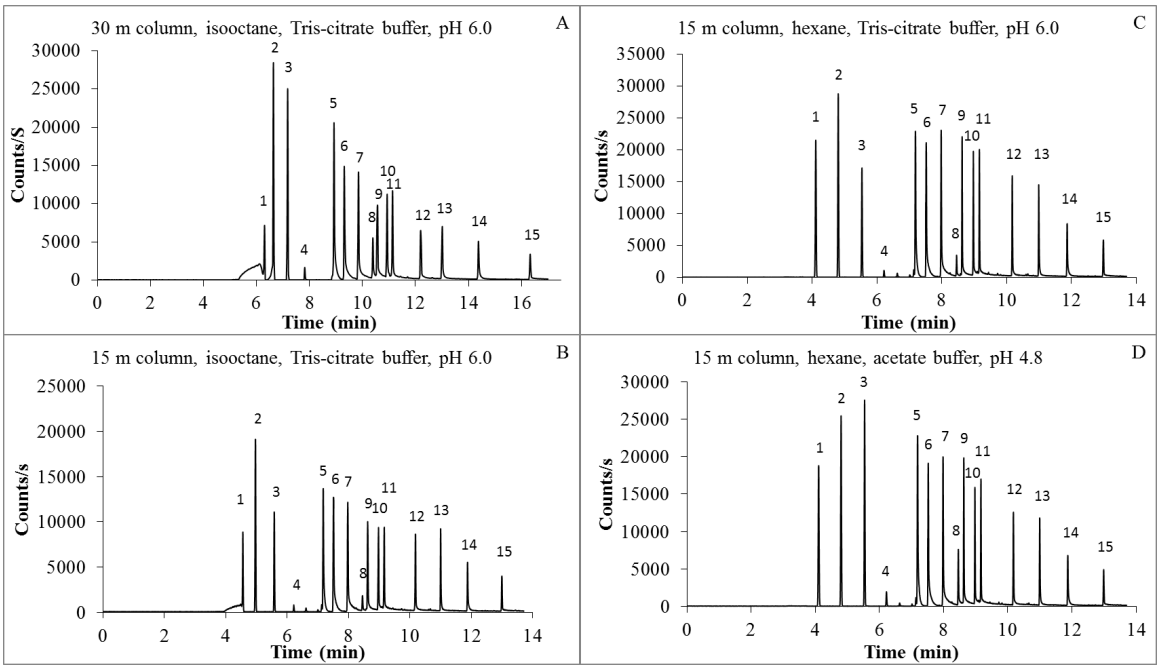

Figure 5: *Separation efficiencies of methy-, butyl-, phenyl- and octy-tin compounds in (A) iso-octane on 30 m GC column, Tris-citrate buffer, pH 6.0, (B) iso-octane on 15 m GC column, Tris-citrate buffer, pH 6.0, (C) hexane on 15 m GC column, Tris-citrate buffer, pH 6.0 (D) hexane on 15 m GC column, acetate buffer, pH 4.8. Concentrations of OTCs were 33.3 ng Sn L^{-1}.*
Legend: (1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn , 5 – MBT, 6 – TPrT, 7 – DBT, 8 – MPhT, 9 – TBT, 10 – MOcT, 11 – TeBuT, 12 – DPhT, 13 – DOcT, 14 – TPhT, 15 – TOcT)

In Figures 5A and 5B separation of 12 OTCs (as well as TPrT, TeBuT and inorganic Sn) in iso-octane on 30 and 15 m GC column, respectively are presented. Tris-citrate buffer (pH 6.0) was used. It can be seen that, with the exception of TMeT, OTCs investigated were selectively separated on 30 m column (Figure 5A), suggesting that shorter GC column (15 m) can also be used. GC conditions of the separation that were carefully optimised for both column lengths (30 m versus 15 m) are reported in the section 3.1. From Figures 5A and 5B, it is further evident that by applying shorter GC column, the column efficiency is not compromised. The main difference between both column lengths was found in the duration of the separation. On 15 m GC column the total separation time was 12.88 min and on 30 m column 18.65 min, which means about 30 % reduction in separation time.

For selective separation of TMeT from solvent front, hexane which has lower boiling point (69 °C) (Donard et al., 1986) than iso-octane (99 °C) (Michel and Averty, 1991) and hence elutes faster from GC column was applied (Figure 5C). In this experiment Tris-citrate buffer and 15 m GC column were used. It is evident from Figure 5C that TMeT and other OTCs were selectively separated.

Comparison of Tris-citrate and acetate buffers at optimal pH (pH 6.0 and pH 4.8, respectively), using 15 m column and extraction into hexane for separation of 12 OTCs (as well as TPrT, TeBuT and inorganic Sn) is presented in Figures 5C and 5D. It is evident that both buffers can be efficiently applied for adjustment of the pH of ethylation. The differences can be observed in signal intensities. They were higher for MMeT and MPhT when acetate buffer was used, while for all other OTCs investigated, signal intensities were higher when Tris-citrate buffer was used.

4.1.3 Signal stability of OTCs in hexane

Due to high volatility of hexane, special attention must be paid to prevent its evaporation during the measurements. To prove that no loss of analytes occurred during the

determination by GC-ICP-MS, the signal stability of 12 OTCs investigated (ethylated at pH 6 using Tris-citrate buffer) was monitored. For this purpose, stability test with sixty subsequent determinations (15 consecutive parallel samples each in 4 replicates) was carried out on 15 m GC column. Results of this study are presented in Figure 6.

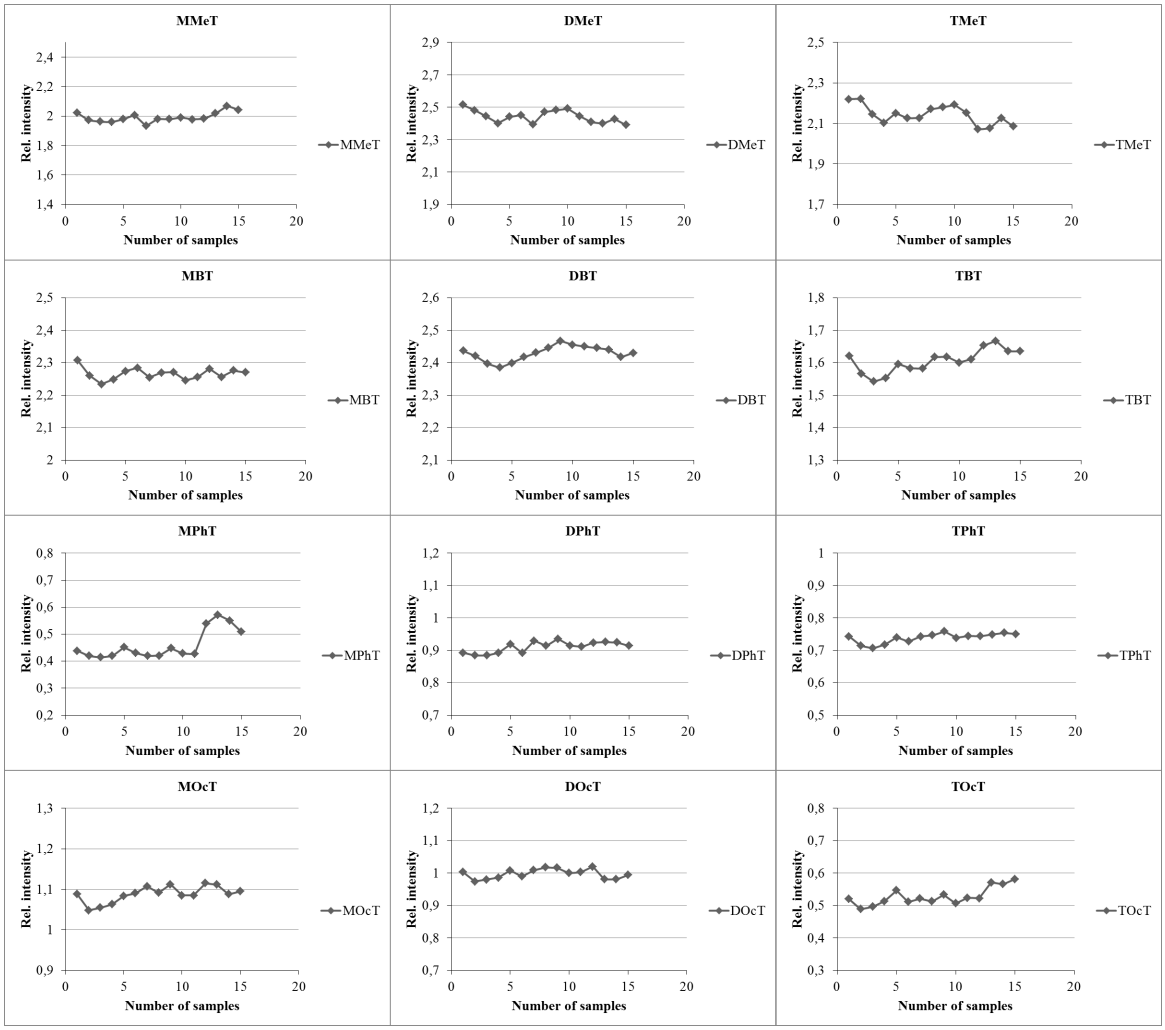

Figure 6: *Stability of OTC signals (hexane used for extraction of ethylated OTCs into organic phase, 15 m GC column, concentrations of OTCs 33.3 ng Sn L^{-1}).*

Each point on the chart represents an average of 4 consecutive measurements of the same sample. As it can be seen from the Figure 6, analytical signal was constant during the course of the determinations proving that there was no evaporation of hexane. Henceforth, hexane was used for extraction of ethylated OTCs.

4.1.4 Evaluation of the analytical method for determination of OTCs in water

The performances of analytical method (Tris-citrate buffer pH 6, ethylation by $NaBEt_4$, extraction into hexane, separation on 15 m GC column, and ICP-MS detection) for the simultaneous determination of 12 OTCs were evaluated. The limits of detection (LOD) and limits of quantification (LOQ) were calculated by the analyses of six replicates of uncontaminated fresh and salty water samples as three times the standard deviation of the

uncontaminated sample (3*s*) and LOQ as ten times the standard deviation of the uncontaminated sample (10*s*), respectively. Mixtures of calibration standards of OTCs in the range from 0.6 to 33.3 ng Sn L^{-1} in organic phase were prepared and calibration graphs obtained for methyl-tins (MMeT, DMeT, TMeT), butyl-tins (MBT, DBT, TBT), phenyl-tins (MPhT, DPhT and TPhT), and octyltins (MOcT, DOcT and TOcT). Repeatability of measurements was examined by 6 consecutive determinations of fresh or salty water sample with OTCs concentration of 33.3 ng Sn L^{-1} in hexane. The reproducibility of measurement was checked by 6 consecutive determinations of the same fresh and salty water sample on two different days. The results obtained for LOD, LOQ, correlation coefficient, repeatability and reproducibility for salty water are listed in Table 2.

Table 2: *LOD, LOQ, correlation coefficient, repeatability and reproducibility of measurements of analytical method for simultaneous determination of 12 OTCs in salty water samples by GC-ICP-MS.*

OTC	LOD (ng Sn L^{-1})	LOQ (ng Sn L^{-1})	r^2	Repetability RSD (%)	Reproducibility RSD (%)
MMeT	0.05	0.17	0.9997	2.2	3.0
DMeT	0.06	0.20	0.9999	2.7	7.9
TMeT	0.05	0.17	0.9995	4.3	8.0
MBT	0.45	1.50	0.9999	1.1	1.9
DBT	0.11	0.37	0.9995	1.7	3.7
TBT	0.15	0.50	0.9964	4.8	6.5
MPhT	0.16	0.53	0.9984	14.7	4.8
DPhT	0.12	0.40	0.9996	5.2	2.3
TPhT	0.11	0.37	0.9994	3.2	1.9
MOcT	0.10	0.33	0.9989	3.7	3.0
DOcT	0.07	0.23	0.9996	3.0	3.9
TOcT	0.10	0.33	0.9992	7.2	3.5

Data from Table 2 indicate good linearity of measurement (regression coefficients for all OTCs $r^2 > 0.9964$). Measurements were sensitive, (LOD for particular OTC ranged from 0.05 to 0.45 ng Sn L^{-1} and LOQ from 0.17 to 1.50 ng Sn L^{-1}) repeatable and reproducible (for 80 % of measurements better than 5 %). For fresh water sample almost the identical or even better performances of analytical method than for salty water were obtained.

4.1.5 Quantification of OTCs in water samples

Quantification of OTCs was performed by applying standard addition calibration using 15.15 ng Sn L^{-1} of TPrT as an internal standard. Since no certified reference material exists for water samples, marine water with no measurable concentrations of determined OTCs were spiked with known amounts of all 12 OTCs. Calibration curves were prepared and samples analysed by applying developed analytical method (Tris-citrate buffer pH 6, ethylation by $NaBEt_4$, extraction into hexane, separation on 15 m GC column, and ICP-MS detection). Spike recovery test was performed at concentrations 10 and 20 ng Sn L^{-1} in samples to confirm the efficiency of the developed analytical method. Analysis was made in 6 replicates. Recoveries of spiked target compounds in samples ranged from 97 % to 103 % for methyl-tins, from 85 % to 99 %, for butly-tins, from 87 % to 109 % for phenyl-tins, and from 103 % to 126 % for octyltins. From the results it was concluded that analytical method developed was suitable for its intended use.

4.1.5.1 Analysis of marine water samples

To assess the applicability of the developed analytical method the concentrations of OTCs in marine water samples from the Northern Adriatic Sea were determined. Sampling was performed monthly at five different locations in time period from January to June 2009. In these samples in general MMeT, DMeT and butyl-tins were detected. The concentrations of all other OTCs were below LOD of the applied analytical method. Results that show concentration ranges of of the commonly present OTCs determined in marine water samples are presented in Table 3.

Table 3: *Concentration ranges of the commonly present OTCs in water samples from the Northern Adriatic Sea determined by GC-ICP-MS.*

Sample No.	MMeT (ng Sn L^{-1})	DMeT (ng Sn L^{-1})	MBT (ng Sn L^{-1})	DBT (ng Sn L^{-1})	TBT (ng Sn L^{-1})
I	<0.05 - 5.8	0.6 - 6.0	<0.45 - 2.6	0.7 - 3.8	<0.15 - 0.3
II	<0.05 - 5.4	1.3 - 17.0	0.5 - 7.3	0.6 - 5.7	<0.15 - 0.3
III	1.8 - 7.3	2.7 - 17.4	<0.45 - 2.4	0.4 - 6.0	0.2 - 2.3
IV	<0.05 - 2.9	1.1 - 9.6	<0.45 - 1.4	0.5 - 3.3	<0.15 - 16.5
V	<0.05 - 34.2	1.4 - 16.9	<0.45 - 3.5	0.7 - 3.8	<0.15 - 1.6

For comparison of the performance of Tris-citrate buffer to most commonly applied acetate buffer, the results are presented in Table 4 while the representative GC-ICP-MS chromatograms of the sample III are shown in Figure 7.

Table 4: *Concentrations of OTCs in marine water sample III from the Northern Adriatic Sea by GC-ICP-MS, using (A) Tris-citrate, pH 6.0 and (B) acetate, pH 4.8 buffers. Representative chromatograms are presented in Figure 7.*

OTC compound	(A) Concentration (ng Sn L^{-1})	(B) Concentration (ng Sn L^{-1})
MMeT	0.19 ± 0.01	0.20 ± 0.01
DMeT	0.29 ± 0.02	0.30 ± 0.02
TMeT	0.20 ± 0.01	0.20 ± 0.01
MBT	1.9 ± 0.1	2.3 ± 0.1
DBT	2.5 ± 0.1	2.5 ± 0.1
TBT	0.19 ± 0.01	<0.15

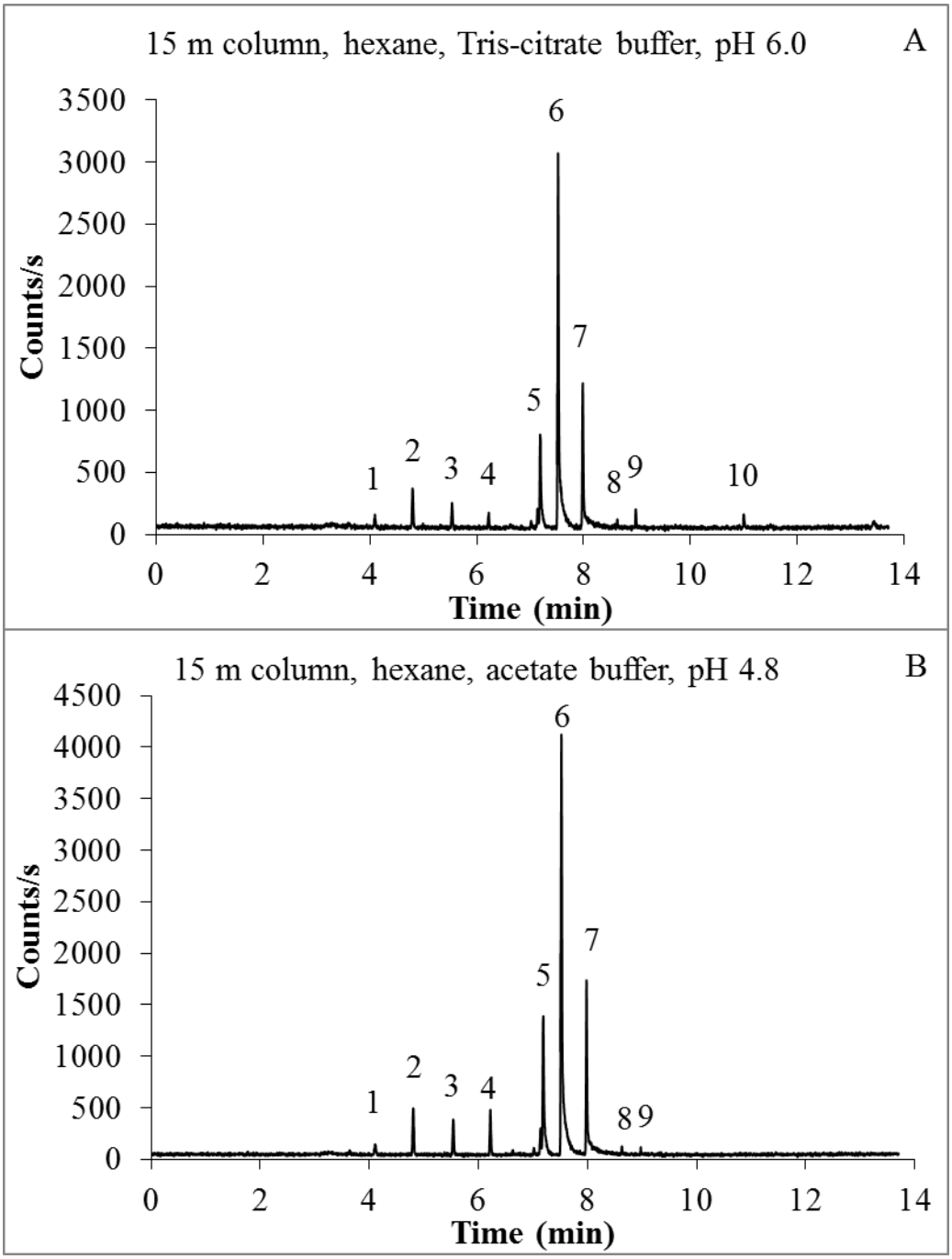

Figure 7: *GC-ICP-MS chromatogram of ethylated methy- and butyl-tin species determined in a marine water sample III: (A) hexane, 15 m GC column, Tris-citrate buffer, pH 6.0, (B) hexane, 15 m GC column, acetate buffer, pH 4.8.*
Legend: 1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn , 5 – MBT, 6 – TPrT, 7 – DBT, 8 – TBT, 9 – MOcT, 10 – DOcT

It is evident from Figure 7 that the OTC signals are in general similar for both buffers applied. Data from Table 4 further demonstrate good agreement of results in OTCs that were quantified by the use of both buffers. Tris-citrate buffer indicates slightly better selectivity in determination of TBT, MOcT and DOcT, that were not quantified in the sample analysed when acetate buffer was applied.

4.2 Development of analytical procedure for determination of OTCs in landfill leachates

In comparison to fresh and sea waters, landfill leachates that contain high amounts of organic matter, proteins, fats and inorganic and organic pollutants are samples with much higher degree of matrix complexity. So, optimization of extraction parameters and pH of derivatisation was necessary for both ethylation and propylation procedures. Before GC-ICP-MS determination of OTCs it was also necessary to remove the dispersed emulsion that appeared in the organic phase (hexane). To separate the emulsion 1 mL of concentrated HCl or 25 % KOH in methanol was added to 2 mL of hexane. The mixture was shaken for 5 min and centrifuged for 20 min at 4200 x *g*. The volume of recovered hexane from the emulsion was on average 1 mL when concentrated HCl was applied and 1.5 mL when 25 % KOH in methanol was added. The centrifugation time for 25 % KOH in methanol (20 min) was shorter than for HCl (40 min). Analyses of OTCs in clear organic phase (hexane) indicated that application of HCl or 25 % KOH in methanol for separation of the dispersed emulsion did not influence the speciation of OTCs. The same concentrations were determined regardless the agent used. Due to the shorter time necessary for separation of emulsion and higher recovery of the clear organic phase, the use of 25 % KOH in methanol is recommended and was applied in further experiments.

4.2.1 Optimization of the extraction procedure from landfill leachates

In order to develop optimal extraction procedure for OTCs determination in landfill leachate samples, several parameters that influence the overall extraction efficiency were critically evaluated.

4.2.1.1 Comparison of mechanical and ultrasonic shaking

To optimize the extraction procedure different extraction modes were compared: ultrasonic extraction and mechanical shaking. Landfill leachate sample was spiked with 100 ng Sn (TPrT, butyltins, methyltins, phenyltins and octyltins). Tris-citrate buffer (pH 6.0) was used as extractant. For derivatisation, ethylation with $NaBEt_4$ was applied. Ultrasonic extractions were carried out at 40 °C for 15, 30 and 60 minutes, while mechanical shaking was performed at room temperature for 1, 4, 6, and 16 hours. The results of these experiments indicated, that the optimal time for extraction using mechanical shaking was 16 hours, while ultrasonic extraction did not give comparable results even after 60 min. It was further demonstrated that ultrasonic extraction was far less efficient than mechanical shaking. As can be seen from Fig. 8., signal intensities of ultrasonic extraction were appreciably lower. Due to that reason, mechanical shaking (16 hours) was applied in further experiments.

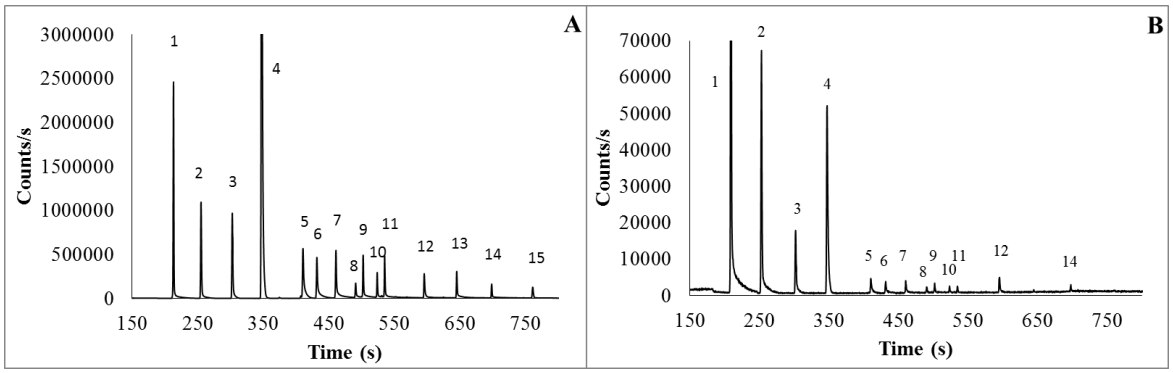

Figure 8: *Chromatograms of OTCs in spiked (100 ng Sn) landfill leachate (Sample No. 3) (Tris-citrate buffer, pH 6.0, derivatisation with $NaBEt_4$) for (A) extraction by mechanical shaking (16 h) and (B) ultrasonic extraction (1 h).*
Legend:
1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn, 5 – MBT, 6 – TPrT, 7 – DBT, 8 – MPhT, 9 – TBT, 10 – MOcT, 11 – TeBuT, 12 – DPhT, 13 – DOcT, 14 – TPhT, 15 – TOcT

4.2.1.2 Comparison of ethylation and propylation efficiencies

In order to optimize alkylation efficiency for methyl-, butyl-, phenyl- and octyltins, $NaBEt_4$ and $NaBPr_4$ were used for derivatisation. Spiked landfill leachate (100 ng Sn, extraction with Tris-citrate buffer, pH 6.0) was subjected to mechanical shaking for 1 to 16 hours. The results of these experiments are presented in Fig. 9.

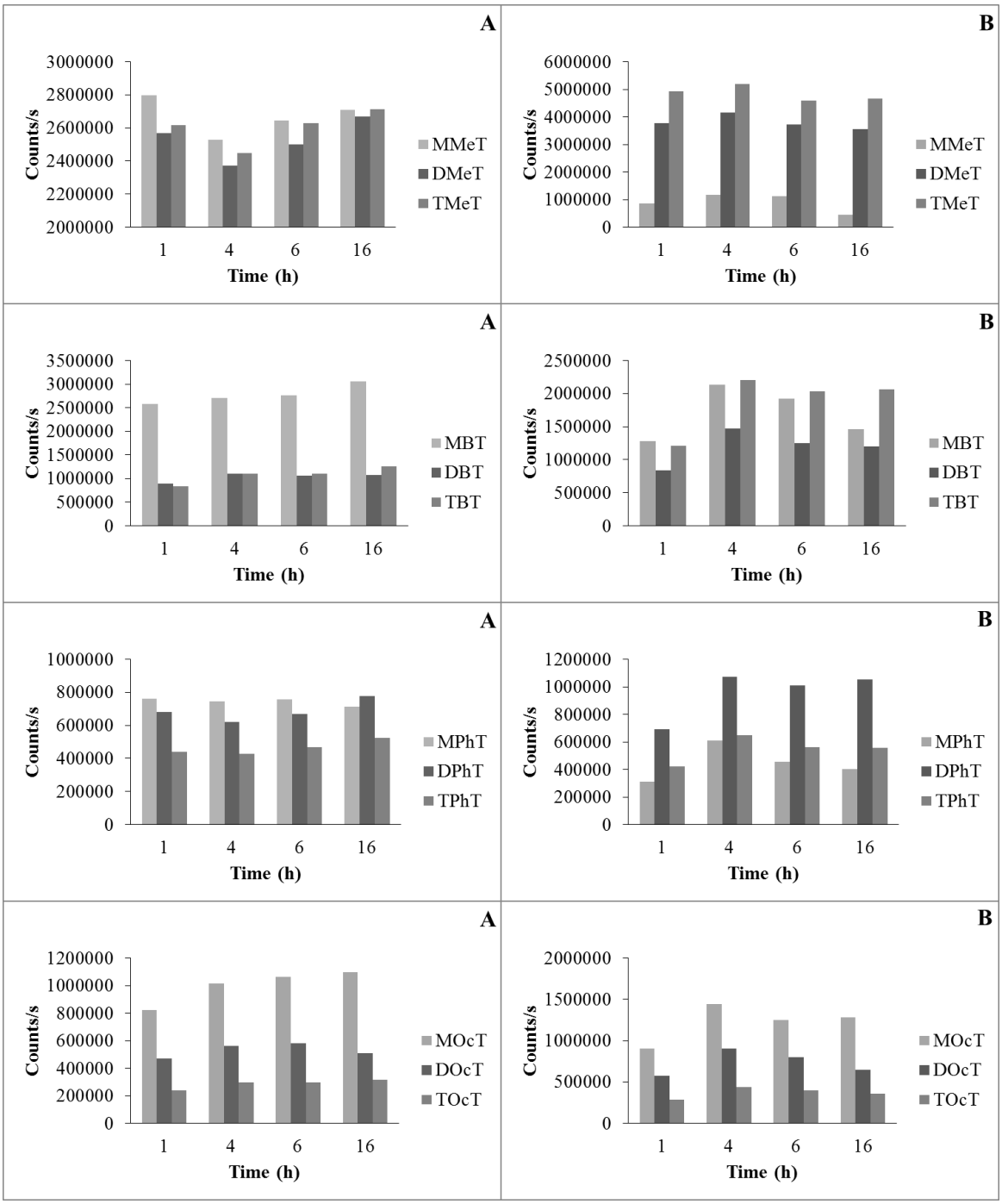

Figure 9: *Variation of signals for (A) ethylated and (B) propylated OTCs in spiked (100 ng Sn) landfill leachate (Sample No. 3) with time. Mechanical shaking, Tris-citrate buffer (pH 6.0).*

Data from Fig. 9 indicates that maximal signal for OTCs investigated are obtained after 4 hours for propylation and after 16 hours for ethylation procedure. Despite shorter time necessary for propylation, further experiments demonstrated, that propylation provoked splitting of phenyltin chromatographic peaks, resulting in higher limits of quantification and worse repeatability and reproducibility of measurements. Therefore, ethylation was applied throughout of following experiments with exception, when ethyltins were investigated.

4.2.1.3 The application of methanol as co-extracting agent

To facilitate the extraction procedure, different concentrations of methanol were applied as co-extracting agent to spiked landfill leachate with methyl-, butyl-, phenyl- and octyltins (100 ng Sn) prior to addition of Tris-citrate buffer (pH 6.0), ethylation reagent and hexane. The results are shown in Fig. 10.

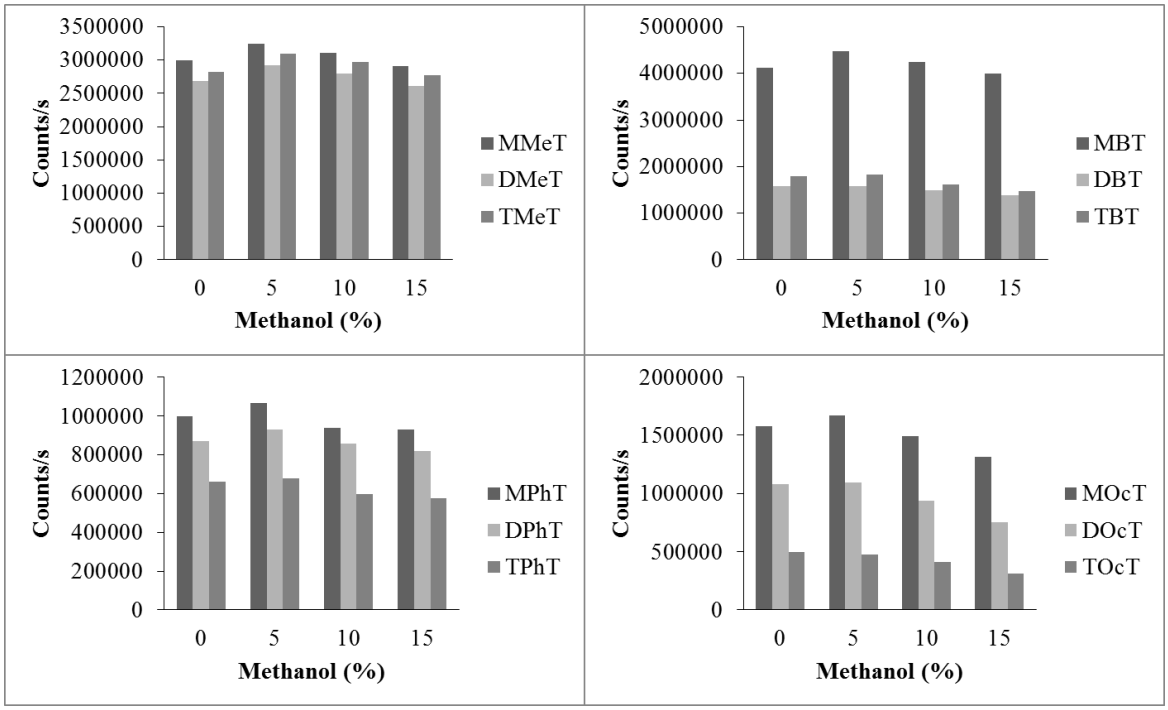

Figure 10: *Influence of methanol addition (%) as co-extracting agent on signals of OTCs in spiked (100 ng Sn)landfill leachate (Sample No. 3). Mechanical shaking (16 h), Tris-citrate buffer (pH 6.0), derivatisation with* $NaBEt_4$.

Data from these experiments indicate that addition of 5 % methanol as co-extracting agent is optimal for all OTCs investigated. Since there were reports in the literature (Mersiowsky et al., 2001) that application of 25 % KOH in methanol improved the extraction efficiency, 5 % methanol and 5 % methanol added along with 25 % KOH in methanol as co-extracting agents was applied prior to extraction of landfill leachate.

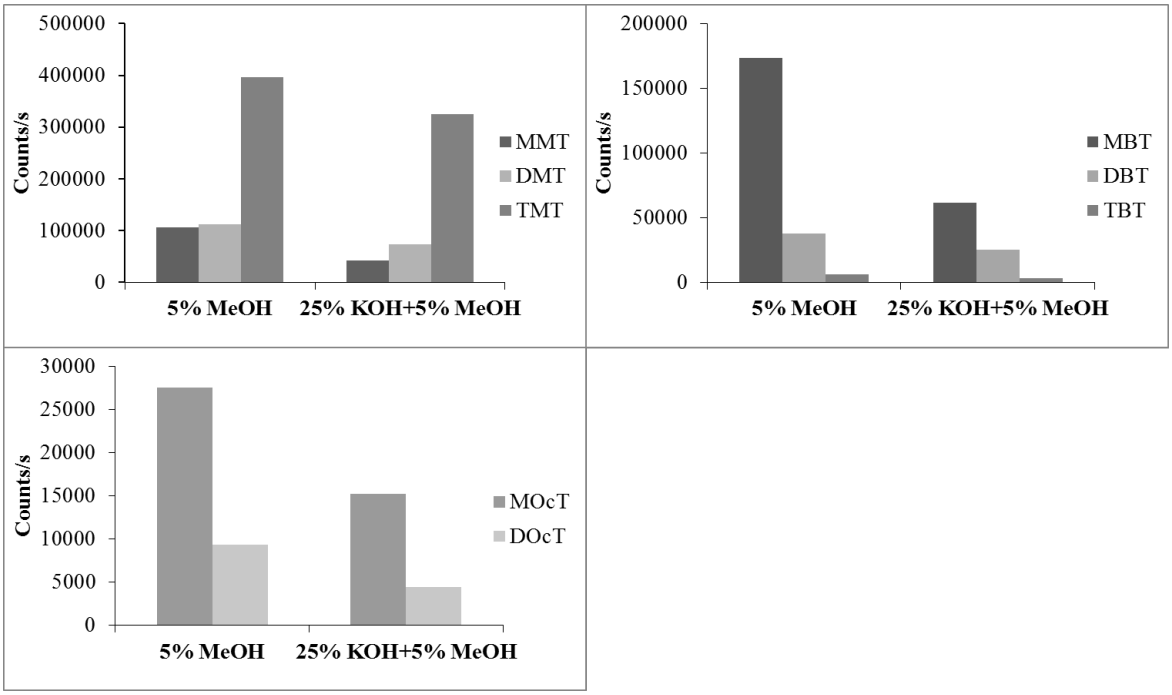

Figure 11: *Influence of 5 % methanol addition and 5 % methanol addition along with 25 % KOH in methanol as co-extracting agents on signals of OTCs in landfill leachate (Sample No. 3). Mechanical shaking (16 h), Tris-citrate buffer (pH 6.0), derivatisation with* $NaBEt_4$.

The outcomes of these experiments shown in Fig. 11 demonstrated that addition of 25 % KOH in methanol has deleterious effect on signal intensities, most probably due to degradation of OTCs at high pH values. Therefore, the addition of KOH was omitted.

To further improve the extraction efficiency different modes of extraction as presented in Fig. 12 were applied.

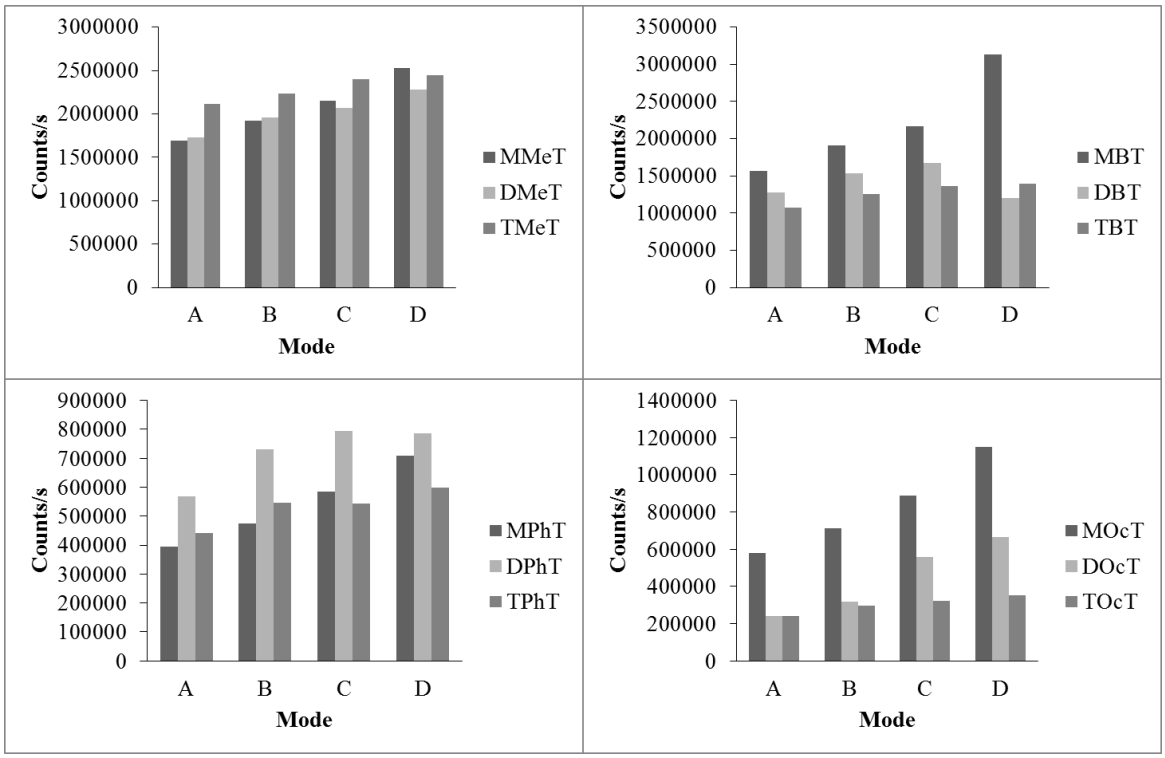

Figure 12: *Influence of different modes of extraction on OTCs signals of spiked (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking, Tris-citrate buffer (pH 6.0), derivatisation with* $NaBEt_4$.

Legend:

Mode A: Buffer, ethylation reagent and hexane were added to the sample. Mixture was then shaken at 300 rpm for 16 hours.

Mode B: 5 % methanol, buffer, ethylation reagent and hexane were added to the sample. Mixture was then shaken at 300 rpm for 16 hours.

Mode C: 5 % methanol and buffer were added to the sample and the mixture was shaken at 300 rpm for 2 hours. After that ethylation reagent and hexane were added and the mixture was shaken for additional 16 hours.

Mode D: 5 % methanol was added to sample and the mixture was shaken at 300 rpm for 2 hours. After that buffer, ethylation reagent and hexane were added and the mixture was shaken at 300 rpm for additional 16 hours.

It is evident that co-extraction with methanol is the most effective, when 5 % methanol is first added to the sample and mixture shaken for 2 hours, followed by the simultaneous addition of Tris-citrate buffer (pH 6.0), derivatisation reagent and hexane and application of mechanical shaking for next 16 hours. This positive effect on signals of OTCs is the most pronounced for monosubstituted OTCs, in particular for MBT and MOcT (Fig. 12, Mode D). So, the latter mode of extraction was used in the continuation of the experiments.

4.2.1.4 Optimization of pH of extraction buffer

In the analysis of OTCs the pH of extraction contributes to the derivatisation efficiency (Cai et al., 1994). To optimize the pH of extraction landfill leachate was spiked with methyl-, butyl-, phenyl- and octyltins (100 ng Sn). 5 % methanol was added to spiked sample and the mixture was shaken at 300 rpm for 2 hours. After that Tris-citrate buffer (pH from 3 to 8), alkylation reagent and hexane were added and the mixture was shaken at 300 rpm for additional 16 hours (Fig. 12, Mode D). As alkylation reagents $NaBEt_4$ and $NaBPr_4$ were used. It was found experimentally that the overall extraction efficiency for both alkylation reagents was poor within the pH range of the extraction buffer from 3 to 4 (due to decomposition of the alkylation reagents) and from 7 to 8 (due to formation of hydroxo species). So, the pH range between 4.5 and 6.5 was carefully investigated. The data of these experiments for ethylated and propylated OTCs are shown in Fig. 13.

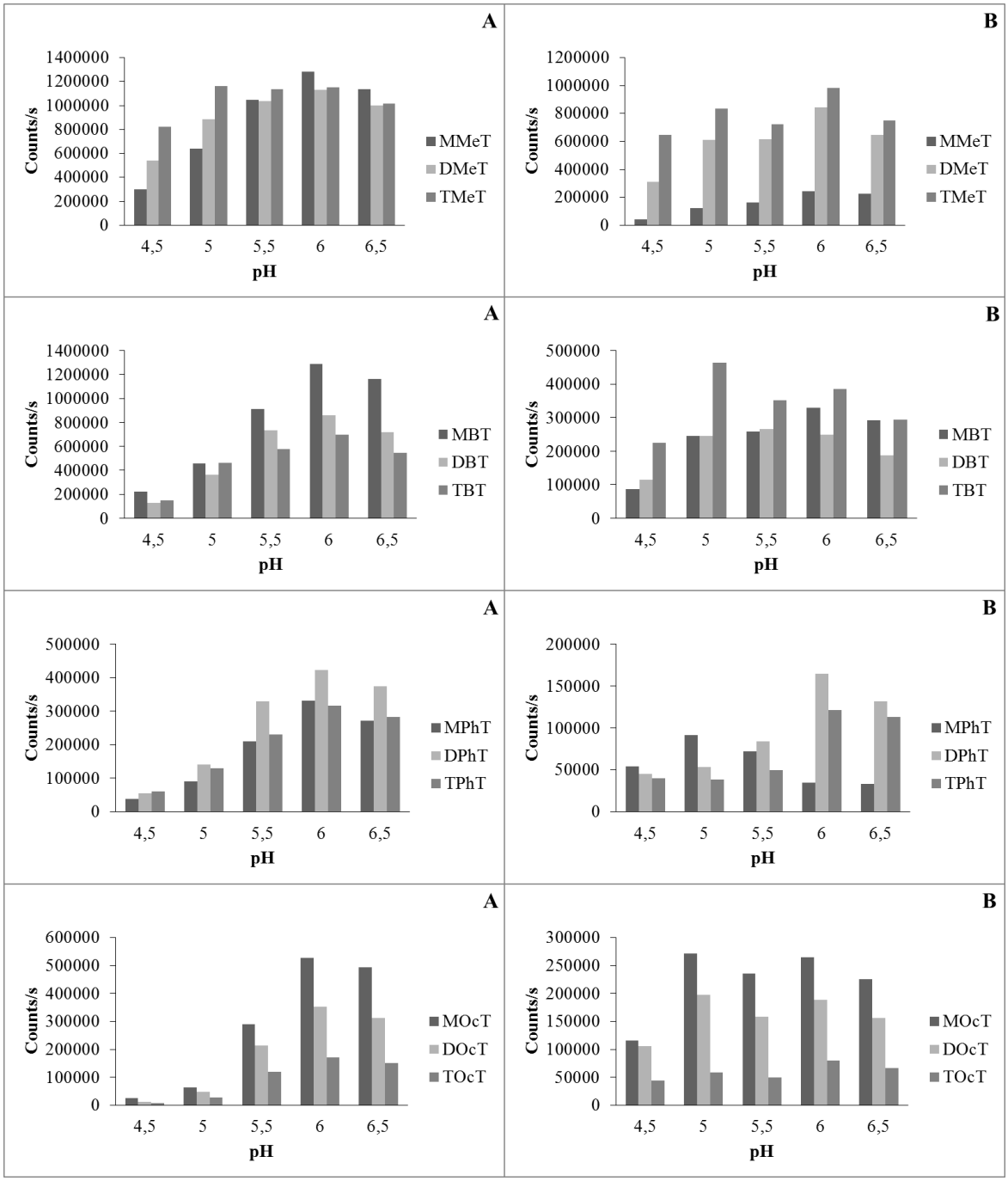

Figure 13: *Variation of OTCs signals for (A) ethylated and (B) propylated OTCs in spiked (100 ng Sn) landfill leachate (Sample No. 3) with pH of Tris-citrate buffer. Mechanical shaking (16 h, mode D as presented in Fig.12).*

It is evident from Fig. 13 that extraction with buffer having pH 6.0 is the best compromise for the derivatisation of all OTCs investigated. Maximal signal intensities are in general observed both for propylation and ethylation derivatisation procedures. Therefore, the pH of buffer was adjusted to 6 in the following experiments.

4.2.1.5 Comparison of extraction efficiencies using Tris-citrate and acetate buffers

The most commonly used buffer in derivatisation of ionic OTCs is acetic acid – acetate buffer with optimal pH of derivatisation 4.8 (Heroult et al., 2008; R. Morabito, 2000; Nemanic et al., 2007; Oliveira et al., 2010; Scancar et al., 2007; Zuliani et al., 2010; Zuliani et al., 2006; Zuliani et al., 2008). However, as was reported in the literature, optimal pH can be quite higher, due to the complex sample matrix that can change prevalent mechanism of alkylation, especially in the presence of high carbon content and high ionic strength (Cai et al., 1994). In order to demonstrate that Tris-citrate buffer facilitates the solubilisation of OTCs from complex landfill leachate matrix, samples were spiked with methyl-, butyl-, phenyl- and octyltins (100 ng Sn) and the ethylation extraction procedure was applied at optimal conditions using Tris-citrate (pH 6.0) and acetate (pH 4.8) buffers. The results of these experiments presented in Fig. 14 for all OTCs investigated were higher when Tris-citrate buffer was applied in the extraction procedure.

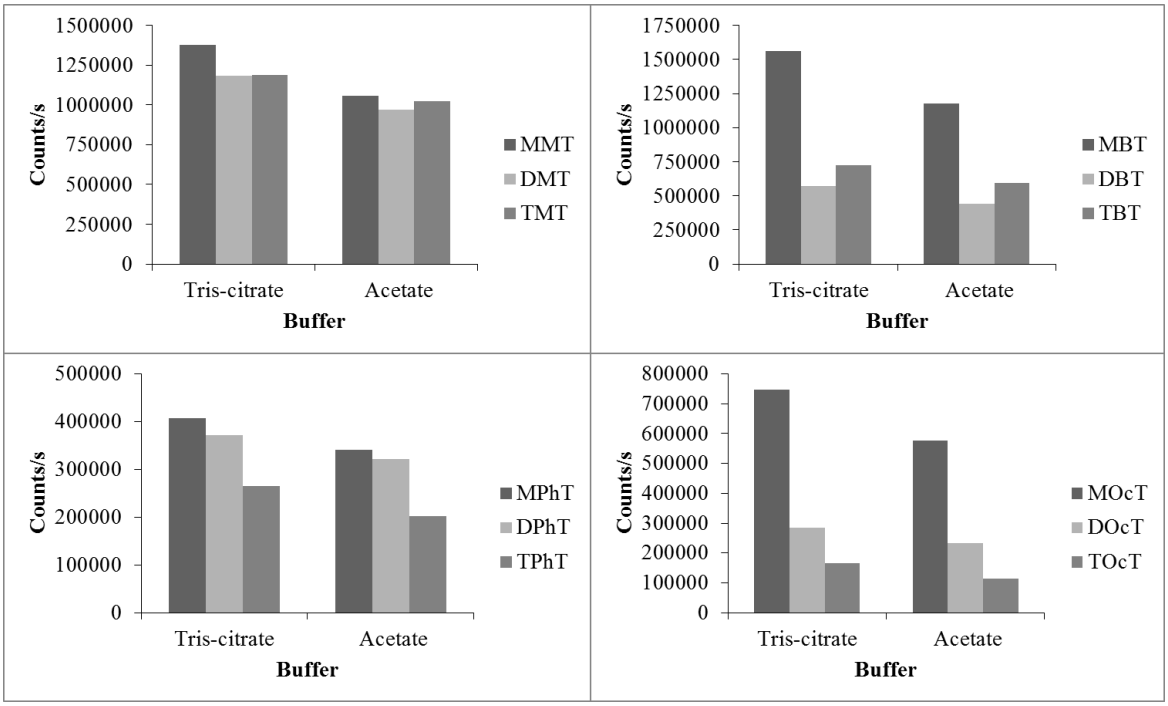

Figure 14: *Comparison of OTCs signals obtained for Tris-citrate buffer (pH 6.0) and acetate buffer (pH 4.8) for ethylated OTCs in spiked (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking (16 h, mode D as presented in Fig.12).*

The same positive effect of Tris-citrate in comparison to acetate buffer was observed in the unspiked landfill leachate sample. Based on outcomes of our experiments the use of Tris-citrate buffer in the extraction procedure is highly recommended in complex sample matrices such as landfill leachates.

4.2.2 Concentration of the alkylation reagent used for derivatisation

Mersiowsky et al. used large amounts of $NaBEt_4$ (3 mL of 20 % (m/V) $NaBEt_4$, sample volume 100 mL) for derivatisation of OTCs from landfill leachates. To check the concentration of the alkylation reagent necessary for quantitative derivatisation of OTCs in landfill leachates, the same amounts of $NaBEt_4$ and/or $NaBPr_4$ as reported by Mersiowsky et al. were added to sample extracts and OTCs signals compared to the addition of the amount of alkylation reagents recommended in this work (2 mL of 2 % (m/V) $NaBEt_4$ or $NaBPr_4$)(Mersiowsky et al., 2001). Experimental data demonstrated that 2 mL of 2 % (m/V) $NaBEt_4$ or $NaBPr_4$ added to the 200 mL of such (sample) extracts ensured quantitative derivatisation of OTCs in the landfill leachates. The later amount of alkylation reagents was sufficient to compensate also for the consumption of the reagent by other species present in the sample (e.g. sulfur, mercury, lead).

4.2.3 The performance of analytical procedure for determination of OTCs in landfill leachates

The performances of analytical procedure (Tris-citrate buffer pH 6, ethylation by $NaBEt_4$ and propylation by $NaBPr_4$, extraction into hexane, separation on 15 m GC column and ICP-MS detection) for simultaneous determination of OTCs in landfill leachate were evaluated. To estimate the linearity of the calibration curves landfill leachate was spiked with mixtures of calibration standards containing methyl-, butyl-, phenyl- and octyltins in concentration range 0.5 to 100 ng Sn L^{-1}. The limits of detection (LODs) and limits of quantification (LOQs) were calculated according to procedures based on EPA document 40 CFR 136 (Sivakumar et. al., 2006) and LOD Guidance Document PUBL-TS-056-96. LODs were determined on the basis of the standard deviations of spiked landfill leachate sample with methyl-, butyl-, phenyl- and octyltins as well as triethyltin (TEtT) (20 ng Sn), measured in eight replicates. To calculate LODs the Student's t-value was multiplied by standard deviations of spiked sample. LOQs were calculated as ten times the standard deviation (10*s*) of spiked sample. Repeatability of measurements was tested for six consecutive determinations of spiked landfill leachate (20 ng Sn) analyzed on the same day, while the reproducibility of measurements was tested after two days for the spiked sample by six consecutive OTCs determinations. The results of the performances of analytical methods are presented in Table 5 for ethylation and in Table 6 for propylation.

Table 5: *LODs, LOQs, correlation coefficients, repeatability and reproducibility of analytical method for simultaneous determination of methyl-, butyl- phenyl- and octyltins in landfill leachate by GC-ICP-MS under the optimal analytical procedure by the use of $NaBET_4$ for derivatisation.*

OTC	LOD (ng Sn L^{-1})	LOQ (ng Sn L^{-1})	r^2	Repeatability RSD (%)	Reproducibility RSD (%)
MMeT	2.2	7.3	0.9978	1.3	1.5
DMeT	0.9	3.0	0.9994	1.3	1.5
TMeT	2.2	7.2	0.9989	1.6	1.7
MBT	0.9	2.9	0.9989	0.9	2.8
DBT	0.7	2.4	0.9992	1.2	5.7
TBT	1.0	3.4	0.9987	3.4	5.2
MPhT	0.1	1.5	0.9955	1.6	1.5
DPhT	0.2	3.0	0.9995	3.1	6.6
TPhT	0.2	4.6	0.9993	5.4	7.9
MOcT	1.0	3.2	0.9996	2.1	3.3
DOcT	0.8	2.7	0.9994	2.0	6.9
TOcT	0.5	1.9	0.9994	1.9	6.3

Table 6: *LODs, LOQs, correlation coefficients, repeatability and reproducibility of analytical method for simultaneous determination of methyl-, butyl- phenyl- and octyltins as well as TEtT in landfill leachate by GC-ICP-MS under the optimal analytical procedure by the use of $NaBPr_4$ for derivatisation.*

OTC	LOD (ng Sn L^{-1})	LOQ (ng Sn L^{-1})	r^2	Repeatability RSD (%)	Reproducibility RSD (%)
MMeT	1.7	5.7	0.9944	2.6	6.9
DMeT	1.9	6.2	0.9964	2.4	5.3
TMeT	2.8	9.4	0.9908	1.6	3.4
TEtT	1.0	3.3	0.9905	2.5	3.0
MBT	3.5	11.6	0.9915	3.9	8.4
DBT	3.1	10.2	0.9910	4.1	6.9
TBT	2.3	7.6	0.9861	5.4	7.7
MPhT	2.8	9.2	0.9979	6.3	10.4
DPhT	1.1	3.7	0.9947	4.6	10.8
TPhT	0.8	2.6	0.9995	2.9	8.9
MOcT	2.1	7.1	0.9954	3.8	8.0
DOcT	2.8	9.4	0.9876	6.4	6.7
TOcT	2.4	8.0	0.9921	7.3	7.7

Data from Tables 5 and 6 indicate that performances of analytical methods are slightly better when $NaBEt_4$ was used for derivatisation. However, the use of $NaBPr_4$ enables also determination of ethyltins in landfill leachates. Since only TEtT is commercially available as standard, data for diethyltin (DEtT) and monoethyltin (MEtT) are not reported. LODs from Tables 2 and 3 are approximately 100 times lower than reported by Mersiowsky et al. (Mersiowsky et al., 2001) and 10 to

40 times lower than those obtained by Pinel-Raffaitin (Pinel-Raffaitin et. al., 2007). It is further evident from Tables 5 and 6 that correlation coefficients for calibration curves (r^2) are better than 0.995 for ethylation and in general better than 0.990 for propylation. Considering the complex matrix of landfill leachate the repeatability of measurement for OTCs analyzed is very good for ethylation (RSD around 2 %) and propylation (RSD around 5 %), while the reproducibility of measurement is slightly worse.

4.2.4 Quantification of OTCs in landfill leachates and the accuracy check

Quantification of OTCs was performed by applying standard addition calibration. In order to minimize uncertainty of chromatographic separation, ethylation yields and extraction efficiency, internal standards were used to normalize chromatographic peaks of OTCs. Thus TPrT (20 ng Sn) and TeBuT (20 ng Sn) were used as internal standards for ethylation and propylation respectively. Since there is no certified reference material for landfill leachates available, spike recovery test was used for the evaluation of the accuracy of the analytical procedures. For this purpose landfill leachate was spiked with methyl-, butyl-, phenyl- and octyltins (20 ng Sn) using $NaBEt_4$ and $NaBPr_4$ for derivatisation and samples analyzed by recommended analytical procedure described in section 3.5.2. Analyses were made in six replicates. Recoveries of spiked target compounds in samples ranged from 89 % to 107 % for methyl-tins, from 90 % to 105 % for butly-tins, from 81 % to 121 % for phenyl-tins, and from 97 % to 117 % for octyltins for ethylation and propylation procedures, respectively. From the results of the recovery tests, it may be concluded that analytical procedure developed was suitable for its intended use in landfill leachates, samples with extremely complex matrix.

4.2.4.1 Analysis of landfill leachate samples

In order to assess the applicability of the analytical method developed, concentrations of OTCs in landfill leachates from municipal non-hazardous waste Landfill Barje, Ljubljana, Slovenia, were determined according to the analytical procedure (3.5.2). To check the presence of ethyltins in samples analyzed, propylation was used for the derivatisation of OTCs. There were no measurable concentrations of ethyltins detected. Therefore, the ethylation was applied for the derivatisation in the analysis of OTCs, since the repeatability and reproducibility of measurements were found to be better (Tables 5 and 6). The results of the analysis of landfill leachates are presented in Table 7, while the corresponding chromatogram for Sample No. 2 is shown in Fig. 15.

Table 7: *Concentrations of OTCs* (methyl-, butyl- and octyltins) in landfill leachates from municipal non-hazardous waste Landfill Barje, (ng Sn L-1).*

Sample No.	MMT (ng Sn L^{-1})	DMT (ng Sn L^{-1})	TMT (ng Sn L^{-1})	MBT (ng Sn L^{-1})	DBT (ng Sn L^{-1})	TBT (ng Sn L^{-1})	MOcT (ng Sn L^{-1})	DOcT (ng Sn L^{-1})
1	156	43	121	124	29	5	30	11
2	128	158	340	132	72	21	41	31
3	229	69	160	109	33	4	35	24

*Results represent the mean value of two parallel determinations of particular OTC with deviation of measurements (± 3-6 %)

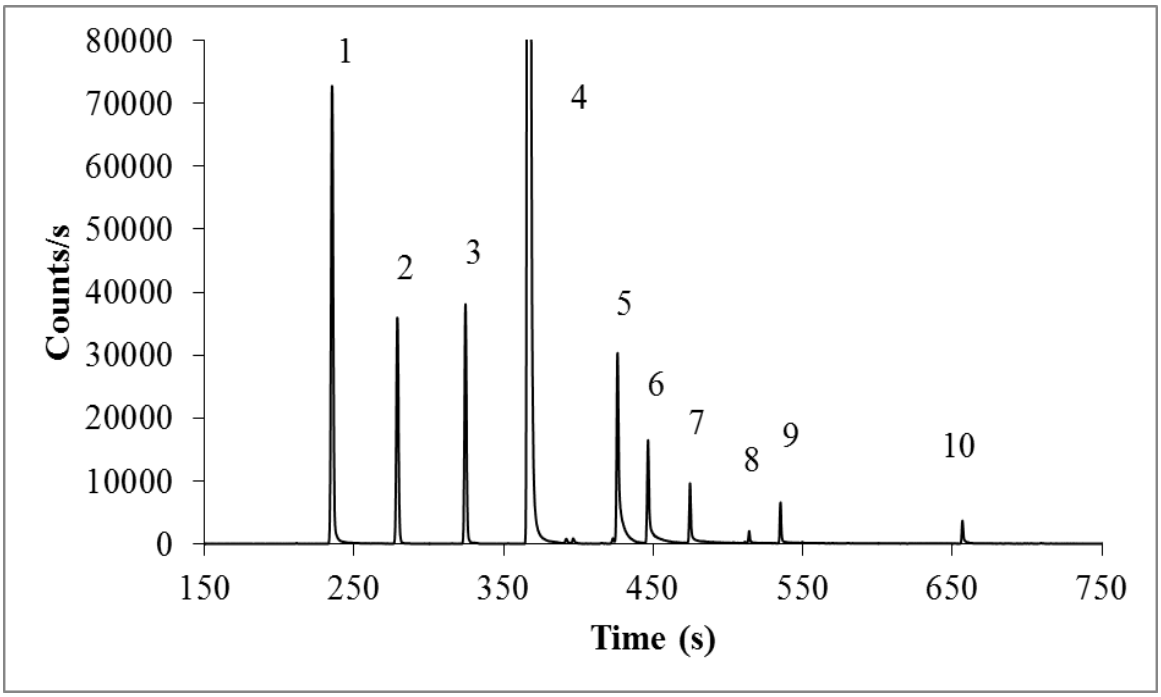

Figure 15: *Chromatograph of OTCs in landfill leachate (Sample No. 2) (Tris-citrate buffer, pH 6.0, derivatisation with $NaBEt_4$, mechanical shaking (16 h, mode D as presented in Fig.12).*
Legend:
1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn, 5 – MBT, 6 – TPrT, 7 – DBT, 8 – TBT, 9 – MOcT, 10 – DOcT

It is evident that on sampling sites investigated methyltins were present in the highest concentrations, followed by the concentrations of butyltins and octyltins. In addition to ethyltins, TOcT and phenyltins were not detected. Concentrations of OTCs determined were the highest in the active landfill basin (Sample No. 2), followed by the concentrations in the joint basin, collecting the leachates from the entire landfill (Sample No. 3) and the lowest in the abandoned, non active landfill basin (Sample No. 1), Table 8. Data further indicate on the highest degree of degradation of OTCs in the abandoned basin (MMT is higher than TMT, MBT is higher than TBT and MOcT is higher than DOcT). In comparison to the reported concentrations of OTCs (MMT, DMT, MBT, DBT, TBT, MOcT, DOcT) in landfill leachates in Sweden, Germany and Italy (Mersiowsky, et al., 2001), the amounts of OTCs determined in Landfill Barje were in general 10 to 20 times lower. Regarding the data reported for French landfills (Pinel-Raffaitin et al., 2007), where in addition to methyltins and butyltins, MEtT and DEtT were also detected, the concentrations of methyltins, MBT and DBT in Landfill Barje were either comparable or about 5 times lower, while the amounts of TBT were up to 50 times lower.

To evaluate the proportion of OTCs regarding the total Sn content, microwave assisted digestion of landfill leachates was performed and the concentrations of total Sn determined by ICP-MS. The results for total amount of Sn, sum of OTCs concentrations (as Sn) and the partitioning of particular OTC in landfill leachates are presented in Table 8.

Table 8: *Concentrations of total Sn* (ng L^{-1}) in landfill leachates from municipal non-hazardous waste Landfill Barje, determined by ICP-MS and concentrations of OTCs** (methyl-, butyl- and octyltins) determined by GC-ICP-MS (ng Sn L^{-1}) and their partitioning (%) in landfill leachates.*

Sample No.	Total concentration of Sn (ng L^{-1})	Total concentration of OTC (ng Sn L^{-1})	Total OTC (%)	MMT (%)	DMT (%)	TMT (%)	MMT (%)	MBT (%)	DBT (%)	TBT (%)	MOcT (%)	DOcT (%)
1	16000	520	3.2	0.98	0.27	0.75	0.98	0.77	0.18	0.03	0.19	0.07
2	9770	925	9.5	1.3	1.6	3.5	1.3	1.3	0.7	0.2	0.4	0.3
3	12020	660	5.5	1.9	0.6	1.3	1.9	0.9	0.3	0.0	0.3	0.2

*Results represent the mean value of two parallel determinations with deviation of measurements (± 2 %)

**Results represent the mean value of two parallel determinations of particular OTC with deviation of measurements (± 3-6 %)

It is evident that the highest concentration of total Sn (16000 ng L^{-1}) was present in the abandoned landfill basin (Sample No. 1), while the total OTCs content was the lowest (520 ng Sn L^{-1}), representing about 3 % of total Sn. On the contrary, the active landfill basin (Sample No. 2) contained the lowest total Sn concentration (about 10000 ng L^{-1}) and the highest content of OTCs (925 ng Sn L^{-1}), that represent 9.5 % of the total amount of Sn. Sample No. 3 from a joint basin, contained the middle concentrations of total Sn and OTCs. It is further evident that the highest proportion of OTCs represents methyltins (6.4, 3.8 and 2.0 %) for samples No. 2, 3 and 1, respectively. The proportion of total OTCs to total Sn content in samples from landfill Barje is comparable to samples from French landfills (Pinel-Raffaitin et al., 2007). Data of the present study demonstrated that the methyltins are the prevailing OTCs species on landfill Barje, representing 60 to 70 % of all OTCs.

5 Conclusions

In the presented doctoral dissertation, new analytical methods were developed with the aim to reliable and simultaneously determine 12 OTCs (methyl-, butyl-, phenyl- and octyltins) in fresh and salty water and landfill leachate samples.

The analytical method for simultaneous determination of 12 OTCs in fresh and salty water samples consists of the following steps: *in situ* derivatisation (by using $NaBEt_4$) of OTCs in water sample matrix adjusted to pH 6.0 with Tris-citrate buffer, extraction of ethylated OTCs into hexane, separation of OTCs in organic phase on 15 m GC column and quantitative determination of separated OTCs by ICP-MS.

In method development, the applicability of phosphate, carbonate and Tris-citrate buffer for the adjustment of pH of the derivatisation was critically evaluated. It was observed that in salty water phosphate and carbonate buffer formed hydroxo- precipitates at neutral and alkaline pH, and carbonate buffer CO_2 bubbles at acidic pH. No such problems were observed when Tris-citrate buffer was applied for pH adjustment in fresh or salty water samples. Thus, Tris-citrate buffer at pH 6.0, which was found to be optimal for ethylation of all OTCs determined, was recommended in derivatisation step of the developed analytical method. Results from our investigation also confirmed the comparability of Tris-citrate buffer to commonly used acetic acid-acetate buffer.

For extraction of ethylated OTCs from sample matrix iso-octane and hexane were used. Among OTCs extracted into iso-octane, only TMeT was not separated efficiently by GC. Separation of TMeT was improved by applying hexane, which also enabled selective separation of all other OTCs. Despite of relatively high volatility of hexane signals remain stable and reproducible during determination by GC-ICP-MS over time period of 48 hours.

The use of GC column of 15 m in length considerably shortened the time of analysis by GC-ICP-MS and enables larger series of water samples to be analysed. For example, the time of analysis of 12 OTCs in 60 water samples by GC-ICP-MS is shortened by 6 hours, representing positive economical effects.

Method was successfully applied for the analyses of marine water samples from the Northern Adriatic Sea. Results demonstrated that sea water from this area is in general contaminated with methyl- and butyl-tin compounds.

The development of the analytical method for simultaneous determination of 12 OTCs in landfill leachate samples partly based on the results that were obtained in the development of the method for the determination of OTCs in environmental water samples (described above). It consists of the following steps: co-extraction of OTCs with 5 % methanol, *in situ* derivatisation of OTCs in landfill leachate matrix adjusted to pH 6.0 with Tris-citrate buffer, extraction of derivatised (ethylated or propylated) OTCs into hexane, separation of OTCs in organic phase on 15 m GC column and quantitative determination of separated OTCs by ICP-MS, applying standard addition calibration method.

The use of 5 % methanol as a co-extraction reagent facilitated the solubilisation of OTCs and was more efficient than the application of 25 % KOH in methanol, proposed

by other investigators.

$NaBEt_4$ was found to be better derivatisation reagent in complex matrix of landfill leachates than $NaBPr_4$, since the latter exhibited lower derivatisation efficiencies and poorer peak resolution for phenyltins. The use of $NaBPr_4$ is mandatory, when ethyltin compounds are analysed.

Tris-citrate buffer adjusted to pH 6.0 enables more efficient extraction and *in situ* derivatisation of OTCs from complex matricies of landfill leachates in comparison to commonly applied acetic acid-

acetate buffer at pH 4.8.

In comparison to the literature data, the LODs for OTCs investigated are 10 to 100 times lower for the developed method, than those obtained by other investigators. One of the reasons for that is higher pre-concentration factor due to larger sample volume used in extraction procedure.

The simplicity, high sensitivity, rapidness and reliability are the main advantages of the developed analytical method in comparison to those that use cleaning of the organic phase through specially prepared silica gel column.

Method was successfully applied for the analyses of landfill leachates from municipal non-hazardous waste Landfill Barje, Ljubljana, Slovenia. Results indicated that leachates contained methyl-, butyl-, and octyltins in concentrations that were lower than those reported from different European municipal landfills.

The developed analytical procedures represent important progress in the field of OTCs speciation. They enable reliable, selective, sensitive and fast simultaneous determination of OTCs in fresh and marine water and landfill leachate samples and can be applied also for routine analyses.

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Appendix 1: Publications

Scientific paper: Rapid and sensitive analytical method for monitoring of 12 organotin compounds in natural waters

Scientific paper: Development of analytical procedure for the determination of methyltin, butyltin, phenyltin and octyltin compounds in landfill leachates by GC-ICP-MS – accepted, in press

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Technical paper

Rapid and Sensitive Analytical Method for Monitoring of 12 Organotin Compounds in Natural Waters

Mitja Vahčič, Radmila Milačič and Janez Ščančar*

Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

** Corresponding author: E-mail: janez.scancar@ijs.si*
Phone: + 386 1 477 3846, Fax: + 386 1 251 9385

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Abstract

A rapid analytical method for the simultaneous determination of 12 different organotin compounds (OTC): methyl-, butyl-, phenyl- and octyl-tins in natural water samples was developed. It comprises of in situ derivatisation (by using $NaBEt_4$) of OTC in salty or fresh water sample matrix adjusted to pH 6 with Tris-citrate buffer, extraction of ethylated OTC into hexane, separation of OTC in organic phase on 15 m GC column and subsequent quantitative determination of separated OTC by ICP-MS. To optimise the pH of ethylation, phosphate, carbonate and Tris-citrate buffer were investigated alternatively to commonly applied sodium acetate – acetic acid buffer. The ethylation yields in Tris-citrate buffer were found to be better for TBT, MOcT and DOcT in comparison to commonly used acetate buffer. Iso-octane and hexane were examined as organic phase for extraction of ethylated OTC. The advantage of hexane was in its ability for quantitative determination of TMeT. GC column of 15 m in length was used for separation of studied OTC under the optimised separation conditions and its performances compared to 30 m column. The analytical method developed enables sensitive simultaneous determination of 12 different OTC and appreciably shortened analysis time in larger series of water samples. LOD's obtained for the newly developed method ranged from 0.05–0.06 ng Sn L^{-1} for methyl-, 0.11–0.45 ng Sn L^{-1} for butyl-, 0.11–0.16 ng Sn L^{-1} for phenyl-, and 0.07–0.10 ng Sn L^{-1} for octyl-tins. By applying the developed analytical method, marine water samples from the Northern Adriatic Sea containing mainly butyl- and methyl-tin species were analysed to confirm the proposed method's applicability.

Keywords: Organotin compounds, simultaneous determination, gas chromatography-inductively coupled plasma mass spectrometry, larger series of natural water samples

1. Introduction

Organotin compounds (OTC) made their mark in 1950s first as stabilizers for polyvinyl chloride (PVC) polymers and a decade latter as highly effective biocides in antifouling paint formulations.[1] Today, other major industrial uses of OTC include production of polyurethane foam, mono- and polyesters, agrochemicals, electro and glass coatings, etc. Due to their intensive industrial and commercial use, OTC are today present globally in the environment. They bio-accumulate in living organisms and are for them toxic at extremely low concentration levels. Several defects of marine invertebrates caused by OTC at sub ng L^{-1} concentrations like imposex in dogwhelk, oyster shell malformation and mussel larvae mortality have been observed. In mammals including humans, OTC are endocrine disruptors, neurotoxic, hepatotoxic and immunotoxic.[3–9] The most toxic forms of OTC are their tri-substituted forms: trimethyltin (TMT), tributyltin (TBT), triphenyltin (TPhT), and trioctyltin (TOcT). Toxicity decreases towards di- and mono-substituted OTC, while inorganic tin is non toxic.

The toxicity of OTC was first discovered in the early 1980s, when a severe decrease in the production of oysters in Arcachon Bay (France) was observed. As a consequence French authorities banned the use of antifouling paints containing butyl-tins on pleasure boats in 1982.[10] Monitoring data (1995–2001) on the presence of organotin compounds in marine sediments of the North-western Mediterranean Spanish Coast demonstrated that organotin regulations on the use of TBT-based antifouling paints have been effective in marinas, but revealed a significant

TBT contamination in commercial and fishing harbours.[11] European Commission prohibited the use of TBT containing antifouling paints on the hulls of boats of less than twenty-five meters and vessels of any length used predominantly on inland waters (Commission Directive, 2002).[12] According to the International Convention on the control of harmful anti-fouling systems on ships (AFS Convention, 2003),[13] from 1 January 2008 any OTC should be either removed from the surfaces of the ships, or efficient sealing should be performed to prevent OTC leaching into the water. TBT and DBT were also included to the list of priority pollutants in the field of water policy in the EU Water Framework Directive – integrated river basin management for Europe (Commission Directive, 2000).[14] Legislative ban on OTC has led to subsequent control of OTC in the environment at low level concentrations. The pollution of marine ecosystems still persists mainly because of long half lives of OTC in sediments and illegal use of OTC containing paints.[15–16]

Because of wide spectrum of commercial and industrial applications the use of OTC is still increasing and they will remain environmental problem for the foreseeable future. Therefore, the development of highly sensitive, selective and accurate analytical methods for the determination of OTC in environmental samples is of crucial importance.[17] Different analytical methods for determination of OTC in aquatic environments,[18,19] sediments,[20,21] mussels,[22,23] soil,[25,24] and sewage sludge[25,26] have already been developed. Trace levels of OTC in these samples were commonly determined by applying a hyphenation of selective separation technique, such as gas (GC)[27,28] or liquid chromatography (LC),[29] to sensitive element or species specific detection system, such as atomic absorption (AAS), atomic emission (AES), mass (MS) or inductively coupled-plasma mass (ICP-MS) spectrometry, and flame (FPD) or pulsed flame (PFPD) photometric detection. Analytical techniques based on GC separation are widely used, mostly because of a very good separation power of GC columns, commercial availability of instruments, and the possibilities to use highly developed detectors or hyphenation to various detection techniques. Prior to GC separation and post-column detection, extraction and derivatisation steps are necessary to transform ionic OTC into their volatile forms. The most commonly used extraction procedures include liquid – liquid,[18,19,30] solid-phase[20–26,28,30] and supercritical fluid extraction.[31] Derivatisation step can be accomplished by different methods, such as alkylation by Grignard reagents,[18,28] hydrogenation by sodium borohydride[28] or ethylation by sodium tetraethyl borate ($NaBEt_4$).[19–26,28,30,32]

The aim of this study was to develop a rapid analytical method for the simultaneous determination of 12 different OTC (methyl-, butyl-, phenyl- and octyl-tins) in larger series of water samples. For this purpose OTC were in situ derivatised with $NaBEt_4$, then extracted into organic phase and in final step determined by GC coupled to ICP-MS. The applicability of phosphate, carbonate and Tris-citrate buffer for the adjustment of pH for derivatisation of OTC in fresh and seawater samples was systematically investigated and compared to commonly applied sodium acetate – acetic acid buffer. Liquid – liquid extraction of ethylated OTC into iso-octane or hexane followed. GC column of 15 m in length was used for the first time for separation of OTC and its performances compared to that of standard 30 m. Finally, OTC were determined in the sea water samples from the Northern Adriatic Sea (Gulf of Trieste) by applying the newly developed GC-ICP-MS analytical method.

2. Experimental

2. 1. Instrumentation

The determination of OTC was carried out on an Agilent 6890 gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, USA) equipped with Agilent 6890 Series Autosampler Injector that was coupled to Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan) via heated transfer line and fitted with 30 m × 0.25 mm or 15 m × 0.25 mm DB-5MS capillary column (film thickness 0.25 μm) coated with 5% phenyl-methylpolysiloxane (Agilent J&W Scientific, Palo Alto, CA, USA). Control and operation of the coupled system was achieved by using Agilent ChemStation Software.

For the separation of OTC on 15 m column the following GC temperature program was applied: at the start the column temperature was held at 50 °C for 0.8 min, then raised to 200 °C at heating rate of 20 °C min^{-1} and held there for 2 min, then raised to 220 °C at heating rate of 40 °C min^{-1} and held there for 0.5 min and, in final step, raised to 280 °C at heating rate of 50 °C min^{-1} and held at this temperature for 2 minutes. The temperature program of GC separation on 30 m column was as follows: at the start the column temperature was held at 60 °C for one minute, then raised to 180 °C at heating rate of 18 °C min^{-1}, then raised to 280 °C at heating rate of 40 °C min^{-1} and held at final temperature for 6.5 minutes. In both separations, inlet temperature was held at 240 °C and transfer – line at 280 °C, helium at flow rate of 1 mL min^{-1} was used as carrier gas, injection mode was split-less and injection volume 1μL.

ICP-MS operated under conditions listed in Table 1.

Table 1. ICP-MS operating parameters

Parameter	Unit
RF power	960 W
Sample Depth	8.0 mm
Carrier Gas	0.69 L min^{-1}
Optional Gas (O_2)	5.5% (v/v in carrier gas)
Integration time per isotope	0.1 s
Isotopes measured	^{118}Sn and ^{120}Sn
Tune gas	100 ppm Xe in Ar
Total acquisition time	778 s

Mechanical shaking during the extraction procedure was performed on orbital shaker Vibromix 40 (Tehtnica, Železniki, Slovenia).

2. 2. Reagents and Materials

All reagents used were of analytical-reagent grade. Milli-Q water (18.2 MΩ) (Milipore, Bedford, MA, USA) was used for the preparation of all aqueous solutions. Monomethyltin trichloride ($MMTCl_3$, 98%), dimethyltin dichloride ($DMTCl_2$, 95%), and trimethyltin chloride (TMTCl, 99%), were purchased from Acros Organics, (New Jersey, NY, USA). Monobutyltin trichloride ($MBTCl_3$, 95%), tributyltin chloride (TBTCl, 96%), monophenyltin trichloride ($MPhTCl_3$, 98%), diphenyltin dichloride ($DPhTCl_2$, 96%) and triphenyltin chloride (TPhTCl, 97%) were purchased from Aldrich (Milwaukee, WI, USA).

Dibutyltin dichloride ($DBTCl_2$, 98%), tetrabutyltin (TeBuT), that was included in the speciation procedure as a standard which does not need alkylation, and tripropyltin chloride (TPrTCl, 98%) were obtained from Merck (Darmstadt, Germany). Monooctyltin trichloride ($MOcTCl_3$, 99%) and dioctyltin dichloride ($DOcTCl_2$, 99%) were purchased from LGC Promochem (Wesel, Germany) and trioctyltin chloride (TOcTCl, > 90%) from Fluka (Buchs, Switzerland). OTC standard stock solutions containing 1000 mg (expressed as Sn) L^{-1} were prepared in methanol. Stored in the dark at 4 °C, they were stable for 6 months. Working OTC standard solutions were prepared daily.

Iso-octane, hydrochloric acid, sodium chloride and citric acid monohydrate were obtained from Merck (Darmstadt, Germany). Acetic acid, nitric acid and anhydrous sodium acetate were purchased from Carlo Erba (Milan, Italy), hexane and methanol from J. T. Baker (Deventer, Holland), sodium tetraethyl borate ($NaBEt_4$, 98%) from Strem Chemicals (Newburyport, MA, USA), potassium di-hydrogen phosphate and di-potassium hydrogen phosphate from Riedel-de Haen (Seeize, Germany), and Tris (hydroxymethyl)aminomethane (Tris), sodium hydrogen carbonate and di-sodium carbonate from Kemika (Zagreb, Croatia). An aqueous solution of sodium tetraethyl borate ($NaBEt_4$) (2% (w/v)) was prepared just before derivatisation. All buffers were prepared daily.

2. 3. Analytical Procedure

A newly developed liquid – liquid extraction procedure was used prior to the determination of OTC in different water samples by GC-ICP-MS. Briefly, 300 mL aliquot of water sample (deionised water, salt water containing 3.8% NaCl, sea water from the Northern Adriatic Sea) was transferred into 500 mL dark glass reactor vessel along with 100 mL of selected 0.2 M buffer solution. Phosphate, carbonate and Tris-citrate buffers were used to optimise the pH of derivatisation of OTC in fresh or salty water samples. Their applicability was critically evaluated and compared to acetate buffer. Phosphate buffer was prepared from potassium di-hydrogen phosphate and di-potassium hydrogen phosphate salt in appropriate ratios to match pH range from 4.4 to 9.0, carbonate buffer from di-sodium carbonate and sodium hydrogen carbonate salts to match pH range from 4.0–10.0 (pH was adjusted with HCl), and Tris-citrate buffer from Tris and citric acid salts to match pH range from 3.0 to 10.0 (pH was adjusted with citric acid or ammonia). Sodium acetate – acetic acid buffer was prepared at pH of 4.8. All samples were spiked with internal standard solution TPrT, TeBuT and methyl-, butyl-, phenyl- and octyl-tin standard solutions in concentration range from 0.06 to 33.3 ng Sn L^{-1}. To spiked samples 0.5 mL of 2% (m/V) $NaBEt_4$ for derivatisation and 1 mL of iso-octane or hexane as an extraction agent for ethylated OTC species were added. Samples were mechanically shaken for 45 min and after that organic phase for analysis collected into 2 mL dark vials using Pasteur pipette. Blank samples were spiked only with internal standard (TPrT) and determined after applying the same analytical procedure as for samples. All the analyses were made in triplicate.

2. 4. Cleaning Procedure

To avoid contamination all glassware were rinsed three times with tap water, soaked in 20% nitric acid for 48 hours, rinsed three times with tap water, three times with deionised water and heated at 400 °C for at least 4 hours.

3. Results and Discussion

3. 1. Optimisation of Derivatisation

For "in situ" derivatisation of ionic OTC in water samples by $NaBEt_4$, pH that was adjusted to around 5 with sodium acetate-acetic acid buffer was frequently chosen as optimal. In these conditions, the yield of ethylation of OTC depends on the degree of substitution and the nature of the alkyl groups linked to the tin atom.[33] However, as was found reported in the literature, optimal pH can be quite higher than this value, due to the sample matrix that can retard ethylation rate, and pH effect on stability of $NaBEt_4$, which decomposed more rapidly at lower pH.[33] Therefore, in the present study, optimisation of pH for "in situ" ethylation of ionic methyl-, butyl-, phenyl- and octyl-tin compounds was first performed in Milli Q water at pH ranging from 4 to 10. For this purpose, phosphate, carbonate, and Tris-citrate buffers were chosen as an alternative to commonly used acetate buffer. Ethylated OTC were extracted into iso-octane and determined by GC-ICP-MS as described previously in section 2.3. For separation 15 m GC column was used. Experimental results of

this preliminary study demonstrated that for all buffers investigated optimal pH of ethylation lied between pH 5 and 7.5 (data not shown). This pH range was then studied more in details. The analytical signal intensities normalised to TPrT that correspond to ethylation yields of OTC in Milli Q water samples spiked with methyl-, buthyl-phenyl- and octyl-tin compounds (33.3 ng Sn L^{-1}) in pH range from 5 to 7.5 are shown in Figures 1–4. It was experimentally proven that normalisation to TeBuT gave similar results.

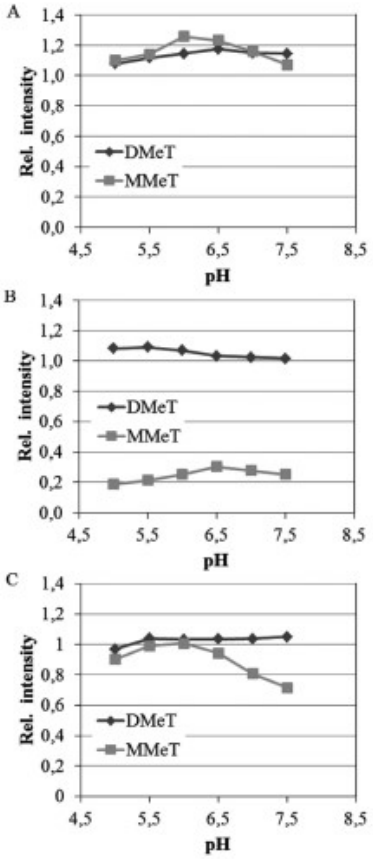


Figure 1. Effect of pH on the relative analytical signal intensities of spiked methyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

Results of optimisation of pH for ethylation of methyl-tin compounds (Figure 1) show that when carbonate buffer was used (Figure 1A), ethylation of monomethyl-tin (MMeT) and dimethyl-tin (DMeT) was optimal at around pH 6.0. Similarly, maximum ethylation yield for MMeT and DMeT was observed at around pH 6.0 when phosphate (Figure 1B) or Tris-citrate (Figure 1C) buffer was used for pH adjustment. Results for optimisation of ethylation pH are not shown for trimethyl-tin (TMeT) since TMeT was not separated quantitatively in

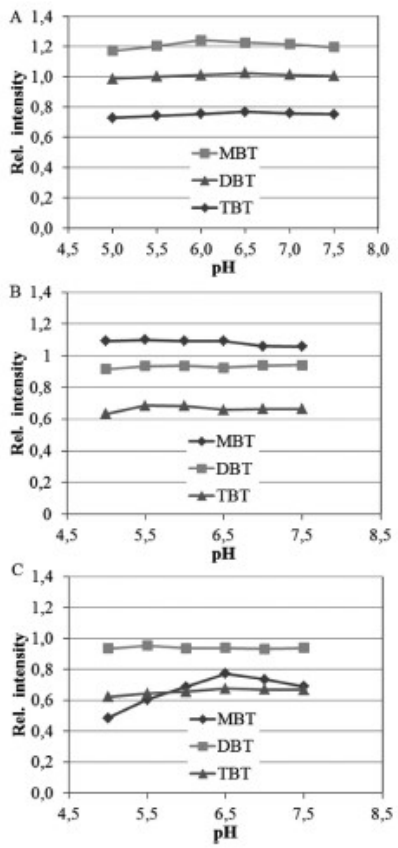


Figure 2. Effect of pH on the relative analytical signal intensities of spiked butyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

iso-octane which was used as organic phase for extraction of ethylated OTC.

In Figure 2 ethylation yields (as analytical signal intensities) for monobutyl-tin (MBT), dibutyl-tin (DBT) and tributyl-tin TBT are presented. When carbonate buffer was used (Figure 2A), maximum ethylation yield for all buty-tin compounds lied between pH 6.0 and 7.0. It is also evident that the ethylation of ionic butyl-tin compounds was almost constant over the whole investigated pH range. Similarly, more or less constant ethylation yield over the whole pH range investigated was obtained with the phosphate (Figure 2B) and Tris-citrate (Figure 2C) buffers.

Figure 3 presents ethylation yields of monophenyl-tin (MPhT), diphenyl-tin, (DPhT) and triphenyl-tin TPhT. As it can be seen from Figure 3A, when carbonate buffer was used for pH adjustment at pH below 7, ethylation of phenyl-tin compounds didn't depend significantly on pH while at higher pH ethylation yield for phenyl-tin started to decline. Applying phosphate buffer (Figure 3B), the

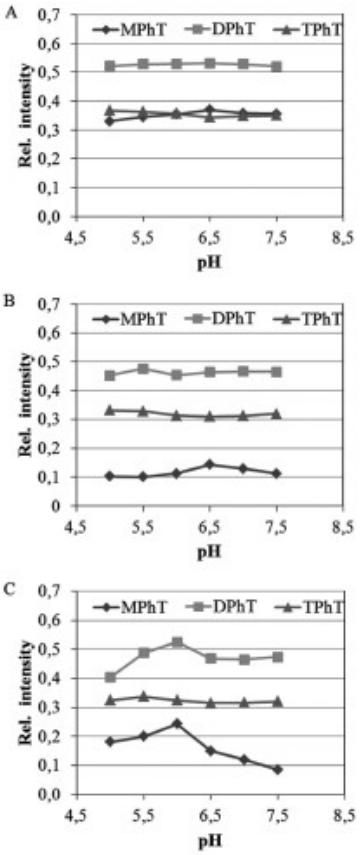


Figure 3. Effect of pH on the relative analytical signal intensities of spiked phenyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

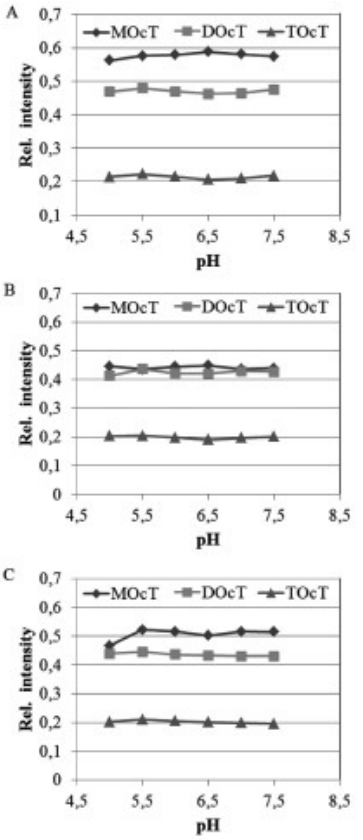


Figure 4. Effect of pH on the relative analytical signal intensities of spiked octyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

ethylation of MPhT and TPhT was constant in pH range between pH 5.0 and 8.0, and for DPhT optimal between pH 5.0 and 6.0. In Tris-citrate buffer (Figure 3C) maximum ethylation yield for MPhT and DPhT was observed at pH 6.0, while for TPhT it remained relatively constant over the whole pH range investigated.

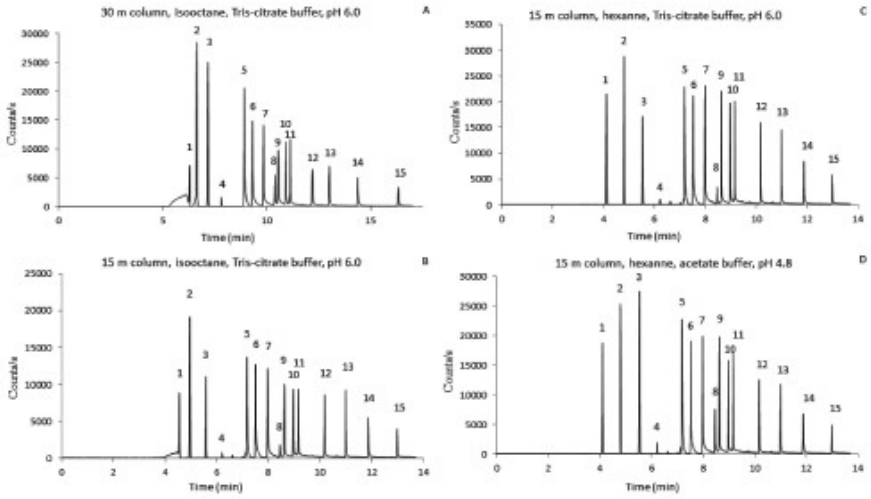
Results of optimisation of pH for ethylation of octyl-tin compounds are presented in Figure 4. In carbonate buffer (Figure 4A) ethylation of all tested octyl-tin compounds was optimal between pH 6.0 and 7.0. Similar were results when phosphate (Figure 4B) or Tris-citrate (Figure 4C) buffer was applied.

In general, experimental results has shown that the effect of pH on the ethylation of OTC tested was the most pronounced for methy-tin compounds and progressively decreased with the size of the alkyl- (butyl- and octyl-tin compounds) or aryl- (phenyl-tin compounds) functional groups. It should be also pointed out that above described studies were carried out in Milli Q water due to the fact that in salty water (artificial salt water with 3.8% NaCl or sea water) at pH higher than 6, as expected, visible precipitates in phosphate and carbonate buffers, and bubbles in carbonate buffer at pH lower than 4 have started to form. Precipitates or bubbles were not observed when Tris-citrate buffer was applied. Among buffers studied Tris-citrate buffer was found to be the most appropriate for adjustment of pH in different water samples. This buffer can be used for pH adjustment for ethylation of OTC in fresh and salty water samples in wide pH range. Experimental results also demonstrated that for particular OTC the optimal pH of ethylation slightly varies, but overall optimal ethylation yields for all OTC can be achieved at pH 6.0.

3. 2. Performances of GC Columns with Different Length, Applicability of Iso-octane and Hexane, and Comparison of Tris-citrate and Acetate Buffer

Survey of the relevant published literature indicates that GC columns of 30 m in length were mostly applied for the separation of derivatised OTC in various environmental samples. In Figure 5 signal intensities of spiked OTC (33.3 ng Sn L^{-1}) in water samples, extracted into iso-octane or hexane, separated on 15 m or 30 m GC columns, using Tris-citrate or acetate buffer, are shown.

In Figures 5A and 5B separation of 12 OTC (as well as TPrT, TeButT and inorganic Sn) in iso-octane on 30 and 15 m GC column, respectively are presented. Tris-citrate



1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn , 5 – MBT, 6 – TPrT, 7 – DBT, 8 – MPhT, 9 – TBT, 10 – MOcT, 11 – TeBuT, 12 – DPhT, 13 – DOcT, 14 – TPhT, 15 – TOcT

Figure 5. Separation efficiencies of methy-, butyl-, phenyl- and octy-tin compounds in (A) iso-octane on 30 m GC column, Tris-citrate buffer, pH 6.0, (B) iso-octane on 15 m GC column, Tris-citrate buffer, pH 6.0, (C) hexane on 15 m GC column, Tris-citrate buffer, pH 6.0 (D) hexane on 15 m GC column, acetate buffer, pH 4.8. Concentrations of OTC were 33.3 ng Sn L^{-1}.

buffer (pH 6.0) was used. It can be seen that, with the exception of TMeT, OTC investigated were selectively separated on 30 m column (Figure 5A), suggesting that shorter GC column (15 m) can also be used. GC conditions of the separation that were carefully optimised for both column lengths (30 m versus 15 m) are reported in the Instrumental section. From Figures 5A and 5B, it is further evident that by applying shorter GC column, the column efficiency is still good enough to resolve the peaks of the 12 OTC investigated. The main difference between both column lengths was found in the duration of the separation. On 15 m GC column the total separation time was 12.88 min and on 30 m column 18.65 min, which means about 30% reduction in separation time.

For selective separation of TMeT from solvent front, hexane which has lower boiling point (69 °C)[34] than isooctane (99 °C)[35] and hence elutes faster from GC column was applied (Figure 5C). In this experiment Tris-citrate buffer and 15 m GC column were used. It is evident from Figure 5C that TMeT and other OTC were selectively separated.

Comparison of Tris-citrate and acetate buffers at optimal pH (pH 6.0 and pH 4.8, respectively), using 15 m column and extraction into hexane for separation of 12 OTC (as well as TPrT, TeBuT and inorganic Sn) is presented in Figures 5C and 5D. It is evident that both buffers can be efficiently applied for adjustment of the pH of ethylation. The differences can be observed in signal intensities. They were higher for MMeT and MPhT when acetate buffer was used, while for all other OTC investigated, signal intensities were higher when Tris-citrate buffer was used.

3. 3. Signal Stability of OTC in Hexane

Due to high volatility of hexane, special attention must be paid to prevent its evaporation during the measurements. To prove that no loss of analytes occurred during the determination by GC-ICP-MS, the signal stability of 12 OTC investigated (ethylated at pH 6 using Tris-citrate buffer) was monitored. For this purpose, stability test with sixty subsequent determinations (15 consecutive parallel samples each in 4 replicates) was carried out on 15 m GC column. Results of this study are presented in Figure 6.

Each point on the chart represents an average of 4 consecutive measurements of the same sample. As it can be seen from the Figure 6, analytical signal was constant during the course of the determinations proving that there was no evaporation of hexane. Henceforth, hexane was used for extraction of ethylated OTC.

3. 4. Evaluation of the Analytical Method

The performances of analytical method (Tris-citrate buffer pH 6, ethylation by $NaEt_4B$, extraction into hexane, separation on 15 m GC column, and ICP-MS detection) for the simultaneous determination of 12 OTC were evaluated. The limits of detection (LOD) and limits of quantification (LOQ) were calculated by the analyses of six replicates of uncontaminated fresh and salty water samples as three times the standard deviation of the uncontaminated sample (3*s*) and LOQ as ten times the standard deviation of the uncontaminated sample (10*s*), respectively. Mixtures of calibration standards of OTC in the range from 0.6 to 33.3 ng Sn L^{-1} in organic phase were prepared

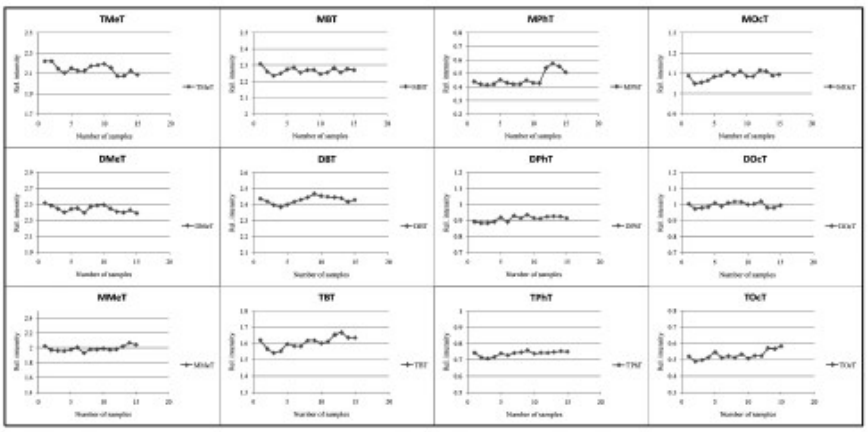


Figure 6. Stability of analytical signal (hexane used for extraction of ethylated OTC into organic phase, 15 m GC column, concentrations of OTC 33.3 ng Sn L^{-1}).

and calibration graphs obtained for methyl-tins (MMeT, DMeT, TMeT), butyl-tins (MBT, DBT, TBT), phenyl-tins (MPhT, DPhT and TPhT), and octyltins (MOcT, DOcT and TOcT). Repeatability of measurements was examined by 6 consecutive determinations of fresh or salty water sample with OTC concentration of 33.3 ng Sn L^{-1} in hexane. The reproducibility of measurement was checked by 6 consecutive determinations of the same fresh and salty water sample on two different days. The results obtained for LOD, LOQ, correlation coefficient, repeatability and reproducibility for salty water are listed in Table 2.

Table 2. LOD, LOQ, correlation coefficient, repeatability and reproducibility of measurements of analytical method for simultaneous determination of 12 OTC in salty water samples by GC-ICP-MS.

OTC	LOD (ng Sn L^{-1})	LOQ (ng Sn L^{-1})	r^2	Repeatability RSD (%)	Reproducibility RSD (%)
MMeT	0.05	0.17	0.9997	2.2	3.0
DMeT	0.06	0.20	0.9999	2.7	7.9
TMeT	0.05	0.17	0.9995	4.3	8.0
MBT	0.45	1.50	0.9999	1.1	1.9
DBT	0.11	0.37	0.9995	1.7	3.7
TBT	0.15	0.50	0.9964	4.8	6.5
MPhT	0.16	0.53	0.9984	14.7	4.8
DPhT	0.12	0.40	0.9996	5.2	2.3
TPhT	0.11	0.37	0.9994	3.2	1.9
MOcT	0.10	0.33	0.9989	3.7	3.0
DOcT	0.07	0.23	0.9996	3.0	3.9
TOcT	0.10	0.33	0.9992	7.2	3.5

Data from Table 2 indicate good linearity of measurement (regression coefficients for all OTC $r^2 > 0.9964$). Measurements were sensitive, (LOD for particular OTC ranged from 0.05 to 0.45 ng Sn L^{-1} and LOQ from 0.17 to 1.50 ng Sn L^{-1}) repeatable and reproducible (for 80% of measurements better than 5%). For fresh water sample almost the identical or even better performances of analytical method than for salty water were obtained.

3. 5. Quantification of OTC

Quantification of OTC was performed by applying standard addition calibration using 15.15 ng Sn L^{-1} of TPrT as an internal standard. Since no certified reference material exists for water samples, marine water with no measurable concentrations of determined OTC were spiked with known amounts of all 12 OTC. Calibration curves were prepared and samples analysed by applying developed analytical method (Tris-citrate buffer pH 6, ethylation by $NaEt_4B$, extraction into hexane, separation on 15 m GC column, and ICP-MS detection). Spike recovery test was performed at concentrations 10 and 20 ng Sn L^{-1} in samples to confirm the efficiency of the developed analytical method. Analysis was made in 6 replicates. Recoveries of spiked target compounds in samples ranged from 97% to 103% for methyl-tins, from 85% to 99%, for butly-tins, from 87% to 109% for phenyl-tins, and from 103% to 126% for octyltins. From the results it was concluded that analytical method developed was suitable for its intended use.

3. 6. Analysis of Marine water Samples

To assess the applicability of the developed analytical method the concentrations of OTC in marine water samples from the Northern Adriatic Sea were determined. Sampling was performed monthly at five different locations in time period from January to June 2009. In these samples in general MMeT, DMeT and butyl-tins were detected. The concentrations of all other OTC were below LOD of the applied analytical method. Results that show concentration ranges of of the commonly present OTC determined in marine water samples are presented in Table 3.

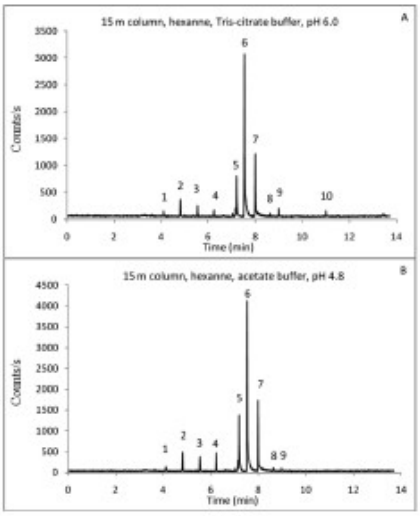
Table 3. Concentration ranges of the commonly present OTC in water samples from the Northern Adriatic Sea determined by GC-ICP-MS.

Sample No.	MMeT (ng Sn L^{-1})	DMeT (ng Sn L^{-1})	MBT (ng Sn L^{-1})	DBT (ng Sn L^{-1})	TBT (ng Sn L^{-1})
I	<0.05–5.8	0.6–6.0	<0.45–2.6	0.7–3.8	<0.15–0.3
II	<0.05–5.4	1.3–17.0	0.5–7.3	0.6–5.7	<0.15–0.3
III	1.8–7.3	2.7–17.4	<0.45–2.4	0.4–6.0	0.2–2.3
IV	<0.05–2.9	1.1–9.6	<0.45–1.4	0.5–3.3	<0.15–16.5
V	<0.05–34.2	1.4–16.9	<0.45–3.5	0.7–3.8	<0.15–1.6

For comparison of the performance of Tris-citrate buffer to most commonly applied acetate buffer, representative GC-ICP-MS chromatograms of the sample III are shown in Figure 7, while the results are presented in Table 4.

Table 4. Concentrations of OTC in marine water sample III from the Northern Adriatic Sea by GC-ICP-MS, using (A) Tris-citrate, pH 6.0 and (B) acetate, pH 4.8 buffers. Representative chromatograms are presented in Figure 7.

OTC compound	(A) Concentration (ng Sn L^{-1})	(B) Concentration (ng Sn L^{-1})
MMeT	0.19 ± 0.01	0.20 ± 0.01
DMeT	0.29 ± 0.02	0.30 ± 0.02
TMeT	0.20 ± 0.01	0.20 ± 0.01
MBT	1.9 ± 0.1	2.3 ± 0.1
DBT	2.5 ± 0.1	2.5 ± 0.1
TBT	0.19 ± 0.01	<0.15
MOcT	0.36 ± 0.2	<0.10
DOcT	0.41 ± 0.2	<0.07



1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn , 5 – MBT, 6 – TPrT, 7 – DBT, 8 – TBT, 9 – MOcT, 10 – DOcT

Figure 7. A representative GC-ICP-MS chromatogram of ethylated methy- and butyl-tin species determined in a marine water sample III: (A) hexane, 15 m GC column, Tris-citrate buffer, pH 6.0, (B) hexane, 15 m GC column, acetate buffer, pH 4.8.

It is evident from Figure 7 that the sensitivities of analytical signals are in general similar for both buffers applied. Data from Table 4 further demonstrate good agreement of results for MMeT, DMeT, TMeT, MBT and DBT that were quantified by the use of both buffers. For TBT, MOcT and DOcT Tris-citrate buffer exhibits better sensitivity. Namely, in acetate buffer the latter compounds were not quantified.

4. Conclusions

A new analytical method for simultaneous determination of 12 OTC (methyl-, butyl-, phenyl and octyltins) in fresh and salty water samples was developed. It consists of several steps: in situ derivatisation (by using $NaEt_4B$) of OTC in water sample matrix adjusted to pH 6.0 with Tris-citrate buffer, extraction of ethylated OTC into hexane, separation of OTC in organic phase on 15 m GC column and quantitative determination of separated OTC by ICP-MS.

The applicability of phosphate, carbonate and Tris-citrate buffer was critically compared for the adjustment of pH of the derivatisation. It was experimentally observed that in salty water phosphate and carbonate buffer formed hydroxo- precipitates at neutral and alkaline pH, and carbonate buffer CO_2 bubbles at acidic pH. No such problems were observed when Tris-citrate buffer was applied for pH adjustment in fresh or salty water samples. Thus, Tris-citrate buffer at pH 6.0, which was found to be optimal for ethylation of all OTC determined was recommended in derivatisation step of the developed analytical method. In addition, the ethylation yields in Tris-citrate buffer were better for TBT, MOcT and DOcT in comparison to commonly applied acetate buffer.

For extraction of ethylated OTC from sample matrix iso-octane and hexane were used. Among OTC extracted into iso-octane, TMeT was not separated efficiently by GC. Separation of TMeT was significantly improved by applying hexane, which enabled also selective separation of all other OTC. Despite of its relatively high volatility analytical signals remain stable and reproducible during determination by GC-ICP-MS over time period of 48 hours.

The use of GC column of 15 m in length considerably shortened the time of analysis by GC-ICP-MS and enables larger series of water samples to be analysed. This is of great importance in commercial monitoring analysis. For example, the time of analysis of OTC in 60 water samples by GC-ICP-MS is shortened by 6 hours, representing positive economical effects.

The developed analytical method enables reliable, selective, sensitive and faster simultaneous determination of 12 OTC in environmental water samples. It was applied for the analyses of marine water samples from the Northern Adriatic Sea. Results demonstrated that sea water from this area is in general contaminated with methyl- and butyl-tin compounds.

5. Acknowledgements

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Povzetek

Razvili smo hitro analizno metodo za simultano določitev 12 različnih organokositrovih spojin (OTC): metil-, butil-, fenill- in oktil-kositrovih spojin v različnih vzorcih vod. Metodo sestavljajo sledeči koraki: derivatizacija OTC v slanih ali površinskih vodah z $NaBEt_4$, uravnavanje pH na vrednost 6 s Tris-citratnim pufrom, ekstrakcija etiliranih OTC v heksan, ločba OTC v organski fazi na 15 m GC koloni in kvantitativna določitev ločenih OTC z ICP-MS. Alternativo, v praksi splošno uporabljenem acetatnem pufru, smo pri optimizaciji pH etilacije preučevali uporabo fosfatnega, karbonatnega in Tris-citratnega pufra. Za kvantitativno ekstrakcijo etiliranih OTC v organsko fazo smo preiskali uporabnost izo-oktana in heksana. Preučevane OTC smo ločili na 15 m GC koloni in ločbo primerjali s 30 m GC kolono. Razvita analizna metoda omogoča simultano določitev 12 različnih OTC in znatno skrajša čas analize v večjih serijah vzorcev vod. Uporabnost metode smo preizkusili z analizo vsebnosti OTC v vzorcih morskih vod severnega Jadrana. Rezultati so pokazali, da je morska voda severnega Jadrana onesnažena predvsem z butil- in metil-kositrovimi spojinami.

Development of analytical procedure for the determination of methyltin, butyltin, phenyltin and octyltin compounds in landfill leachates by GC-ICP-MS

Mitja Vahčič, Radmila Milačič, Janez Ščančar*
Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

*Corresponding author. Tel.: +386 1 4773846

E-mail address: janez.scancar@ijs.si (J. Scancar)

A B S T R A C T

Landfilling is the most common disposal of municipal waste. During the decomposition of different waste materials, several toxic compounds are leached. Although organotin compounds (OTC) represent an important group of pollutants in landfill leachates, there are only few analytical procedures reported for their analysis. These procedures are complex or recommend the use of enriched stable isotopes that are available only for butyltins. In the present work analytical procedure for simultaneous routine speciation analysis of methyl-, butyl-, phenyl- and octyl-tins in landfill leachates by GC-ICP-MS was developed. For this purpose the applicability of methanol as co-extraction reagent and Tris-citrate buffer for adjustment of pH for derivatization of OTC in landfill leachates was carefully investigated. The use of $NaBEt_4$ and $NaBPr_4$ as derivatization reagents for liquid-liquid extraction into hexane was critically evaluated. 15 m GC column was used for rapid separation of OTC. The developed analytical procedure was sensitive (LODs for OTC investigated in general better than 2 ng Sn L^{-1}) with good repeatability of measurement (RSDs mostly better than 3 %) and was successfully applied in the analysis of OTC in landfill leachates using standard addition calibration method. Due to its simplicity and reliability it is appropriate to be used in routine laboratories for monitoring of OTC in landfill leachates.

Keywords: methyltin, butyltin, phenyltin, octyltin compounds; landfill leachates; analytical procedure, gas chromatography-inductively coupled plasma mass spectrometry

1. Introduction

The most predominant route of municipal waste disposal today is landfilling. As a consequence municipal solid waste sites contain great diversity of chemical compounds and elements which are being released and/or produced during the decomposition of waste material, either by microbiological or chemical pathways [1,2]. The release of chemical substances from municipal waste sites to the environment occurs through two main pathways, leachates and biogases [2]. In order to evaluate their environmental, toxicological and sanitary impacts, it is necessary to monitor pollutants in landfill leachates and biogases [2]. Most of the chemical characterization up to date was focused on the occurrence and determination of metals and metalloids in landfill leachates [3-6]. Organotin and other organometallic compounds represent an important group of pollutants in landfill leachates. The presence of organotin compounds (OTC) in landfill leachates is mainly due to wastes which contain OTC and partly a consequence of microbiological transformations [7]. If not adequately built and maintained, landfills could represent latent environmental threat due to potential leaching of different pollutants to surface and underground waters. Since the start of wide industrial use more than 50 years ago, OTC were employed in many important applications. The most significant are the use of OTC as PVC stabilisers, wood preservatives, pesticides, fungicides, polyurethane and silicone catalysts and up until recently, as antifouling agents. Because of their wide industrial applications they are globally present in the environment [7,8]. OTC are highly toxic chemicals (even at ng L^{-1} concentration levels) with bioaccumulation potential, which have wide variety of toxic effects on various organisms. Occurrence of imposex in dogwhelk populations, oyster shell malformation and mussel larvae mortality are only some the effects that have been observed in the marine environment. In mammals including humans, OTC are neurotoxic, immunotoxic and endocrine disrupters [9-15]. The most toxic forms of OTC are their trisubstituted forms, followed by disubstituted and monosubstituted. Because of that, OTC have been listed as priority pollutants in the both, European Union Water Framework Directive [16] and European Pollutant Emission Register [17]. Due to their adverse effects to the environment and human beings it is of fundamental importance to understand the pathways and distribution of OTC in different environmental compartments. To follow their occurrence and fate in the environment, the development of highly sensitive, selective and accurate analytical methods for determination of OTC in various environmental samples is of crucial importance [18]. Different analytical procedures for determination of OTC in aquatic environments [19,20], mussels [21,22], sediments [23,24], soils [22] and sewage sludge [25,26] have been developed in our group. Trace levels of OTC in these samples were most commonly determined by applying analytical techniques such as gas chromatography (GC) coupled to pulsed flame photometric detector (PFPD), mass spectrometry (MS) or inductively coupled plasma mass spectrometry (ICP-MS). Separation techniques are mostly based on GC, because it offers superior separation power, is commercially widely available and can be coupled to many different suitable detectors. But, prior to GC separation and post column detection, extraction and derivatization steps are necessary to transform ionic OTC into their volatile forms. The most commonly used extraction procedures include liquid – liquid [19,20,27], solid-phase [21-28] and supercritical fluid extraction [29]. Derivatization step can be accomplished by different methods, such as alkylation by

Grignard reagents [19,30,31], hydrogenation by sodium borohydride [30,31] or ethylation by sodium tetraethyl borate ($NaBEt_4$) [20-28,30,31]. Despite of a variety of analytical procedures for the determination of OTC in different sample matrices, there is still a lack of reliable analytical methods for OTC speciation in municipal landfill leachates. The main reason is that landfill leachates are very complex matrices with high ionic strength and high content of organic carbon. So, they represent unique analytical problem for reliable and accurate determination of OTC [3]. To facilitate the extraction of OTC from landfill leachates and to break up the organic matrix, Mersiowsky et al. [1] used 25 % KOH in methanol by stirring the sample for two hours in the pre-extraction step. Further analytical steps in general followed the DIN 38407-13 method for determination of selected organotin compounds by GC. pH was adjusted to 4.5 with glacial acetic acid. For in-situ derivatization and liquid-liquid extraction 3 mL of 20 % sodium tetraethylborate ($NaBEt_4$) and 20 mL of n-hexane were added and sample stirred for 16 hours. Retrieval of hexane phase and it's drying with Na_2SO_4 followed. After that hexane was evaporated to 2 ml, cleaned up on activated silica gel and analyzed by GC-FPD. The limit of detection (LOD) was 0.1 µg L^{-1} for monomethyltin (MMT), dimethyltin (DMT), monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monooctyltin (MOcT) and dioctyltin (DOcT). Another analytical procedure for determination of OTC in landfill leachates was reported by Pinel-Raffaitin et. al [3]. Microwave assisted extraction at 40 W using nitric acid for promoting the hydrolysis of organic matter and to solubilise OTC was applied. Liquid-liquid extraction at pH 5 into isooctane and derivatization with sodium tetrapropylborate ($NaBPr_4$) followed. Determination of OTC was performed using isotopically enriched butyltin species with GC-ICP-MS analysis, while methyltin and ethyltin species were quantified using external and internal calibration approaches. LODs for methyltin, ethyltin and butyltin species were between 0.01 and 0.04 µg L^{-1}. The above mentioned methods developed for determination of OTC in landfill leachates are complex with relatively high LODs. In addition, the use of stable isotopes which are not commercially available for all OTC and are also expensive, is a limiting factor for routine speciation. Therefore, the aim of our study was to develop analytical procedure for simultaneous routine determination of different organotin compounds (methyl-, butyl-, phenyl- and octyl-tins) in landfill leachate samples. For this purpose, OTC were in situ derivatized, extracted into organic phase and determined by GC coupled to ICP-MS. The applicability of methanol as co-extraction reagent and Tris-citrate buffer for adjustment of pH for derivatization of OTC in landfill leachate samples was systematically investigated. The use of $NaBEt_4$ and $NaBPr_4$ as derivatization reagents for liquid – liquid extraction into hexane was critically evaluated. 15 m GC column was used for rapid separation of OTC. Finally, OTC from landfill leachates of three different basins of municipal non-hazardous waste disposal were determined using standard addition calibration method.

2. Experimental

2.1. Instruments and reagents

The determination of OTC was carried out on an Agilent 6890 gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, USA) equipped with Agilent 6890 Series Autosampler Injector that was coupled to Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan) via heated transfer line and fitted with 15 m × 0.25 mm DB-5MS capillary column (film thickness 0.25 µm) coated with 5 % phenyl-

methylpolysiloxane (Agilent J&W Scientific, Palo Alto, CA, USA). Control and operation of the coupled system was achieved by using Agilent ChemStation Software.
For the separation of OTC on 15 m column the following GC temperature program was applied: at the start the column temperature was held at 50 °C for 0.8 min, then raised to 200 °C at heating rate of 20 °C min^{-1} and held there for 2 min, then raised to 220 °C at heating rate of 40 °C min^{-1} and held there for 0.5 min and, in final step, raised to 280 °C at heating rate of 50 °C min^{-1} and held at this temperature for 2 minutes. Inlet temperature was held at 240 °C and transfer – line at 280 °C. Helium at flow rate of 1 mL min^{-1} was used as a carrier gas, injection mode was split-less and injection volume 2 μL. The operating ICP-MS conditions can be found in the Supplementary material.
Mechanical shaking of samples during the extraction procedure and organic phase containing dispersed emulsion was performed on orbital shaker Vibromix 40 and Vibromix 10, respectively (Tehtnica, Železniki, Slovenia). For determination of total Sn concentration in sample leachates microwave assisted digestion was performed on a CEM Corporation (Matthews, NC, USA) CEM MARS 5 Microwave Acceleration Reaction System. Centrifugation of samples was performed on Hettich Universal 320 Centrifuge (Hettich GmbH & Co. KG, Tuttlingen, Germany).
All reagents used were of analytical-reagent grade. Milli-Q water (18.2 MΩ) (Milipore, Bedford, MA, USA) was used for the preparation of all aqueous solutions. Monomethyltin trichloride ($MMTCl_3$, 98 %), dimethyltin dichloride ($DMTCl_2$, 95 %), and trimethyltin chloride (TMTCl, 99 %), were purchased from Acros Organics, (New Jersey, NY, USA). Monobutyltin trichloride ($MBTCl_3$, 95 %), tributyltin chloride (TBTCl, 96 %), monophenyltin trichloride ($MPhTCl_3$, 98 %), diphenyltin dichloride ($DPhTCl_2$, 96 %), triphenyltin chloride (TPhTCl, 97 %) and triethylbromide (TETBr, 97 %) were purchased from Aldrich (Milwaukee, WI, USA). Dibutyltin dichloride ($DBTCl_2$, 98 %), tetrabutyltin (TeBuT), that was included in the speciation procedure as a standard which does not need alkylation, and tripropyltin chloride (TPrTCl, 98 %) were obtained from Merck (Darmstadt, Germany). Monooctyltin trichloride ($MOcTCl_3$, 99 %) and dioctyltin dichloride ($DOcTCl_2$, 99 %) were purchased from LGC Promochem (Wesel, Germany) and trioctyltin chloride (TOcTCl, > 90 %) from Fluka (Buchs, Switzerland).
OTC standard stock solutions containing 1000 mg (expressed as Sn) L^{-1} were prepared in methanol. Stored in the dark at 4 ºC, they were stable for 6 months. Working OTC standard solutions were prepared daily.
Hydrochloric acid, 25 % aqueous solution of ammonia, citric acid monohydrate and Tris (hydroxymethyl)aminomethane (Tris) and hydrogen peroxide (s.p.) were obtained from Merck (Darmstadt, Germany). Nitric acid was purchased from Carlo Erba (Milan, Italy), hexane and methanol from J.T. Baker (Deventer, Holland), sodium tetraethyl borate ($NaBEt_4$, 98 %) from Strem Chemicals (Newburyport, MA, USA) and sodium tetrapropyl borate ($NaBPr_4$, 99 %) from ABCR (Karlsruhe, Germany). Aqueous solutions of sodium tetraethyl borate ($NaBEt_4$) (2 % (w/v)) and sodium tetrapropyl borate (($NaBPr_4$) (2 % (w/v)) were prepared just before derivatization. Tris-citrate buffer was prepared daily.

2.2. Sample collection

Samples of leachates were collected in municipal non-hazardous waste Landfill Barje, Ljubljana, Slovenia. Joint surface of the disposal area is 49.300 m^2 and has the capacity of 884.000 m^3. Sampling was performed on three sampling basins. Sampling site No. 1

was abandoned, non active landfill basin (Sample No. 1), sampling site No. 2 (Sample No. 2) was active landfill basin and sampling site No. 3 (Sample No. 3) was a joint basin, collecting the leachates from the entire landfill. Samples of leachates were taken by a sample collector which was thoroughly washed beforehand, transferred into 2.5 L dark glass bottles and immediately transported to laboratory in iceboxes. In the laboratory the leachates were homogenized by shaking at 300 rpm for 5 min at rotary shaker. After that 200 mL aliquots were stored in dark glass containers and frozen at -20 °C to await the analysis. Prior to analysis samples were thawed, equilibrated to room temperature and shaken at 300 rpm for 5 min.

2.3. Cleaning procedure

To avoid contamination all glassware and sampling bottles were rinsed three times with tap water, soaked in 20 % nitric acid for 48 hours, rinsed three times with tap water, three times with water and heated at 400 °C for at least 4 hours.

2.4. Determination of total Sn concentrations in landfill leachates

5 mL of samples were added into Teflon beakers. Then 2 mL of nitric acid and 2 mL of hydrogen peroxide (s.p.) were added and samples subjected to closed vessel microwave digestion at maximal power of 1200 W: ramp to temperature 20 min, 180 ºC, pressure 10 bar, hold 20 min, cooling 20 min. Clear solutions were quantitatively transferred into 20 mL glass volumetric flasks and filed to mark with water. The same procedure, with exception that no sample was added, was applied to determine blank. The total concentrations of Sn in the digested samples were determined by ICP-MS. Analyses were made in duplicates.

2.5. Recommended analytical procedure for the determination of OTC in landfill leactahes

A liquid – liquid extraction procedure was used prior to the determination of OTC in landfill leachate samples by GC-ICP-MS. Briefly, 200 mL aliquots of landfill leachate samples were transferred into 500 mL dark glass reactor vessels along with methanol that was added in concentration 5 % relative to sample volume. Mixtures were mechanically shaken for 2 hours. After shaking 190 mL of 0.2 mol L^{-1} Tris-citrate buffer was added to each sample, so that the total volume (sample, methanol plus buffer) was 400 mL. Tris-citrate buffer was prepared from Tris and citric acid to match pH 6 for derivatization. pH was adjusted with citric acid or ammonia. Addition of TPrT internal standard solution (20 ng Sn) followed. For the standard addition calibration method mixtures of methyl-, butyl-, phenyl- and octyl-tins were added to sample aliquots in concentrations ranging from 0.2 to 100 ng Sn. The amount of standards that were added to prepare the standard addition calibration curve ranged between 50 to 200 μL. For derivatization 2 mL of 2 % (m/V) $NaBEt_4$ were added to the sample extracts, followed by the addition of 2 mL of hexane, as an extraction agent for ethylated OTC species. Samples were then mechanically shaken for 16 hours. After that organic phase was collected into 15 mL dark glass vials using Pasteur pipette. Organic phase contained dispersed emulsion. To separate the emulsion from the organic phase, 1 mL of 25 % KOH in methanol was added, shaken for 5 min and centrifuged for 20 min at 4200 x *g*. Clear organic phase was transferred into a dark glass vials for GC-ICP-MS analysis. For the determination of ethyltins, TeBuT (20 ng Sn) was used as internal standard solution and 2 % (m/V) of $NaBPr_4$ (2 mL) as derivatization agent. To control

the purity of reagents used, the same analytical procedure was applied to Milli Q water. If not stated otherwise, all the analyses were made in duplicates.

3. Results and discussion

3.1. Development of analytical procedure for determination of OTC in landfill leachates

In comparison to fresh and sea waters, landfill leachates that contain high amounts of organic matter, proteins, fats and inorganic and organic pollutants are samples with much higher degree of matrix complexity. So, optimization of extraction parameters and pH of derivatization was necessary for both ethylation and propylation procedures.

It was found experimentally that after derivatization and extraction into organic phase (hexane), dispersed emulsion was formed. Such organic extract was unsuitable for GC separation. So, before GC-ICP-MS determinations, it was necessary to obtain clear hexane phase. For this purpose 1 mL of concentrated HCl or 25 % KOH in methanol was added to 2 mL of hexane. The mixture was shaken for 5 min and centrifuged for 20 min at 4200 x *g*. The volume of recovered hexane from the emulsion was on average 1 mL when concentrated HCl was applied and 1.5 mL when 25 % KOH in methanol was added. The centrifugation time for 25 % KOH in methanol (20 min) was shorter than for HCl (40 min). Analyses of OTC in clear organic phase (hexane) indicated that application of HCl or 25 % KOH in methanol for separation of the dispersed emulsion did not influence the speciation of OTC. The same concentrations were determined regardless the agent used. Due to the shorter time necessary for separation of emulsion and higher recovery of the clear organic phase, the use of 25 % KOH in methanol is recommended and was applied in further experiments.

3.2. Optimisation of the extraction procedure

In order to develop optimal extraction procedure for OTC determination in landfill leachate samples, several parameters that influence the overall extraction efficiency were critically evaluated.

3.2.1. *Comparison of ultrasonic and mechanical shaking*

To optimize the extraction procedure different extraction modes were compared: ultrasonic extraction and mechanical shaking. Landfill leachate sample No. 3 was spiked with 100 ng Sn (TPrT, butyltins, methyltins, phenyltins and octyltins). Tris-citrate buffer was used to adjust pH to 6. For derivatization, ethylation with $NaBEt_4$ was applied. Ultrasonic extractions were carried out at 40 °C for 15, 30 and 60 minutes, while mechanical shaking was performed at room temperature for 1, 4, 6, and 16 hours. The results of these experiments indicated, that the optimal time for mechanical shaking was 16 hours. Ultrasonic extraction was found to be inefficient regardless of the time applied. This can be evidently seen from data of Fig. 1, by comparing the signal intensities of spiked landfill leachate. For ultrasonic the extraction signal intensities were appreciably lower than those obtained by mechanical shaking. Due to that reason, mechanical shaking (16 hours) was applied in further experiments.

3.2.2. *Comparison of ethylation and propylation efficiencies*

In order to optimize alkylation efficiency for methyl-, butyl-, phenyl- and octyltins, $NaBEt_4$ and $NaBPr_4$ were used for derivatization. Spiked landfill leachates (Sample No. 3) were prepared in two parallel samples (100 ng Sn, Tris-citrate buffer, pH 6.0) and

subjected to mechanical shaking for 1 to 16 hours. The results of these experiments are presented in Fig. 2. Data from Fig. 2 indicates that optimal signal intensities for OTC investigated are obtained after 4 hours for propylation and after 16 hours for ethylation procedure. Since propylation provoked splitting of phenyltin chromatographic peaks, ethylation was applied throughout of the following experiments. The exception being only when ethyltins were investigated.

3.2.3. The application of methanol as co-extracting agent

To facilitate the extraction procedure, different concentrations of methanol were applied as co-extracting agent to spiked landfill leachate with methyl-, butyl-, phenyl- and octyltins (100 ng Sn) prior to addition of Tris-citrate buffer (pH 6.0), ethylation reagent and hexane. Spiked leachates (Sample No. 3) were prepared in two parallel samples. The results are shown in Fig. 3. Data from these experiments indicate that addition of 5 % methanol as co-extracting agent is optimal for all OTC investigated. Since there were reports in the literature [1] that application of 25 % KOH in methanol improved the extraction efficiency, 5 % methanol and 5 % methanol added along with 25 % KOH in methanol as co-extracting agents was applied prior to extraction of landfill leachate. The outcomes of these experiments shown in Fig. 4 demonstrated that addition of 25 % KOH in methanol has deleterious effect on signal intensities, most probably due to degradation of OTC at high pH values. Therefore, the addition of KOH was omitted. To further improve the extraction efficiency different modes of extraction as presented in Fig. 5 were applied. It is evident that co-extraction with methanol is the most effective, when 5 % methanol is first added to the sample and mixture shaken for 2 hours, followed by the simultaneous addition of Tris-citrate buffer (pH 6.0), derivatization reagent and hexane and application of mechanical shaking for next 16 hours. This positive effect on signal intensities is the most pronounced for monosubstituted OTC, in particular for MBT and MOcT (Fig. 5, Mode D). DBT is the only species of 12 OTC investigated, that exhibits slightly higher sensitivity in Mode C. Nevertheless, Mode D was found to be the best compromise for all OTC investigated, so it was used in the continuation of the experiments.

3.2.4. Optimization of pH of extraction buffer

In the analysis of OTC the pH of extraction importantly contributes to the derivatization efficiency [31]. To optimize the pH of extraction landfill leachate (Sample No. 3) was spiked (two parallel samples) with methyl-, butyl-, phenyl- and octyltins (100 ng Sn). 5 % methanol was added and the mixture was shaken at 300 rpm for 2 hours. After that Tris-citrate buffer (pH from 3 to 8), alkylation reagent and hexane were added and the mixture was shaken at 300 rpm for additional 16 hours (Fig. 5, Mode D). As alkylation reagents $NaBEt_4$ and $NaBPr_4$ were used. It was found experimentally that the overall extraction efficiency for both alkylation reagents was poor within the pH range of the extraction buffer from 3 to 4 (due to decomposition of the alkylation reagents) and from 7 to 8 (due to formation of hydroxo species). So, the pH range between 4.5 and 6.5 was carefully investigated. The data of these experiments for ethylated and propylated OTC are shown in Fig. 6. It is evident from Fig. 6 that extraction at pH 6.0 is the best compromise for the derivatization of all OTC investigated. Maximal signal intensities are in general observed both for propylation and ethylation derivatization procedures. Therefore, the pH of buffer was adjusted to 6 in the following experiments.

3.2.5. *Comparison of extraction efficiencies using Tris-citrate and acetate buffers*

The most commonly used buffer in derivatization of ionic OTC is acetic acid – acetate buffer with optimal pH of derivatization 4.8 [20-28]. However, as was reported in the

literature, optimal pH can be quite higher, due to the complex sample matrix that can change prevalent mechanism of alkylation, especially in the presence of high carbon content and high ionic strength [31]. In order to demonstrate that Tris-citrate buffer facilitates the solubilisation of OTC from complex landfill leachate matrix, sample No. 3 was spiked (in two parallels) with methyl-, butyl-, phenyl- and octyltins (100 ng Sn) and the ethylation extraction procedure was applied at optimal conditions using Tris-citrate (pH 6.0) and acetate (pH 4.8) buffers. The signal intensities of these experiments presented in Fig. 7 were higher for all OTC investigated when Tris-citrate buffer was applied to adjust the pH. The same positive effect of Tris-citrate in comparison to acetate buffer was observed in the unspiked landfill leachate sample. Based on outcomes of our experiments the use of Tris-citrate buffer is highly recommended in complex sample matrices such as landfill leachates.

3.3. *Concentration of the alkylation reagent used for the derivatization*

Mersiowsky et al. [1] used large amounts of $NaBEt_4$ (3 mL, 20 %, sample volume 100 mL) for derivatization of OTC from landfill leachates. To check the concentration of the alkylation reagent necessary for quantitative derivatization of OTC in landfill leachates, the same amounts of $NaBEt_4$ and/or $NaBPr_4$ as reported by Mersiowsky et al. [1] were added to sample extracts and signal intensities compared to the addition of the amount of alkylation reagents recommended in this work (2 mL of 2 % (m/V) $NaBEt_4$ or $NaBPr_4$). Experimental data demonstrated that 2 mL of 2 % (m/V) $NaBEt_4$ or $NaBPr_4$ added to the 200 mL of sample extracts ensured quantitative derivatization of OTC in the landfill leachates. The later amount of alkylation reagents was sufficient to compensate also for the consumption of the reagent by other species present in the sample (e.g. sulfur, mercury, lead).

3.4. The performances of analytical procedure

The performances of analytical procedure (Tris-citrate buffer pH 6, ethylation by $NaBEt_4$ and propylation by $NaBPr_4$, extraction into hexane, separation on 15 m GC column and ICP-MS detection) for simultaneous determination of OTC in landfill leachate were evaluated. To estimate the linearity of the calibration curves landfill leachate was spiked with mixtures of calibration standards containing methyl-, butyl-, phenyl- and octyltins in concentration range 0.5 to 100 ng Sn L^{-1}. The limits of detection (LODs) and limits of quantification (LOQs) were calculated according to procedures based on EPA document 40 CFR 136 [32] and LOD Guidance Document PUBL-TS-056-96 [33]. LODs were determined on the basis of the standard deviations of spiked landfill leachate sample No. 3 with methyl-, butyl-, phenyl- and octyltins as well as triethyltin (TEtT) (20 ng Sn), measured in eight replicates. To calculate LODs the Student's t-value was multiplied by standard deviations of spiked sample. LOQs were calculated as ten times the standard deviation (10*s*) of spiked sample. For evaluation of the repeatability of measurements six independent spikes of sample No. 3 with methyl-, butyl- phenyl- and octyltins (20 ng Sn) were analyzed on the same day. After two days the same experiment was repeated again by different analyst using the same analytical procedure to estimate the reproducibility of measurements. The results of the performances of analytical methods are presented in Table 1 for ethylation and in Table 2 for propylation.

Insert Table 1 about here

Insert Table 2 about here

Data from Tables 1 and 2 indicate that performances of analytical methods are slightly better when $NaBEt_4$ was used for derivatization. However, the use of $NaBPr_4$ enables also determination of ethyltins in landfill leachates. Since only TEtT is commercially available as standard, data for diethyltin (DEtT) and monoethyltin (MEtT) are not reported. Since in the procedure applied 200 mL aliquots of samples were extracted into 2 mL of hexane (preconcentration factor 100), LODs from Tables 1 and 2 are approximately 100 times lower than reported by Mersiowsky et al. [1] and 10 to 40 times lower than those obtained by Pinel-Raffaitin [3] who used lower amounts of samples. It is further evident from Tables 1 and 2 that correlation coefficients for calibration curves (r^2) are better than 0.995 for ethylation and in general better than 0.990 for propylation. Considering the complex matrix of landfill leachate the repeatability of measurement for OTC analyzed is very good for ethylation (RSD around 2 %) and propylation (RSD around 5 %), while the reproducibility of measurement is slightly worse.

3.5. Quantification of OTC in landfill leachates and the accuracy check

Quantification of OTC was performed by applying standard addition calibration using TPrT (20 ng Sn) for ethylation and TeBuT (20 ng Sn) for propylation as internal standards. Since there is no certified reference material for landfill leachates available, spike recovery test was used for the evaluation of the accuracy of the analytical procedures. For this purpose landfill leachate was spiked with methyl-, butyl-, phenyl- and octyltins (20 ng Sn) using $NaBEt_4$ and $NaBPr_4$ for derivatization and samples analyzed by recommended analytical procedure described in section 2.5. Analyses were made in six replicates. Recoveries of spiked target compounds in samples ranged from 89 % to 107 % for methyl-tins, from 90 % to 105 % for butly-tins, from 81 % to 121 % for phenyl-tins, and from 97 % to 117 % for octyltins for ethylation and propylation procedures, respectively. From the results of the recovery tests, it may be concluded that analytical procedure developed was suitable for its intended use in landfill leachates, samples with extremely complex matrix.

3.6. Analysis of landfill leachate samples

In order to assess the applicability of the analytical method developed, concentrations of OTC in landfill leachates from municipal non-hazardous waste Landfill Barje, Ljubljana, Slovenia, were determined under the Recommended analytical procedure (2.5.). To check the presence of ethyltins in samples analyzed, propylation was used for the derivatization of OTC. There were no measurable concentrations of ethyltins detected. Therefore, the ethylation was applied for the derivatization in the analysis of OTC, since the repeatability and reproducibility of measurements were found to be better (Tables 1 and 2). The results of the analysis of landfill leachates are presented in Table 3, while the corresponding chromatogram for Sample No. 2 is shown in Fig. 8.

Insert Table 3 about here

It is evident that on sampling sites investigated methyltins were present in the highest concentrations, followed by the concentrations of butyltins and octyltins. In addition to

ethyltins, TOcT and phenyltins were not detected. Concentrations of OTC determined were the highest in the active landfill basin (Sample No. 2), followed by the concentrations in the joint basin, collecting the leachates from the entire landfill (Sample No. 3) and the lowest in the abandoned, non active landfill basin (Sample No. 1). Data further indicate on the highest degree of degradation of OTC in the abandoned basin (MMT is higher than TMT, MBT is higher than TBT and MOcT is higher than DOcT). In comparison to the reported concentrations of OTC (MMT, DMT, MBT, DBT, TBT, MOcT, DOcT) in landfill leachates in Sweden, Germany and Italy [1], the amounts of OTC determined in Landfill Barje were in general 10 to 20 times lower. Regarding the data reported for French landfills [3], where in addition to methyltins and butyltins, MEtT and DEtT were also detected, the concentrations of methyltins, MBT and DBT in Landfill Barje were either comparable or about 5 times lower, while the amounts of TBT were up to 50 times lower.
To evaluate the proportion of OTC regarding the total Sn content, microwave assisted digestion of landfill leachates was performed and the concentrations of total Sn determined by ICP-MS. The results for total amount of Sn, sum of OTC concentrations (as Sn) and the partitioning of selected OTC in landfill leachates are presented in Table 4.

Insert Table 4 about here

It is evident that the highest concentration of total Sn (16000 ng L^{-1}) was present in the abandoned landfill basin (Sample No. 1), while the total OTC content was the lowest (520 ng Sn L^{-1}), representing about 3 % of total Sn. On the contrary, the active landfill basin (Sample No. 2) contained the lowest total Sn concentration (about 10000 ng L^{-1}) and the highest content of OTC (925 ng Sn L^{-1}), that represent 9.5 % of the total amount of Sn. Sample No. 3 from a joint basin, contained the middle concentrations of total Sn and OTC. It is further evident that the highest proportion of OTC represent methyltins (6.4, 3.8 and 2.0 %) for samples No. 2, 3 and 1, respectively. The proportion of total OTC to total Sn content in samples from landfill Barje is comparable to samples from French landfills [3]. Data of the present study demonstrated that the methyltins are the prevailing OTC species on landfill Barje, representing 60 to 70 % of all OTC.

4. Conclusions

A new analytical procedure for the simultaneous determination of methyl-, butyl-, phenyl- and octyltins in landfill leachates was developed. It consists of several steps: co-extraction with 5 % methanol, in situ derivatization of OTC in landfill leachate adjusted to pH 6.0 with Tris-citrate buffer, extraction of alkylated (ethylated or propylated) OTC into hexane, separation of OTC in organic phase on 15 m GC column and quantitative determination of separated OTC by ICP-MS, applying standard addition calibration method.
The use of 5 % methanol as co-extraction reagent facilitated the solubilisation of OTC and was more efficient than the application of 25 % KOH in methanol, proposed by other investigators.
$NaBEt_4$ was found to be better derivatization reagent in complex matrix of landfill leachates than $NaBPr_4$, since the latter exhibited lower derivatization efficiencies and poor peak resolution for phenyltins. However, when ethyltin compounds are analyzed, the use of $NaBPr_4$ is mandatory.

Tris-citrate buffer adjusted to pH 6.0 enables more efficient extraction and derivatization of OTC from complex matrices of landfill leachates in comparison to commonly applied acetic acid-acetate buffer at pH 4.8.
The developed analytical procedure enables reliable simultaneous determination of OTC in landfill leachate samples and can be applied for routine analysis. LODs for OTC investigated are, due to higher preconcentration factor, 10 to 100 times lower than those obtained by other investigators. In comparison to the reported analytical procedures that use cleaning of the organic phase through specially prepared silica gel column the developed analytical procedure is simpler, more sensitive and faster. In addition, it does not depend on the use of enriched stable isotopes that are available only for butyltins. The procedure developed was successfully applied in the analyses of landfill leachates from municipal non-hazardous waste Landfill Barje, Ljubljana, Slovenia. Results indicated that leachates contained methyl-, butyl-, and octyltins in concentrations that were lower than those reported from different European municipal landfills.

Acknowledgements
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Captions to the Figures

Fig. 1 Signal intensities of OTC in spiked (100 ng Sn) landfill leachate (Sample No. 3) (Tris-citrate buffer, pH 6.0, derivatization with $NaBEt_4$) for (A) extraction by mechanical shaking (16 h) and (B) ultrasonic extraction (1 h).
Legend:
1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn, 5 – MBT, 6 – TPrT, 7 – DBT, 8 – MPhT, 9 – TBT, 10 – MOcT, 11 – TeBuT, 12 – DPhT, 13 – DOcT, 14 – TPhT, 15 – TOcT

Fig. 2 Variation of signal intensities for (A) ethylated and (B) propylated OTC in spiked (100 ng Sn) landfill leachate (Sample No. 3) with time. Mechanical shaking, Tris-citrate buffer (pH 6.0). Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.

Fig. 3 Influence of methanol addition (%) as co-extracting agent on signal intensities of spiked OTC (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking (16 h), Tris-citrate buffer (pH 6.0), derivatization with $NaBEt_4$. Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.

Fig. 4 Influence of 5 % methanol addition and 5 % methanol addition along with 25 % KOH in methanol as co-extracting agents on signal intensities of landfill leachate (Sample No. 3). Mechanical shaking (16 h), Tris-citrate buffer (pH 6.0), derivatization with $NaBEt_4$. Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.

Fig. 5 Influence of different modes of extraction on signal intensities of spiked (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking, Tris-citrate buffer (pH 6.0), derivatization with $NaBEt_4$. Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.
Legend:
Mode A: Buffer, ethylation reagent and hexane were added to the sample. Mixture was then shaken at 300 rpm for 16 hours.
Mode B: 5 % methanol, buffer, ethylation reagent and hexane were added to the sample. Mixture was then shaken at 300 rpm for 16 hours.
Mode C: 5 % methanol and buffer were added to the sample and the mixture was shaken at 300 rpm for 2 hours. After that ethylation reagent and hexane were added and the mixture was shaken for additional 16 hours.
Mode D: 5 % methanol was added to sample and the mixture was shaken at 300 rpm for 2 hours. After that buffer, ethylation reagent and hexane were added and the mixture was shaken at 300 rpm for additional 16 hours.

Fig. 6 Variation of signal intensities for (A) ethylated and (B) propylated OTC in spiked (100 ng Sn) landfill leachate (Sample No. 3) with pH of Tris-citrate buffer. Mechanical shaking (16 h, mode D as presented in Fig.5). Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.

Fig. 7 Comparison of signal intensities obtained for Tris-citrate buffer (pH 6.0) and acetate buffer (pH 4.8) for ethylated OTC in spiked (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking (16 h, mode D as presented in Fig.5). Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.

Fig. 8 Signal intensities of OTC in landfill leachate (Sample No. 2) (Tris-citrate buffer, pH 6.0, derivatization with $NaBEt_4$, mechanical shaking (16 h, mode D as presented in Fig.5).
Legend:
1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn, 5 – MBT, 6 – TPrT, 7 – DBT, 8 – TBT, 9 – MOcT, 10 – DOcT

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Figures:

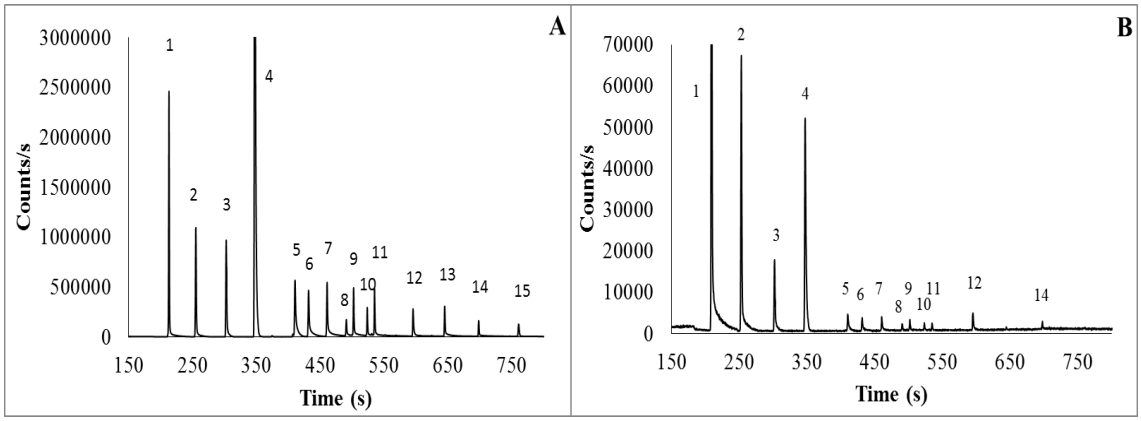
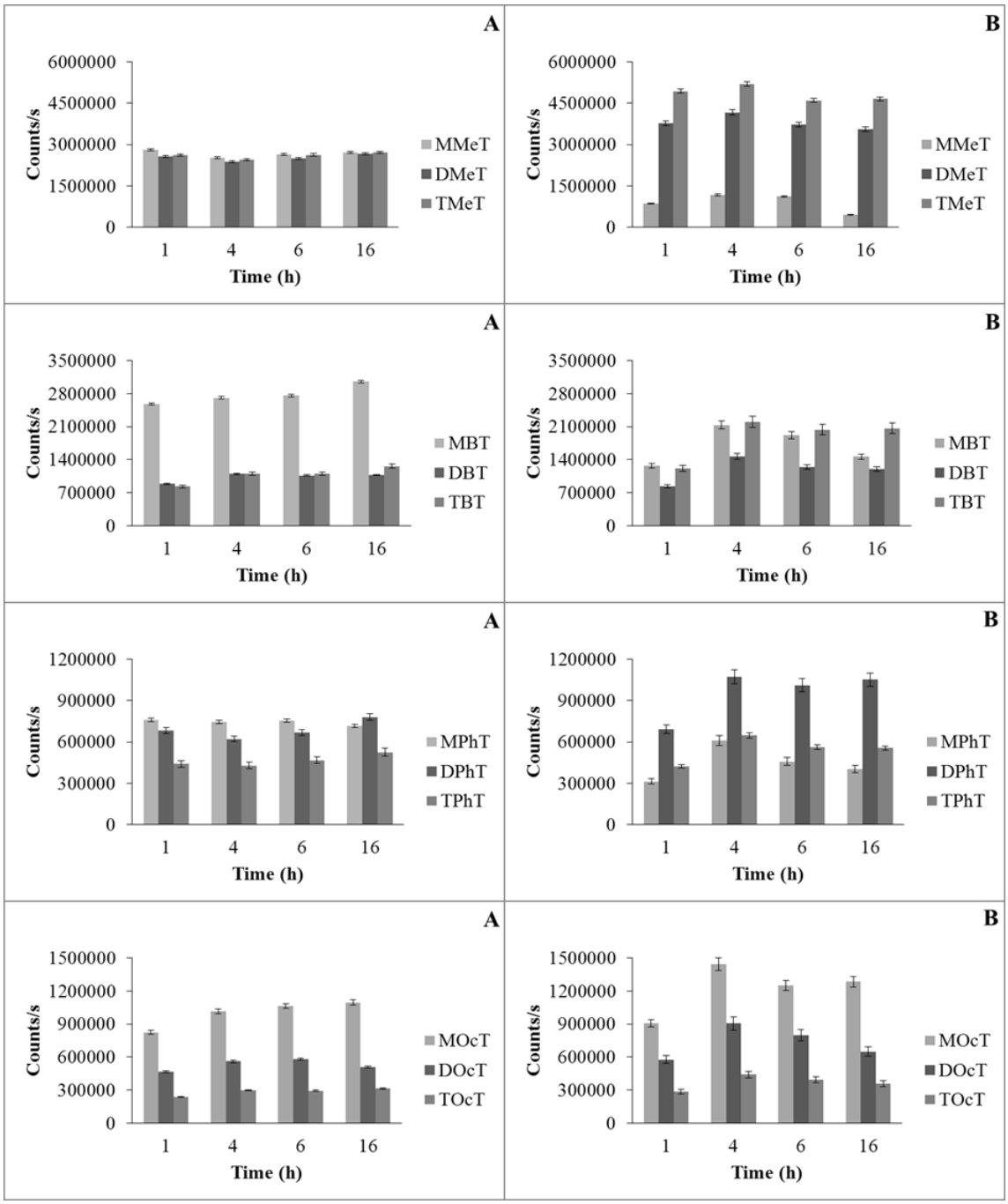


Fig. 1 Signal intensities of OTC in spiked (100 ng Sn) landfill leachate (Sample No. 3) (Tris-citrate buffer, pH 6.0, derivatization with $NaBEt_4$) for (A) extraction by mechanical shaking (16 h) and (B) ultrasonic extraction (1 h).
Legend:
1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn, 5 – MBT, 6 – TPrT, 7 – DBT, 8 – MPhT, 9 – TBT, 10 – MOcT, 11 – TeBuT, 12 – DPhT, 13 – DOcT, 14 – TPhT, 15 – TOcT



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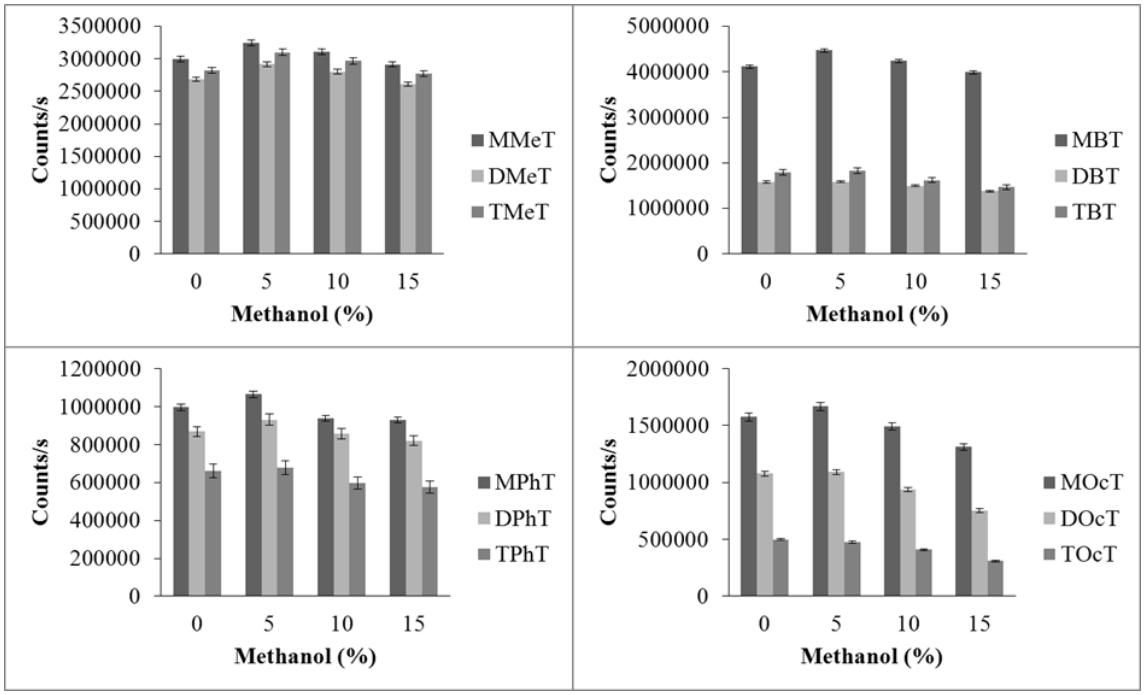
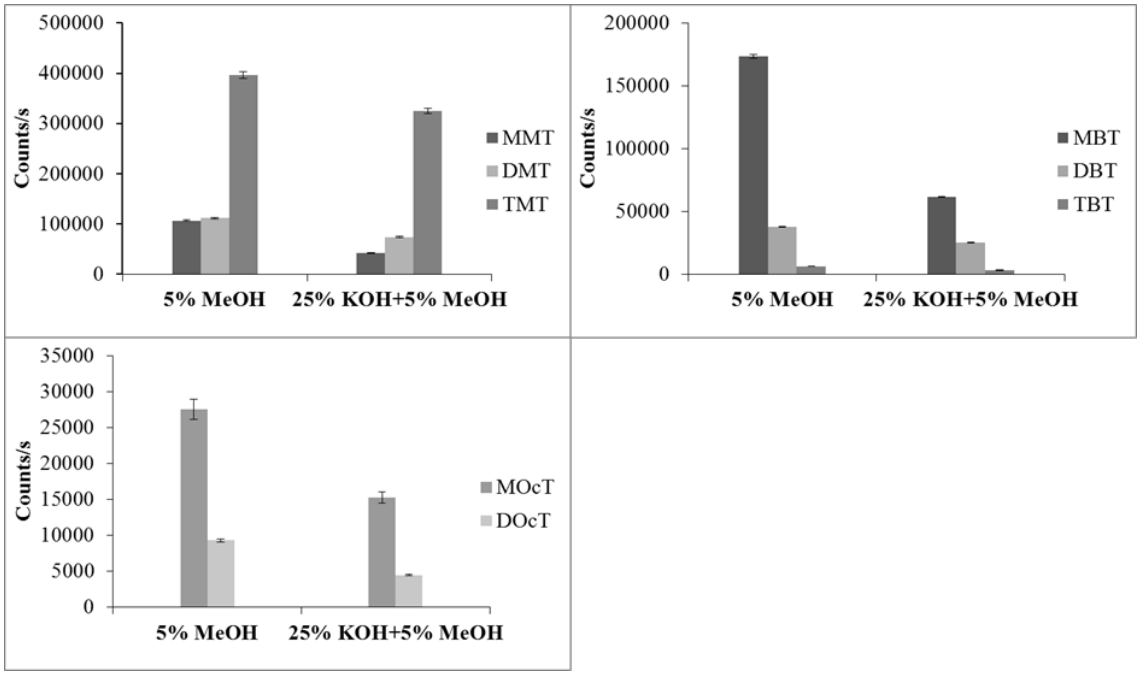


Fig. 3 Influence of methanol addition (%) as co-extracting agent on signal intensities of spiked OTC (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking (16 h), Tris-citrate buffer (pH 6.0), derivatization with $NaBEt_4$. Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.



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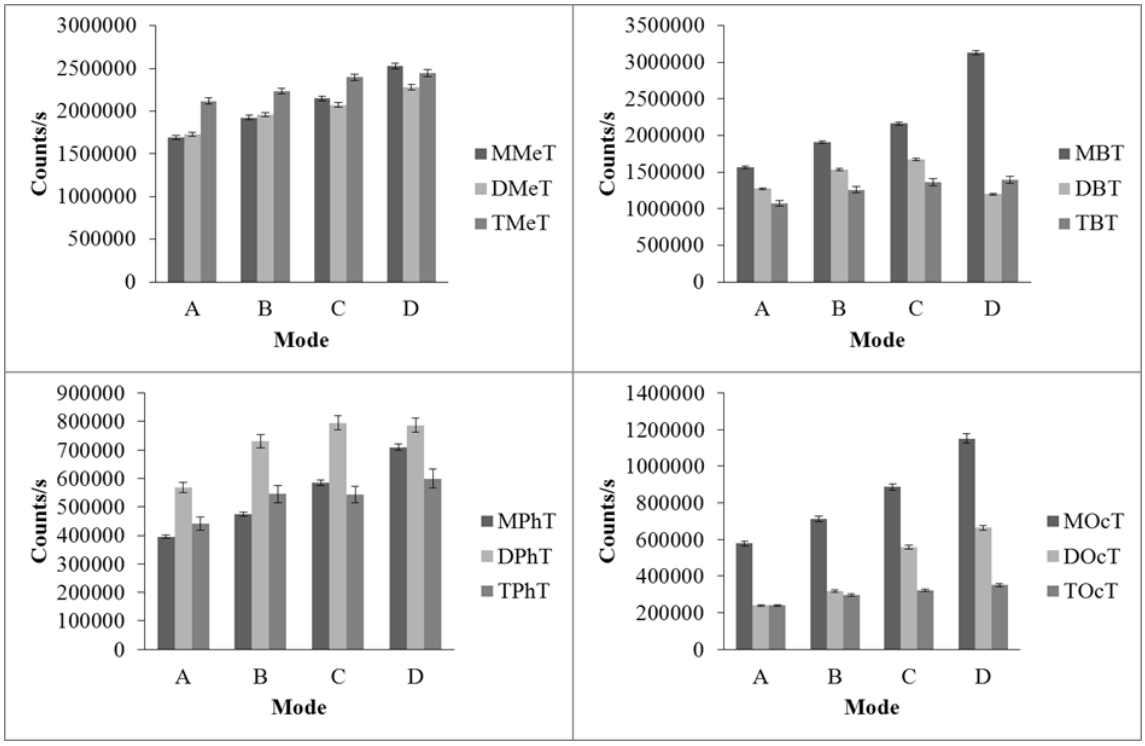
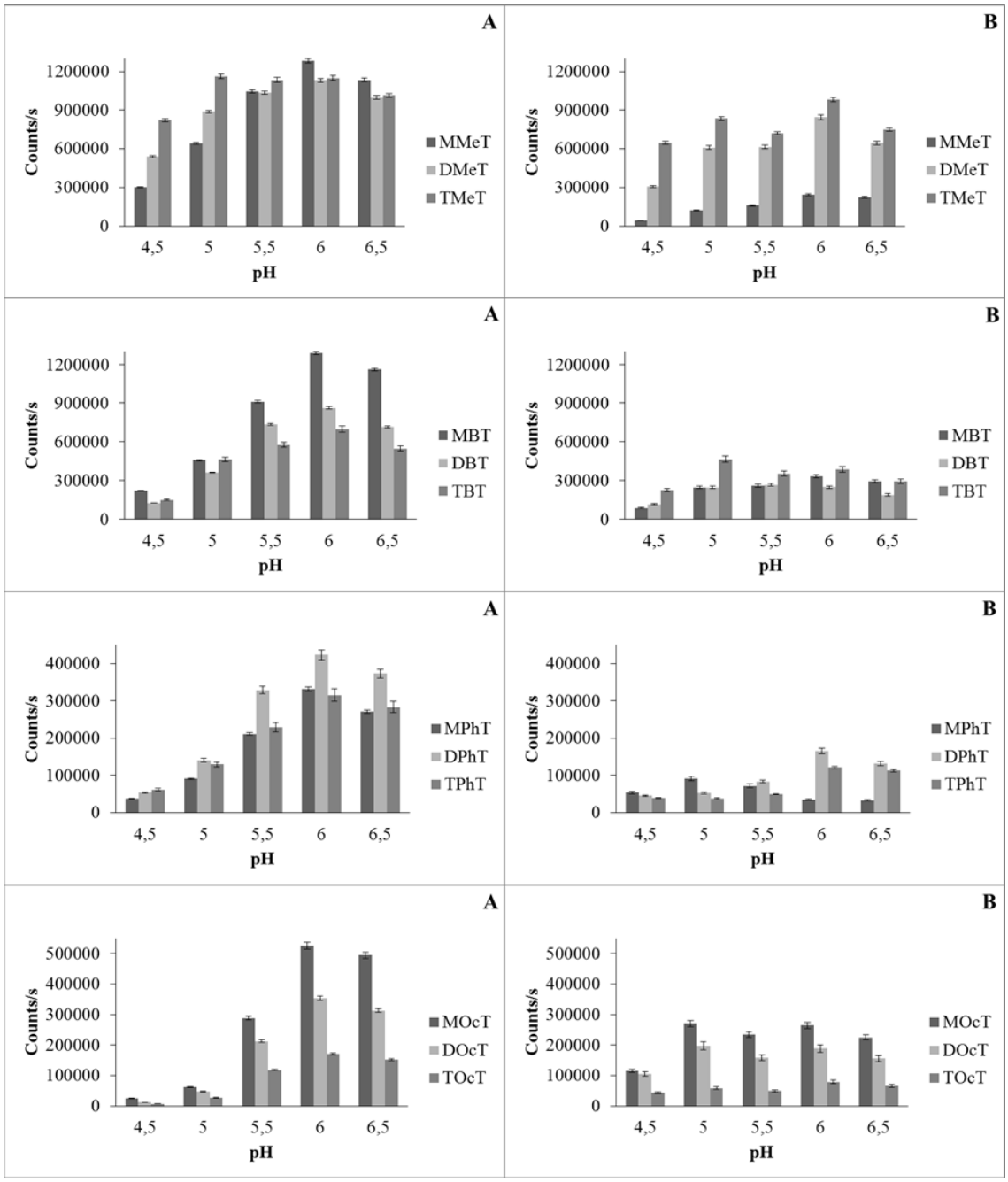


Fig. 5 Influence of different modes of extraction on signal intensities of spiked (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking, Tris-citrate buffer (pH 6.0), derivatization with $NaBEt_4$. Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.
Legend:
Mode A: Buffer, ethylation reagent and hexane were added to the sample. Mixture was then shaken at 300 rpm for 16 hours.
Mode B: 5 % methanol, buffer, ethylation reagent and hexane were added to the sample. Mixture was then shaken at 300 rpm for 16 hours.
Mode C: 5 % methanol and buffer were added to the sample and the mixture was shaken at 300 rpm for 2 hours. After that ethylation reagent and hexane were added and the mixture was shaken for additional 16 hours.
Mode D: 5 % methanol was added to sample and the mixture was shaken at 300 rpm for 2 hours. After that buffer, ethylation reagent and hexane were added and the mixture was shaken for additional 16 hours.



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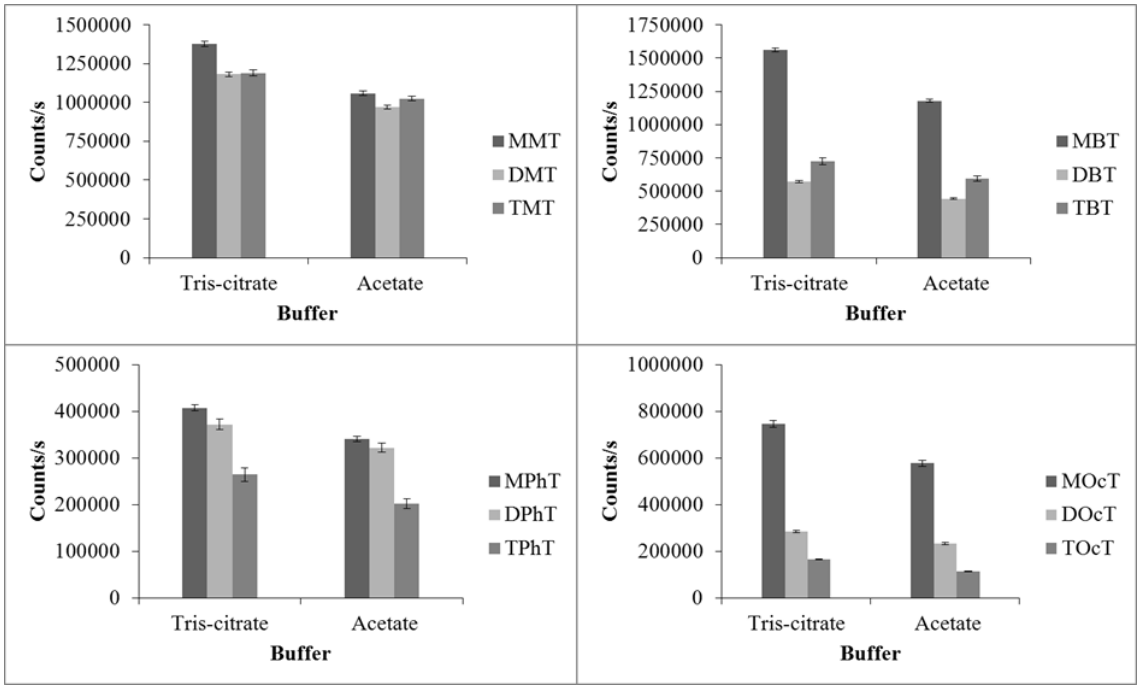

Fig. 7 Comparison of signal intensities obtained for Tris-citrate buffer (pH 6.0) and acetate buffer (pH 4.8) for ethylated OTC in spiked (100 ng Sn) landfill leachate (Sample No. 3). Mechanical shaking (16 h, mode D as presented in Fig.5). Results represent the average of two parallel samples. In each bar the two concentrations that characterize the mean value are indicated.

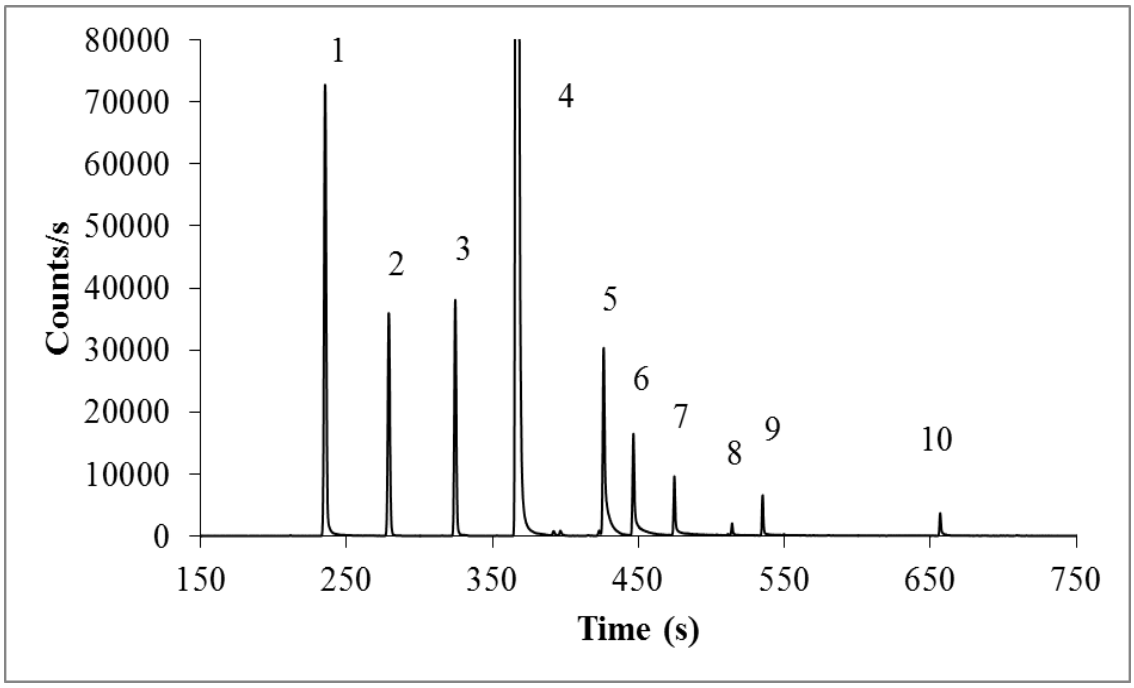

Fig. 8 Signal intensities of OTC in landfill leachate (Sample No. 2) (Tris-citrate buffer, pH 6.0, derivatization with $NaBEt_4$, mechanical shaking (16 h, mode D as presented in Fig.5).
Legend:
1 – TMeT, 2 – DMeT, 3 – MMeT, 4 – Sn, 5 – MBT, 6 – TPrT, 7 – DBT, 8 – TBT, 9 – MOcT, 10 – DOcT

Tables:

Table 1
LODs, LOQs, correlation coefficients, repeatability and reproducibility of measurements of analytical method for simultaneous determination of methyl-, butyl-phenyl- and octyltins in landfill leachate by GC-ICP-MS under the optimal analytical procedure by the use of $NaBET_4$ for derivatization.

OTC	LOD* (ng Sn L^{-1})	LOQ* (ng Sn L^{-1})	r^2	Repeatability** RSD (%)	Reproducibility** RSD (%)
MMeT	2.2	7.3	0.9978	1.3	1.5
DMeT	0.9	3.0	0.9994	1.3	1.5
TMeT	2.2	7.2	0.9989	1.6	1.7
MBT	0.9	2.9	0.9989	0.9	2.8
DBT	0.7	2.4	0.9992	1.2	5.7
TBT	1.0	3.4	0.9987	3.4	5.2
MPhT	0.1	1.5	0.9955	1.6	1.5
DPhT	0.2	3.0	0.9995	3.1	6.6
TPhT	0.2	4.6	0.9993	5.4	7.9
MOcT	1.0	3.2	0.9996	2.1	3.3
DOcT	0.8	2.7	0.9994	2.0	6.9
TOcT	0.5	1.9	0.9994	1.9	6.3

* eight replicates
** six replicates

Table 2
LODs, LOQs, correlation coefficients, repeatability and reproducibility of measurements of analytical method for simultaneous determination of methyl-, butyl-phenyl- and octyltins as well as TEtT in landfill leachate by GC-ICP-MS under the optimal analytical procedure by the use of $NaBPr_4$ for derivatization.

OTC	LOD* (ng Sn L^{-1})	LOQ* (ng Sn L^{-1})	r^2	Repeatability** RSD (%)	Reproducibility** RSD (%)
MMeT	1.7	5.7	0.9944	2.6	6.9
DMeT	1.9	6.2	0.9964	2.4	5.3
TMeT	2.8	9.4	0.9908	1.6	3.4
TEtT	1.0	3.3	0.9905	2.5	3.0
MBT	3.5	11.6	0.9915	3.9	8.4
DBT	3.1	10.2	0.9910	4.1	6.9
TBT	2.3	7.6	0.9861	5.4	7.7
MPhT	2.8	9.2	0.9979	6.3	10.4
DPhT	1.1	3.7	0.9947	4.6	10.8
TPhT	0.8	2.6	0.9995	2.9	8.9
MOcT	2.1	7.1	0.9954	3.8	8.0
DOcT	2.8	9.4	0.9876	6.4	6.7
TOcT	2.4	8.0	0.9921	7.3	7.7

* eight replicates
** six replicates

Table 3
Concentrations of OTC* (methyl-, butyl- and octyltins) determined in landfill leachates from municipal non-hazardous waste Landfill Barje, determined by GC-ICP-MS (ng Sn L^{-1}).

Sample No.	MMT (ng Sn L^{-1})	DMT (ng Sn L^{-1})	TMT (ng Sn L^{-1})	MBT (ng Sn L^{-1})	DBT (ng Sn L^{-1})	TBT (ng Sn L^{-1})	MOcT (ng Sn L^{-1})	DOcT (ng Sn L^{-1})
1	156±5	43±1	121±4	124±4	29±2	5±0.3	30±1	11±1
2	128±4	158±5	340±10	132±4	72±4	21±1	41±1	31±2
3	229±7	69±2	160±5	109±3	33±2	4±0.2	35±1	24±1

*Results represent the mean value of two parallel determinations of selected OTC with deviation of measurements

Table 4
Concentrations of total Sn* (ng L^{-1}) in landfill leachates from municipal non-hazardous waste Landfill Barje, determined by ICP-MS and concentrations of OTC** (methyl-, butyl- and octyltins) determined by GC-ICP-MS (ng Sn L^{-1}) and their partitioning (%) in landfill laecahtes.

Sample No.	Total concentration of Sn (ng L^{-1})	Total concentration of OTC (ng Sn L^{-1})	Total OTC (%)	MMT (%)	DMT (%)	TMT (%)	MMT (%)	MBT (%)	DBT (%)	TBT (%)	MOcT (%)	DOcT (%)
1	16000	520	3.2	0.98	0.27	0.75	0.98	0.77	0.18	0.03	0.19	0.07
2	9770	925	9.5	1.3	1.6	3.5	1.3	1.3	0.7	0.2	0.4	0.3
3	12020	660	5.5	1.9	0.6	1.3	1.9	0.9	0.3	0.0	0.3	0.2

*Results represent the mean value of two parallel determinations with deviation of measurements (± 2 %)
**Results represent the mean value of two parallel determinations of selected OTC with deviation of measurements (± 3-6 %)

Appendix 2: Personal bibliography for the period of 2006-2011

ARTICLES AND OTHER COMPONENT PARTS

1.01 Original scientific article

1. VAHČIČ, Mitja, MILAČIČ, Radmila, MLADENOVIČ, Ana, MURKO, Simona, ZULIANI, Tea, ZUPANČIČ, Marija, ŠČANČAR, Janez. Leachability of Cr(VI) and other metals from asphalt composites with addition of filter dust. *Waste manag. (Elmsford)*. [Print ed.], 2008, vol. 28, no. 12, str. 2667-2674. [COBISS.SI-ID 21510183]

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1.08 Published scientific conference contribution

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1.09 Published professional conference contribution

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1.12 Published scientific conference contribution abstract

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7. VAHČIČ, Mitja, MILAČIČ, Radmila, MURKO, Simona, ŠČANČAR, Janez. Leachability of Cr(VI) and other metals from asphalt composites with addition of filter dust. V: 4th International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences, May 25th - 29th 2008, München. *Programme and abstracts*. München: HelmholtzZentrum München, 2008, 2008, str. 66. [COBISS.SI-ID 21770279]

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2.13 Treatise, preliminary study, study

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3.15 Unpublished conference contribution

23. MILAČIČ, Radmila, ZULIANI, Tea, VAHČIČ, Mitja, ŠČANČAR, Janez. *Potencialna uporaba blat iz čistilnih naprav : JP Centralna čistilna naprava Domžale-Kamnik d.o.o., 27.10.2008 Domžale*. 2008. [COBISS.SI-ID 22161191]

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