VILNIUS UNIVERSITY

CENTER FOR PHYSICAL SCIENCES AND TECHNOLOGY

INSTITUTE Of CHEMISTRY

VAIDA ŠMITIENĖ

MICROEXTRACTION AND GAS CHROMATOGRAPHIC DETERMINATION OF ORGANOTIN COMPOUNDS

Summary of doctoral dissertation

Physical sciences, chemistry (03P)

Vilnius, 2015

The research was carried out in Department of Analytical and Environmental Chemistry, Faculty of Chemistry, Vilnius University, in the period of 2010 - 2015.

Scientific supervisor – prof. dr. Vida Vičkačkaitė (Vilnius University, Physical Sciences, Chemistry – 03P)

The defense council – prof. dr. Stasys Tautkus (Vilnius University, Physical Sciences, Chemistry – 03P)

Members:

prof. habil. dr. Eugenijus Norkus (Center of Physical Sciences and Technology, Institute of Chemistry, physical sciences, chemistry – 03P)

dr. Evaldas Naujalis (Center of Physical Sciences and Technology, Semiconductor physics institute, physical sciences, chemistry – 03P)

dr. Germanas Peleckis (Institute for Superconducting and Electronic Materials, University of Wollongong, Australia, physical sciences, chemistry – 03P)

prof. dr. Jūratė Senvaitienė (P. Gudynas Centre for Restoration, physical sciences, chemistry – 03P)

The defense of the dissertation will take place on October 1, 2015 at 2 p. m. at the open meeting of Council of Chemistry science direction at the Auditorium of Physical Chemistry of the Faculty of Chemistry of Vilnius University.

Address: Naugarduko st. 24, LT-03225, Vilnius, Lithuania.

The summary of the doctoral dissertation was sent on August …, 2015.

The dissertation is available at the libraries of Vilnius University and Center for Physical Sciences and Technology Institute of Chemistry and at VU web site: www.vu.lt/lt/naujienos/ivykiu-kalendorius

VILNIAUS UNIVERSITETAS
FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

VAIDA ŠMITIENĖ

ORGANINIŲ ALAVO JUNGINIŲ MIKROEKSTRAKCIJA IR DUJŲ CHROMATOGRAFINIS NUSTATYMAS

Daktaro disertacijos santrauka

Fiziniai mokslai, chemija (03 P)

Vilnius, 2015

Disertacija buvo ruošiama 2010 – 2015 metais Vilniaus universitete, Chemijos fakultete, Analizinės ir aplinkos chemijos katedroje.

Mokslinė vadovė – prof. dr. Vida Vičkačkaitė (Vilniaus universitetas, fiziniai mokslai, chemija – 03P)

Disertacija ginama jungtinėje Vilniaus universiteto ir Fizinių ir technologijos mokslų centro Chemijos mokslo krypties taryboje:

Pirmininkas – prof. dr. Stasys Tautkus (Vilniaus universitetas, fiziniai mokslai, chemija – 03P).

Nariai:

prof. habil. dr. Eugenijus Norkus (Fizinių ir technologijos mokslų centro Chemijos institutas, fiziniai mokslai, chemija – 03P),
dr. Evaldas Naujalis (Fizinių ir technologijos mokslų centro Puslaidininkių fizikos institutas, fiziniai mokslai, chemija – 03P),
dr. Germanas Peleckis (Australijos Superlaidžių ir elektroninių medžiagų institutas, Volongongo universitetas, fiziniai mokslai, chemija – 03P),
prof. dr. Jūratė Senvaitienė (P. Gudyno restauravimo centras, fiziniai mokslai, chemija – 03P).

Disertacija bus ginama viešame Chemijos mokslo krypties tarybos posėdyje 2015 m. spalio mėn. 1 d. 14 val., Vilniaus universiteto Chemijos fakulteto Fizikinės chemijos auditorijoje. Adresas: Naugarduko g. 24, LT-03225 Vilnius, Lietuva.

Disertacijos santrauka išsiuntinėta 2015 m. rugpjūčio mėn. ... d.

Disertaciją galima peržiūrėti Vilniaus universiteto, FTMC Chemijos instituto bibliotekose *ir VU interneto svetainėje adresu:* www.vu.lt/lt/naujienos/ivykiu-kalendorius

.INTRODUCTION

Organotin compounds (OTC) have a wide industrial application. They are used as heat and light stabilizers in polyvinyl chloride products, wood preservatives, pesticides, fungicides, industrial catalysts. Toxicity of tin compounds is strongly dependent on their species. Inorganic tin is considered to be harmless, while organotins are important environmental pollutants, are very toxic and tend to accumulate in living organisms. Because of their widespread use, organotin compounds can be found in different ecosystems: surface water, tap water, waste water, biological samples such as fish and snails and so on. Via water and food chain, OTC get to algae, sea animals and finally to the human body.

All the OTC except methyltins are anthropogenic compounds. Methyltins can also originate from the biomethylation of inorganic and organic tin compounds. Tributyltin (TBT) is the most toxic organotin compound. For many years it has been used as an additive in antifouling paints for ship hulls, so a huge amount of TBT fell to the seas and oceans. Since 2008 International Maritime Organization prohibited to use TBT in anti-fouling paints used on ships however big quantities of TBT is still left in marine environment.

Because of the toxicity and bioaccumulation potential, organotin compounds have been registered as priority pollutants by the European Union in the Pollutant Emission Register (2000/479/EC) and in the Water Framework Directive (2000/60/EC). All the European Union members are obliged to control organotins in the environment. In Lithuania butyltin and phenyltin compounds have been registered as priority pollutants in 2010, and their monitoring has become compulsory. Thus, the development of accurate and sensitive analytical methods for OTC determination is of special importance.

Gas chromatography is the most common approach for OTC determination because it enables to determine different organotin species at low concentrations. However, as OTC present in the environment are in the ionized form, they need to be derivatized before gas chromatographic analysis to obtain their volatile and thermostable forms. In the literature, several derivatization strategies for organotin

compounds are described. The most commonly used derivatization reactions are formation of hydrides by sodium borohydride and alkylation by alkylborates or by Grignard reagents.

As a rule, trace levels of OTC are present in the environmental samples, thus prior to the gas chromatographic determination a preconcentration step is required. For this purpose, liquid-liquid extraction and solid phase extraction are the most widespread techniques, however they are slow, labor intensive, consume large volumes of toxic organic solvents. In order to overcome those shortcomings, miniaturized versions of extraction – solid phase microextraction (SPME) and liquid phase microextrraction (LPME) - have been developed. The methods of LPME, dispersive liquid-liquid microextraction (DLLME), liquid phase microextraction based on the solidification of a floating organic drop and dispersive liquid-liquid microextraction based on the solidification of a floating organic drop are simple, inexpensive and environmentally friendly extraction techniques. On the other hand, the possibilities to apply those microextraction techniques for organotins preconcentration are poorly investigated.

Thus, **the aim of the work** was to examine possibilities of microextraction methods for fast and efficient extraction of organotin compounds, to prepare and apply microextraction techniques for the analysis of aqueous samples.

The main tasks set to achieve the aim were as follows:

1. To optimize derivatization and gas chromatographic-mass spectrometric determination conditions of organotin compounds.
2. To examine the possibilities of dispersive liquid-liquid microextraction, liquid phase microextraction based on the solidification of a floating organic drop and dispersive liquid-liquid microextraction based on the solidification of a floating organic drop for the extraction of organotin compounds and to prepare the techniques for the determination of organotin compounds.
3. To compare the prepared microextraction techniques of organotin compounds.
4. To apply the most appropriate organotin microextraction techniques for the real water sample analysis.

Statements for defense:

1. Gas chromatography-mass spectrometry is a sensitive and selective method for the determination of derivatized organotin compounds.
2. Extraction of organotin compounds can be accomplished using dispersive liquid-liquid microextraction, liquid phase microextraction based on the solidification of a floating drop and dispersive liquid-liquid microextraction based on the solidification of a floating organic drop techniques.
3. Dispersive liquid-liquid microextraction is the most appropriate among the investigated microextraction techniques for the determination of organotin compounds in real water samples.

1.EXPERIMENTAL

Equipment

Gas chromatographic analysis was performed on a PerkinElmer Clarus 580 series gas chromatograph coupled to a PerkinElmer Clarus 560 S mass spectrometer (PerkinElmer, Shelton, USA). The GC system was equipped with Elite-5 MS capillary column (30 m × 0,25 mm id, 0,25 μm film thickness) coated with methylpolysiloxane (5% phenyl). Centrifugation was carried out with a Boeco S-8 centrifuge (Germany). For sample injection 10 μl microsyringe (Hamilton, Reno, NV, USA) was used.

Helium was employed as a carrier gas with a constant flow of 1 ml/min. The injector temperature was held at 250 °C. For methyltin solutions obtained after DLLME the injection port temperature program was: held at 160 °C for 1 min and raised to 250°C at the heating rate of 200 °C/min.

The oven temperature was programmed: for methyltins held at 50 °C for 1 min, from 50 to 90 °C at 15 °C/min, from 90 to 250 °C at 40 °C/min and held at 250 °C for 3 min.; for butyltins held at 80 °C for 3 min, from 80 to 210 °C at 25 °C/min, from 210 to 250 °C at 40 °C/min and held at 250 °C for 3 min.; for phenyltins held at 60 °C for 1 min, from 60 to 250 °C at 30 °C/min and held at 250 °C for 6 min.

The capillary column was connected to the ion source of the mass spectrometer by means of the transfer line maintained at 280 °C. The electron ionization ion source conditions were: electron energy 70 eV and temperature 180 °C.

GC-MS in full scan mode was used for the optimization of the derivatization and extraction conditions. The acquisition was performed in the range of m/z 50 - 500. Selected ion monitoring (SIM) mode was used for the quantitative analysis.

Dispersive liquid-liquid microextraction procedure

To a 10 ml centrifuge tube with a conic bottom 8 ml of organotin compounds aqueous solution adjusted to the required pH and the appropriate amount of $NaBEt_4$ solution (derivatization reagent) were placed. The solution was left for derivatization of organotin compounds. Then the mixture containing of disperser solvent and extraction solvent was rapidly injected to the solution using 1 ml syringe. A cloudy solution formed was centrifuged for 3 min at 5000 rpm. Organic phase with the analytes was sedimented in the bottom of the tube. One µL of the extraction phase was injected into GC-MS.

Liquid phase microextraction based on the solidification of a floating organic drop procedure

To extraction vessel 15 ml of organotin compounds aqueous solution adjusted to the required pH was placed and the appropriate amount of $NaBEt_4$ solution (derivatization reagent) were added. The solution was left for derivatization of organotin compounds. Then the extraction solvent was injected to the solution using microsyringe. The solutinion was stirred on a magnetic stirrer using 800 rpm. Then the vessel was transferred into an ice bath. The solidified solvent was transferred to a conical vial to melt at room temperature. Melted organic phase (1µl) was injected into GC-MS.

Dispersive liquid-liquid microextraction based on the solidification of a floating organic drop procedure

To a 10 ml centrifuge tube with a conic bottom 8 ml of organotin compounds aqueous solution adjusted to the required pH was placed and the appropriate amount of $NaBEt_4$

solution (derivatization reagent) were placed. The solution was left for derivatization of organotin compounds. Then the mixture containing of disperser solvent and extraction solvent was rapidly injected to the solution using 1 ml syringe. A cloudy solution formed was centrifuged for 3 min at 5000 rpm. Then the centrifuge tube was transferred into an ice bath. The solidified organic phase was transferred to a conical vial to melt at room temperature. Melted organic phase (1 µl) was injected into GC-MS.

3. RESULTS AND DISCUSSION

3.1. Microextraction and determination of methyltin compounds

3.1.1. Dispersive liquid-liquid microextraction of methyltin compounds

Derivatization of methyltin compounds

In real samples OTC exist in cationic form, are non-volatile and polar, so before gas chromatographic analysis OTC must be derivatized. Alkylborates obtained by a derivatization procedure using sodium tetraalkylborate ($NaBEt_4$) are stable in the water and a derivatization step can be accomplished in the aqueous phase. Because of that, in this work a derivatization procedure using sodium tetraethylborate was preferred. The variables involved in the derivatization reaction, such as solution pH, reaction time, $NaBEt_4$ concentration were optimized.

To find out the optimum derivatization conditions, liquid-liquid extraction was carried out prior to the GC-MS analysis: to 25 ml of 10 µg/l aqueous methyltin solution 100 µl of 10 % $NaBEt_4$ solution was added (resulting in 0.04 % $NaBEt_4$ concentration in the solution of methyltins) and after 15 min the solution was vigorously extracted with 0.5 ml of *n*-pentane for 2 min. The extract was transferred into the sampling vial and automatically injected into the GC injection port.

In order to chose the optimum pH for methyltins derivatization, derivatization efficiency was studied in the pH range from 1.5 to 5.0. The maximum efficiency was obtained at pH 3 (Fig. 1).

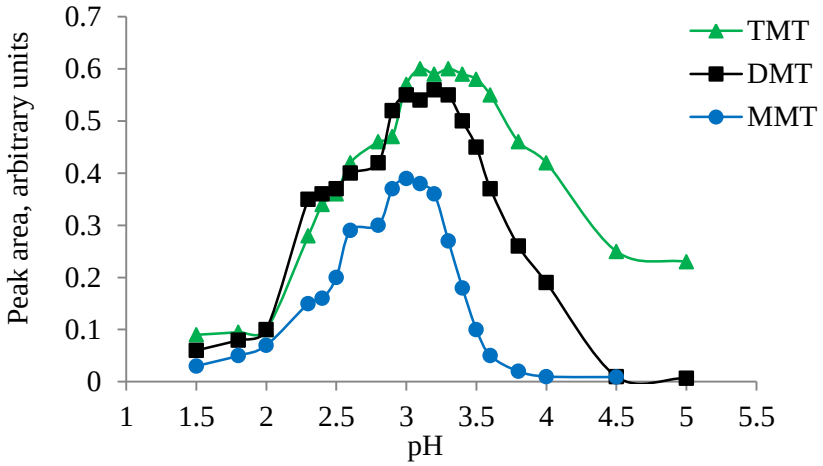

Fig. 1. Effect of pH on the derivatization efficiency. Sample volume 25 ml, concentration of methyltins 10 μg/l, derivatization with 0.04 % $NaBEt_4$, derivatization time 15 min, extraction with *n*-pentane for 2 min.

Derivatization time was studied between 1 and 60 min. The results showed that the peak areas of the analytes increased up to 1 – 10 min (Fig. 2). Thus, 10 min derivatization time was chosen for the further work.

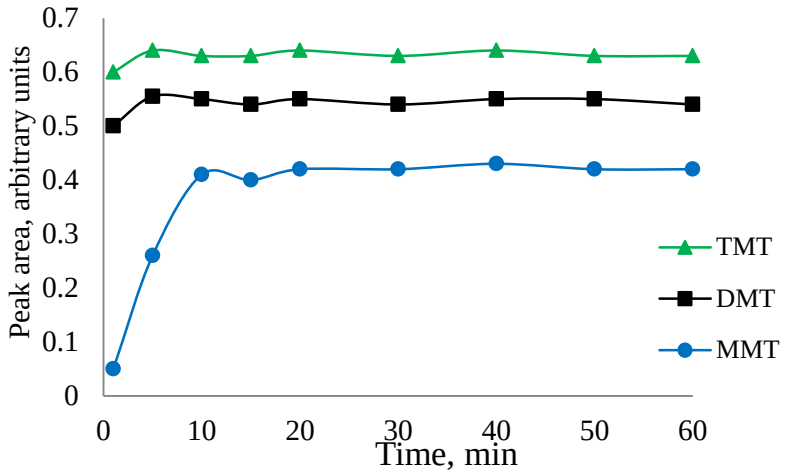

Fig. 2. Effect of the derivatization time on the derivatization efficiency. Sample volume 25 ml, concentration of methyltins 10 μg/l, derivatization with 0.04 % $NaBEt_4$, extraction with *n*-pentane for 2 min.

Optimization of GC-MS conditions

The derivatization conditions described above were optimized using methyltin extracts in *n*-pentane. However, *n*-pentane was not suitable for DLLME. The preliminary investigations showed, that the best extraction solvent for DLLME was 1,2-

dichlorobenzene. However, using 1,2-dichlorobenzene as an extraction solvent and applying sample injection into the hot injection port (250 °C), the peaks of the analytes were asymmetric and broad (Fig. 3A). It seems that in the case of hot injection, 1,2-dichlorobenzene did not condense immediately and thus occupied a broad zone at the beginning of the column. Trapping of the analytes in this broad zone resulted in chromatographic peak broadening.

In order to improve peak shapes, programmed temperature sample introduction was applied. At the initial injection port temperature below the boiling point of the solvent 1,2-dichlorobenzene (180°C), the peaks were sharp and symmetric (Fig. 3B). In order to determine the optimal injection temperature, the efficiencies (expressed by the number of theoretical plates, $N = 16 (t_R/w)^2$) were calculated at different injection temperatures. The results presented in Fig. 4 demonstrate, that 160 – 170°C is the optimal injection temperature. Thus, the following injection port temperature program was applied: the temperature was held at 160 °C for the first 1 min and raised to 250°C at the heating rate of 200 °C/min.

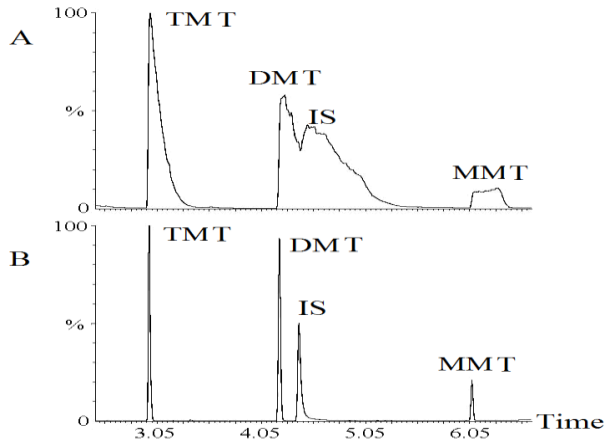

Fig. 3. Chromatograms of ethylated standard mixture of MMT, DMT and TMT (5 µg/l) and internal standard chlorobenzene after DLLME in 1,2-dichlorobenzene at: A - injection port temperature 250 °C, B – programmed injection port temperature (held at 160 °C for 1 min and raised to 250 °C at the heating rate of 200 °C/min). The oven temperature was programmed as follows: 50 °C for 1 min, to 90 °C at 15 °C/min, to 250 °C at 40 °C/min and held for 3 min.

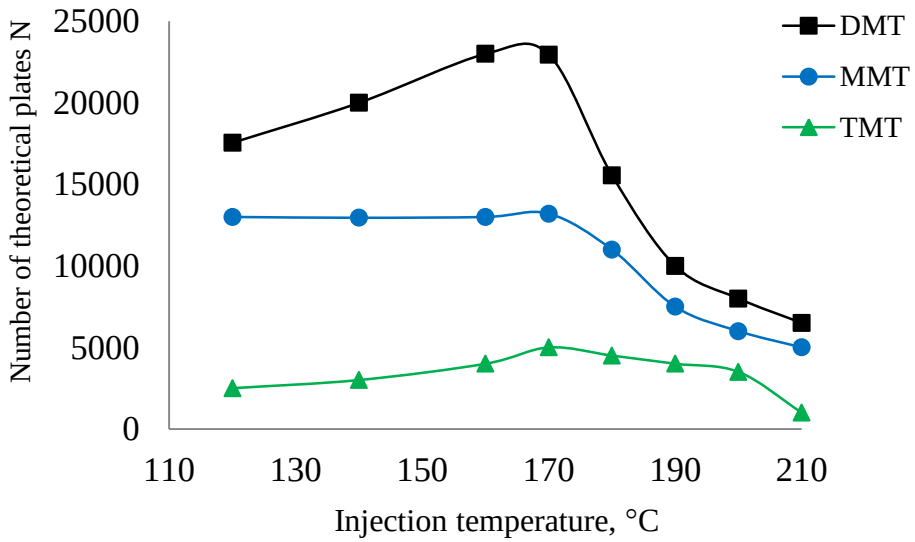

Fig. 4. Effect of initial injection port temperature on the column efficiency.

Optimization of DLLME conditions

Selection of an appropriate extraction solvent plays a main role for DLLME efficiency. An extraction solvent for traditional DLLME should have a higher density than water, should demonstrate a good extraction capability of the compounds of interest and its solubility in water should be low. In the case of subsequent GC analysis, the peak of an extraction solvent should be separated from analyte peaks. Tetrachloromethane, methyl benzoate, chlorobenzene and 1,2-dichlorobenzene were tested as extraction solvents for derivatized methyltins. To investigate the effect of extraction solvent, a mixture containing 500 µl of acetone and 50 µl of extraction solvent was rapidly injected to 8 ml of aqueous solution of derivatized methyltins. A cloudy solution formed was centrifuged at 5000 rpm and 1 µl of the organic phase was manually injected into the GC injection port. The peak of CCl_4 overlapped with the peak of TMT and the peak of chlorobenzene – with the peak of MMT. The peaks of the other two solvents were well separated from the peaks of the analytes with the retention times bigger than those of the analytes. Methyl benzoate showed 1.1–1.2 times higher extraction efficiency in comparison with 1,2-dichlorobenzene. However, the emulsion formed by methyl benzoate was very stable and 10 min of centrifugation were needed to separate the organic and water phases. Moreover, because of the relatively high water solubility of methyl benzoate (2100 mg/l) the volume of the organic phase settled in the bottom of the centrifuge tube was 17-19 µl, whereas in the case of 1,2-dichlorobenzene

(water solubility 160 mg/l) it was 41-43 µl. It means that the volume of 1,2-dichlorobenzene can be decreased twice or even more resulting in the increase of the analytes concentration in the extraction phase. Based on these considerations, 1,2-dichlorobenzene was chosen as an extraction solvent.

The main requirement for disperser solvent is its miscibility with both the extraction solvent and the aqueous phase. Only few solvents, namely acetone, acetonitrile, methanol and ethanol, fulfill this requirement and were studied. The mixture, containing 500 µl of the disperser solvent and 50 µl of 1,2-dichlorobenzene was used for DLLME. As it is demonstrated in Fig. 5, the highest extraction efficiency was achieved using ethanol, thus ethanol was selected as a disperser solvent.

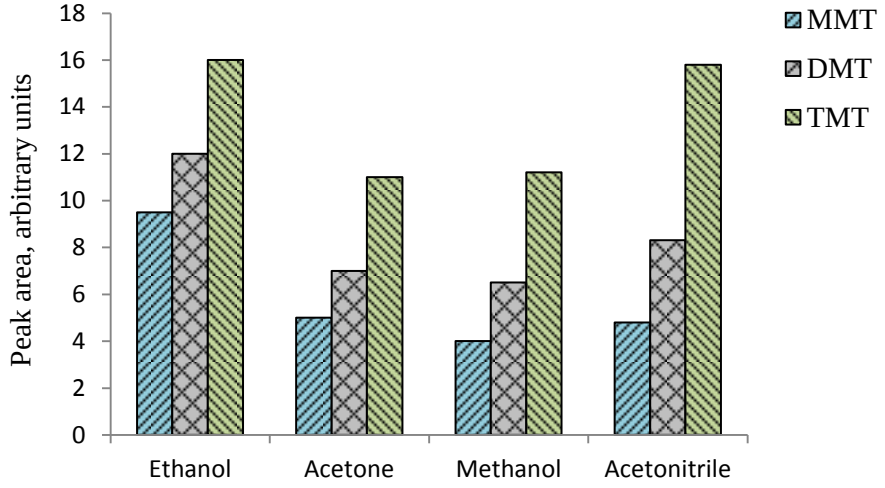

Fig. 5. Effect of the disperser solvent on the DLLME efficiency. Sample volume 8 ml, concentration of methyltins 5 µg/l, derivatization with 0.04 % $NaBEt_4$ for 10 min, extraction solvent 1,2-dichlorobenzene, centrifugation for 3 min at 5000 rpm.

To investigate the effect of the extraction solvent volume, a solution containing 500 µl of ethanol and 15 – 50 µl of 1,2-dichlorobenzene was used. With the increase in extraction solvent volume, peak areas initially increased and reached the maximum at 20 µl (Fig. 6A). Thus, for the further work 20 µl of 1,2-dichlorobenzene was used.

To investigate the effect of the disperser solvent volume, different ethanol volumes (0.1 – 0.9 ml) and 20 µl of extracting solvent were used. At low ethanol volume the cloudy state was not stable and probably this caused lower extraction efficiency.

When the ethanol volume exceeded 0.3 ml, the changes in extraction efficiency were insignificant (Fig. 6B). However, with the increase of ethanol volume also increased the stability of the emulsion. Thus using more than 0.5 ml of ethanol, 3 min centrifugation time was insufficient any more. Because of that 0.3-0.5 ml ethanol volume was considered as the optimum. For the further work, in order to have a convenient ethanol – 1,2-dichlorobenzene mixture volume for the injection (0.4 ml) and considering that the optimum 1,2-dichlorobenzene volume is 20 µL, 0.38 ml of ethanol volume was selected.

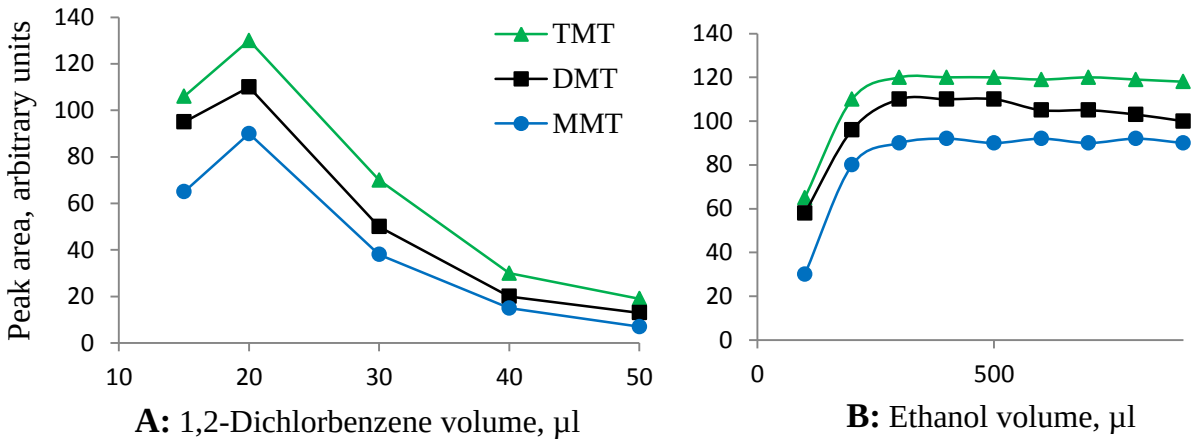

Fig. 6. Effect of the extraction solvent (1,2-dichlorobenzene) volume (A) and disperser solvent (ethanol) volume (B) on the DLLME efficiency. Sample volume 8 ml, concentration of methyltins 5 µg/l, derivatization with 0.04 % $NaBEt_4$ for 10 min, centrifugation for 3 min at 5000 rpm.

At selected DLLME conditions, a concentration of the derivatization reagent was additionally assayed in the range 0.0025–0.1%. For all the methyltins, peak areas increased with the increase of $NaBEt_4$ concentration up to 0.05 – 0.06% (Fig. 7). Based on the results, 0.06% concentration of $NaBEt_4$ was selected.

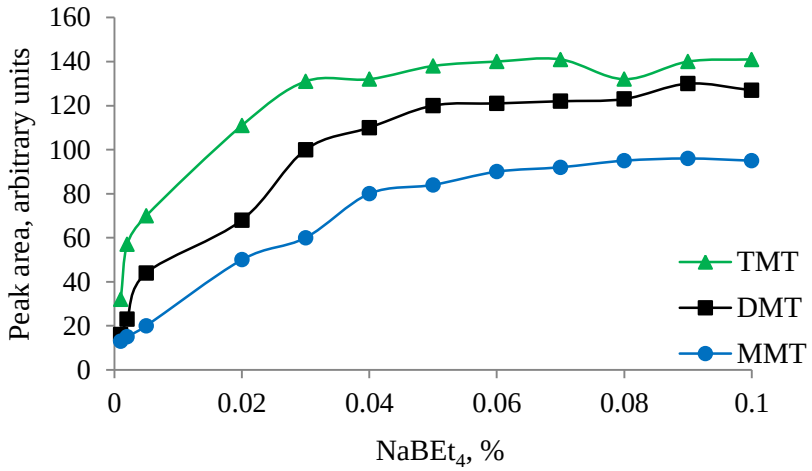

Fig. 7. Effect of $NaBEt_4$ quantity on the derivatization efficiency. Sample volume 8 ml, concentration of methyltins 5 µg/l, extraction solvent 1,2-dichlorobenzene (20 µl), disperser solvent ethanol (380 µl), centrifugation for 3 min at 5000 rpm.

Addition of salt to an aqueous sample solution generally causes a decrease in the solubility of organic compounds in water, and this feature has been widely used to enhance the extraction of the analyte. In our case the aqueous solution contained salts used for the buffer preparation and for derivatization. Further increase of the salt concentration was accomplished by addition of NaCl which is commonly used for this purpose. The addition of up to 0.005 g/ml of NaCl slightly promoted the transport of the analytes to the extracting drop. However, with the further increase of NaCl, the density of the organic phase resulted lower than that of the aqueous phase. Because of that the organic phase formed the upper phase in two-phase system and did not sediment any more. In order to avoid this, in further experiments NaCl was not added to the samples.

Validation of the method

The quality parameters of the suggested method such as linearity, limits of detection and repeatabilities were determined under the optimized extraction conditions. In order to alleviate injected extract volume error, chlorobenzene (1 µg/ml) was applied as an internal standard. For the determination of quality parameters GC-MS in SIM mode was used. The calibration curves were drawn with 8 calibration points with three replicate injections of the extracts obtained after applying DLLME procedure. The linear

ranges were from 0.43, 0.17 and 0.20 ng(Sn)/l up to 2 μg(Sn)/l for TMT, DMT and MMT, respectively. Correlation coefficients were 0.998–0.999. The repeatabilities were determined by five repetitions analysis for 10 and 20 ng(Sn)/l of methyltin compounds. Relative standard deviations were 6.9 – 12.1 % (table 1).

Table 1. Methyltins detection limits and result repeatabilities after DLLME

Analyte	Detection limit, ng(Sn)/l	RSD, % (n=5)	
		10 ng(Sn)/l	20 ng(Sn)/l
MMT	0.06	10.5	8.9
DMT	0.05	11.0	6.9
TMT	0.13	12.1	8.5

3.1.2. Liquid phase microextraction based on the solidification of a floating organic drop of methyltin compounds

Optimization of extraction conditions

An extraction solvent used for liquid phase microextraction based on the solidification of a floating drop (LPME-SFO) should demonstrate a good extraction capability of the compounds of interest; its solubility in water should be low and its melting point should be near room temperature (10 – 30 °C). Five potential extraction solvents: *n*-hexadecane, *n*-heptadecane, 1-undecanol, 1-dodecanol and 2-dodecanol were examined (Fig 8). 1-Undecanol demonstrated the best extraction efficiency and was selected as extraction solvent. The effect of the 1-undecanol volume on the extraction efficiency was also investigated and 20 μl of 1-undecanol was selected as the optimal volume of extraction solvent (Fig 9).

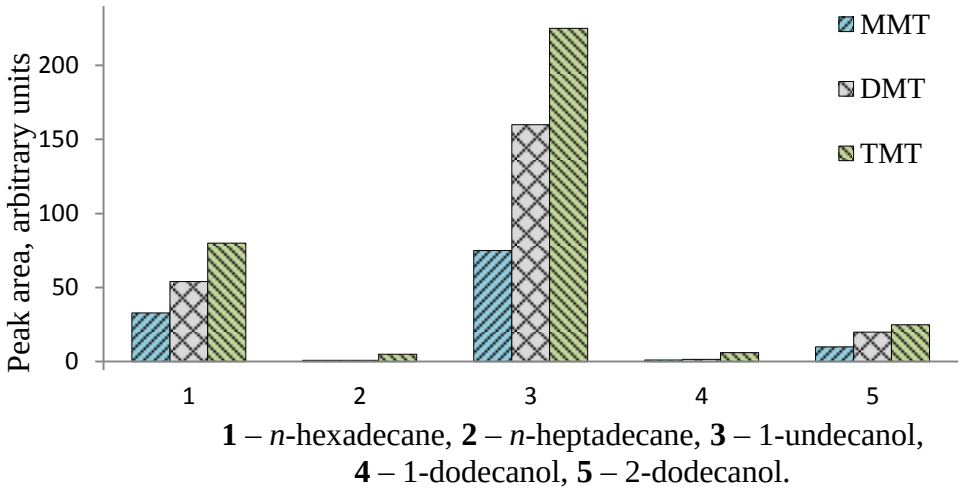

Fig. 8. Effect of the extraction solvent type on the LPME-SFO efficiency. Sample volume 15 ml, concentration of methyltins 1 µg/l, derivatization with 0.06% $NaBEt_4$ for 10 min, extraction time 15 min.

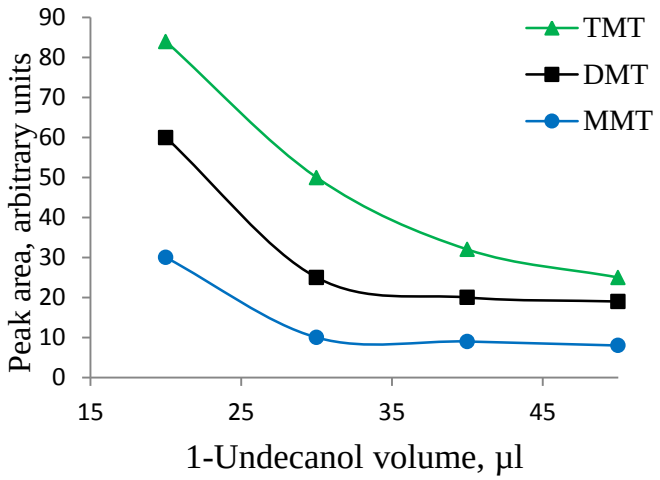

Fig. 9. Effect of the extraction solvent (1-undecanol) volume on the LPME-SFO efficiency. Sample volume 15 ml, concentration of methyltins 1 µg/l, derivatization with 0.06% $NaBEt_4$ for 10 min, extraction time 15 min.

The extraction time was studied between 5 and 50 min and 30 min extraction were selected for the further work.

Validation of the method

The quality parameters of the suggested method such as linearity, limits of detection and repeatabilities were determined under the optimized extraction conditions. In order to alleviate injected extract volume error, *n*-nonane (1 µg/l) was applied as an internal standard. The calibration curves were drawn with 5 calibration points with three

replicate injections of the extracts obtained after applying LME-SFO procedure. The linear ranges were from 521 ng(Sn)/l (MMT), 231 ng(Sn)/l (DMT) and 92 ng(Sn)/l (TMT) up to 1 mg(Sn)/l (for all analytes). Correlation coefficients were 0,994 – 0,995. The repeatabilities were determined by five repetitions analysis for 1.0 and 10.0 µg(Sn)/l of methyltin compounds. Relative standard deviations were 18.1-23.7 % (Table 2).

Table 2. OTC detection limits and result repeatabilities after LPME-SFO

Analyte	**Detection limit, ng(Sn)/l**	**RSD, % (n = 5)**	
		10 µg(Sn)/l	**1,0 µg(Sn)/l**
MMT	158	18.1	23.7
DMT	70	20.5	22.4
TMT	28	18.2	22.8

3.1.3. Application

Comparison of analytical characteristics of DLLME and LPME-SFO techniques demonstrated that dispersive liquid-liquid microextraction is more suitable technique for real samples analysis. Thus the proposed DLLME method was applied for the determination of methyltins in water samples from four rivers in Lithuania, namely Nemunas near Rusnė, Skirvytė near Rusnė, Šventoji in the estuary, and Akmena in the estuary. In all the four samples methyltin compounds were not detected. In order to assess the matrix effect, the standard addition method was applied. The water samples were spiked with 10 and 20 ng(Sn)/l of the studied methyltin compounds. The obtained results were compared with those obtained from spiked distilled water samples. Relative recoveries were determined as the ratio of the concentrations found in real and distilled water samples spiked at the same analyte concentrations. The data (Table 3) indicate that the river water matrix had little effect on the extraction efficiency.

Table 3. Relative recoveries and RSDs of methyltin compounds spiked river water (n = 5)

Analyte	**10 ng(Sn)/l** spiked water, relative recovery, % (RSD, %)				**20 ng(Sn)/l** spiked water, relative recovery, % (RSD, %)			
	Akmena	Nemunas	Skirvyte	Sventoji	Akmena	Nemunas	Skirvyte	Sventoji
MMT	98(11.4)	94 (9.8)	106(11.5)	95 (12.9)	105(9.9)	97 (8.7)	102(9.1)	93(10.6)
DMT	105(12.3)	94 (10.5)	107(12.0)	89 (12.2)	96(11.1)	106 (9.1)	103(7.4)	104(9.3)
TMT	93 (12.5)	108(10.4)	96 (12.6)	98 (10.5)	91 (9.6)	99 (9.4)	93 (6.8)	97 (8.5)

3.2. Microextraction and determination of butyltin compounds

3.2.1. Dispersive liquid-liquid microextraction of butyltin compounds

Derivatization of butyltin compounds

The variables involved in the derivatization reaction, such as solution pH, reaction time, $NaBEt_4$ concentration were optimized.

For derivatization conditions investigation experiments, liquid-liquid extraction was carried out prior to the GC-MS analysis as described in the section ”Derivatization of methyltin compounds”. Derivatization efficiency was studied in the pH range 3.5 - 7.5. The maximum derivatization efficiency was obtained at pH 4.5 (Fig. 10).

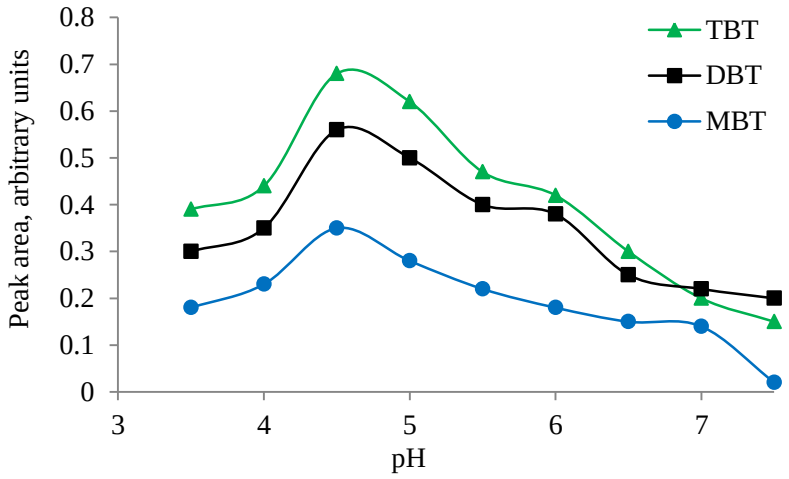

Fig. 10. Effect of pH on the derivatization efficiency. Sample volume 25 ml, concentration of butyltins 10 µg/l, derivatization with 0.04% $NaBEt_4$, derivatization time 15 min, extraction with *n*-hexane for 2 min.

The derivatization time was studied between 1 and 30 min. The results obtained showed that the peak areas of the analytes increased up to 5 min. Thus, 5 min derivatization time was chosen for further work.

Tetrachloromethane, chlorobenzene and bromobenzene were tested for the extraction of derivatized butyltins. To investigate the effect of extraction solvent nature, a mixture containing 500 µl of acetone and 50 µl of extraction solvent was rapidly injected to 8 ml of aqueous solution of derivatized butyltins. A cloudy solution formed was centrifuged for 3 min at 5000 rpm and 1 µl of the organic phase was manually injected into the GC injection port. CCl_4 showed the highest extraction efficiency. Moreover, due to the low boiling point (77 °C) this extraction solvent was easily separated from the analytes. Thus, tetrachloromethane was selected as an optimal extraction solvent.

Two disperser solvents, acetone and methanol, were studied. The mixture, containing 500 µl of the disperser solvent and 50 µl of CCl_4 was used for DLLME. As the extraction efficiency using methanol was 1.1 – 1.3 times higher than using acetone, methanol was selected as a disperser solvent.

To investigate the effect of the extraction solvent volume, a solution containing 500 µL of methanol and 15 – 50 µl of CCl_4 was used. With the increase in extraction solvent volume, peak areas initially increased and reached the maximum at 20 µl. Thus, 20 µl of extracting solvent CCl_4 was selected. (Fig. 11A).

To investigate the effect of the disperser solvent volume, different methanol volumes (0.1 – 1.0 ml) and 20 µl of extracting solvent were used. At low methanol volume the cloudy state was not stable and probably this caused lower extraction efficiency. When the methanol volume exceeded 0.6 ml, the changes in extraction efficiency were insignificant (Fig. 11B). Thus, 0.6 – 1.0 ml methanol volume was considered as the optimum. For the further work, in order to have a convenient methanol – tetrachloromethane mixture volume for the injection and considering that the optimum tetrachloromethane volume is 20 µl, 0.78 ml of methanol volume was selected.

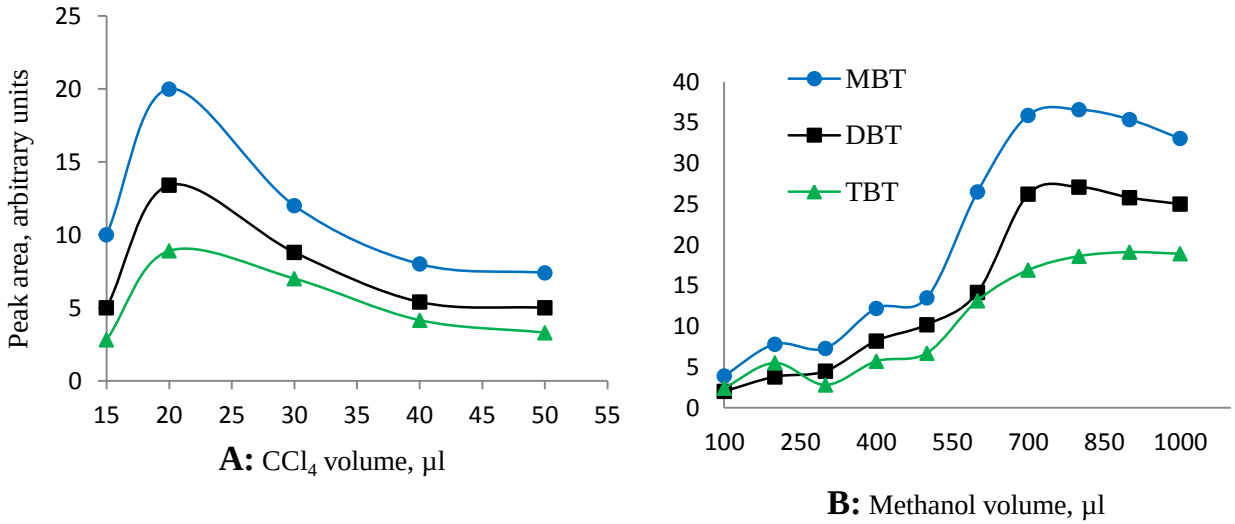

Fig. 11. Effect of the extraction solvent (CCl_4) volume (A) and disperser solvent (methanol) volume (B) on the DLLME efficiency. Sample volume 8 ml, concentration of butyltins 5 µg/l, derivatization with $NaBEt_4$ for 10 min, centrifugation for 3 min at 5000 rpm.

DLLME time was defined as the time between injection of the mixture of disperser solvent and extraction solvent and centrifuge step. Extraction time up to 20 min was investigated. Peak area variations at different extraction time were not significant. Evidently, due to the large surface area between the aqueous and organic phase, 20 – 30 seconds (that take place between the injection and the beginning of the centrifugation) are sufficient for the extraction.

The ionic strength of solution was modified by addition of NaCl which is commonly used for this purpose. However, with the addition of NaCl the extraction efficiency slightly decreased. Thus, in further experiments NaCl was not added to the samples.

At selected DLLME conditions a concentration of the derivatization reagent was additionally assayed in the range 0.005 – 0.08 % and 0.02 % concentration of $NaBEt_4$ was selected.

A chromatogram and mass spectra of derivatized butyltin compounds using the optimized DLLME and GC-MS operating conditions is presented in Fig. 12.

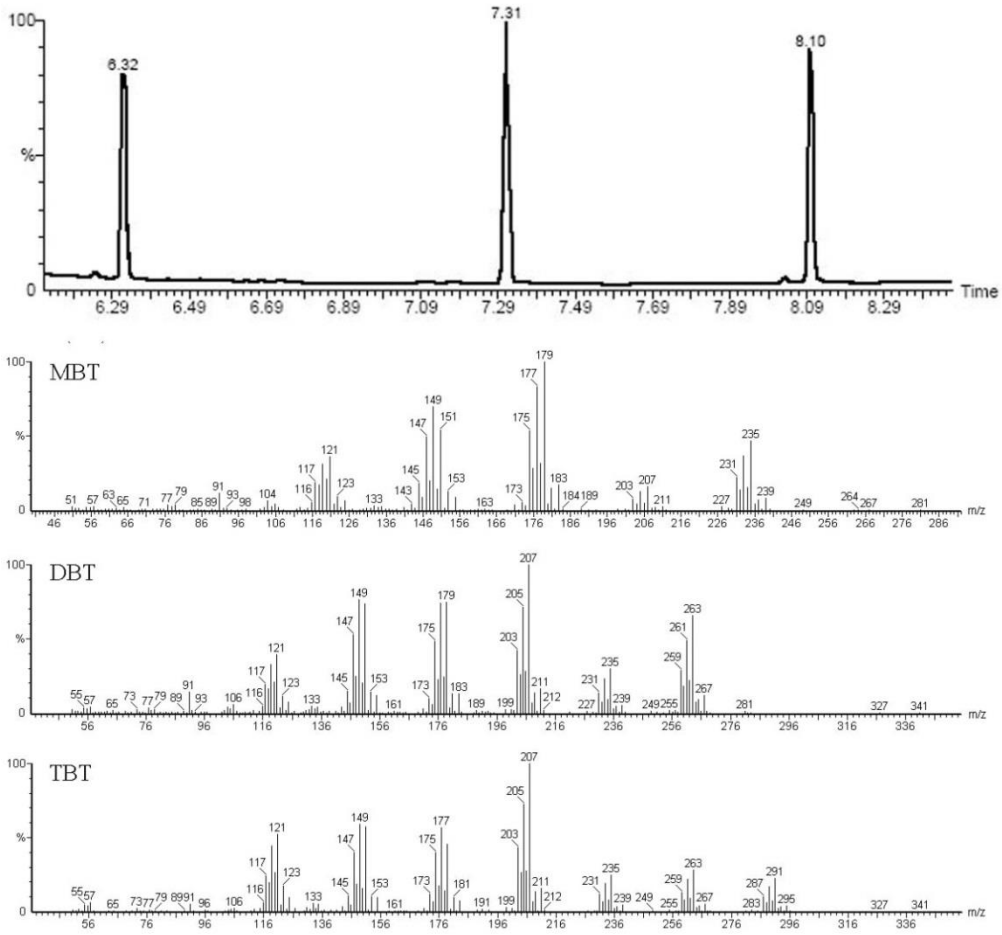

Fig. 12. Total ion GC-MS chromatogram and mass spectra of ethylated standard mixture of MBT, DBT and TBT. For GC-MS conditions see Experimental. RT: 6.32 min MBT, 7.31 min DBT, 8.10 min TBT.

Validation of the method

The quality parameters of the suggested method such as linearity, limits of detection and repeatabilities were calculated under the optimized extraction conditions. For the determination of quality parameters GC-MS determination in SIM mode was used.

In order to alleviate injected extract volume error, *n*-hexadecane (1 µg/l) was applied as an internal standard. The calibration curves were drawn with three replicate injections of the extracts obtained after applying DLLME procedure with 7 calibration points. The linear ranges were from 2.8, 4.2 and 9.8 ng(Sn)/l up to 10 µg(Sn)/l for MBT, DBT and TBT, respectively. Correlation coefficients were 0.996 – 0.999. The

repeatabilities were determined by five repetitions analysis for two concentrations of butyltin compounds. Relative standard deviations (RSDs) were calculated and are summarized in Table 4. These data show that repeatability of the method is satisfactory. Detection limits defined as three times of base-line noise are presented in Table 4.

Table 4. Butyltins detection limits and result repeatabilities after DLLME

Analyte	Detection limit, µg(Sn)/l	RSD, % (n=5)	
		0,1 µg(Sn)/l	10 µg(Sn)/l
MBT	0.0017	17.0	13.0
DBT	0.0025	15.1	3.5
TBT	0.0059	9.0	7.5

3.2.2. Liquid phase microextraction based on the solidification of a floating organic drop of butyltin compounds

Optimization of extraction conditions

Seven organic solvents, namely cyclohexanol, 1-chlorooctadecane, *n*-hexadecane, *n*-heptadecane, 1-undecanol, 1-dodecanol and 2-decanol, were tested as extraction solvents. The highest peak areas of the analytes were obtained using *n*-hexadecane, thus it was selected for the further work. The effect of the *n*-hexadecane volume on the extraction efficiency was also investigated and 20 µl of *n*-hexadecane was selected as the optimal.

The extraction time was studied between 5 and 60 min. and 30 min extraction were selected for the further work.

Validation of the method

The quality parameters such as linearity, limits of detection and repeatabilities were determined under the optimized extraction conditions. In order to alleviate injected extract volume error, 1-chlorooctadecane (1 µg/l) was applied as an internal standard. The calibration curves were drawn with 5 calibration points with three replicate injections of the extracts obtained after applying LME-SFO procedure. The linear ranges were from 1.0, 1.1 and 1.6 µg(Sn)/l up to 500 µg(Sn)/l for MBT, DBT and TBT,

respectively. Correlation coefficients were 0.996 – 0.989. The repeatabilities were determined by five repetitions analysis for 10.0 and 500.0 µg(Sn)/l of buthyltin compounds. Relative standard deviations were 15.6-31.0 % (Table 5).

Table 5. Butyltins detection limits and result repeatabilities after LPME-SFO

Analyte	Detection limit, µg(Sn)/l	RSD, % (n=5)	
		10 µg(Sn)/l	500 µg(Sn)/l
MBT	0.30	20.8	15.6
DBT	0.34	26.0	17.1
TBT	0.50	31.0	19.8

3.2.3. Application

Comparison of analytical characteristics of DLLME and LPME-SFO techniques demonstrated that dispersive liquid-liquid microextraction is more suitable technique for the determination of butyltin compounds. Thus the proposed DLLME method was applied for the determination of butyltins in river water samples. Samples from three rivers, namely Nemunas before Druskininkai, Neris before Paneriai, and Šventoji in the estuary, were taken for the analysis. In all the three samples the studied butyltin compounds were not detected. In order to assess matrix effect, the standard addition method was applied for the determination of butyltins. The water samples were spiked with 0.1 and 1 µg(Sn)/l of the studied butyltin compounds. The obtained results were compared with those obtained from spiked distilled water samples. The resulted relative recoveries are between 90 and 109 % (Table 6). This indicates that river water matrix had little effect on the extraction efficiency.

Table 6. Relative recoveries and RSDs of butyltin compounds spiked river water (n = 3)

Analyte	**0,1 µg(Sn)/l** spiked water, relative recovery, % (RSD, %)			**1,0 µg(Sn)/l** spiked water, relative recovery, % (RSD, %)		
	Nemunas	Neris	Sventoji	Nemunas	Neris	Sventoji
MBT	98 (14.2)	95 (9.9)	91 (10.8)	97 (9.3)	92 (9.0)	99 (8.4)
DBT	94 (11.8)	98 (10.4)	90 (13.2)	97 (10.9)	108 (8.8)	108 (10.1)
TBT	94 (15.1)	105 (8.8)	109 (12.3)	93 (11.2)	100 (6.7)	95 (10.6)

3.3. Microextraction and determination of phenyltin compounds

3.3.1. Dispersive liquid-liquid microextraction of phenyltin compounds

Derivatization of phenyltin compounds

The variables involved in the derivatization reaction, such as solution pH, reaction time, $NaBEt_4$ concentration were optimized. For derivatization conditions investigation experiments, liquid-liquid extraction was carried out prior to the GC-MS analysis as described in the section ” Derivatization of methyltin compounds”.

Derivatization efficiency was studied in the pH range 4–6.5. The maximum peak areas were obtained at pH 4.5. The derivatization time was studied between 5 and 40 min. The extraction efficiency increased up to 15 min and then remained constant. Thus, for the further work 15 min derivatization time was chosen.

DLLME conditions

Tetrachloromethane, chlorobenzene and 1,2-dichlorobenzene were examined for the extraction of derivatized phenyltins. A mixture containing 500 µl of acetone and 50 µl of the extraction solvent was rapidly injected to 8 ml of the aqueous solution of derivatized phenyltins. A cloudy solution formed was centrifuged at 5000 rpm and 1 µl of the sedimented organic phase was manually injected into the GC injection port. Tetrachloromethane showed the highest extraction efficiency (Fig. 13), thus it was selected as an extraction solvent.

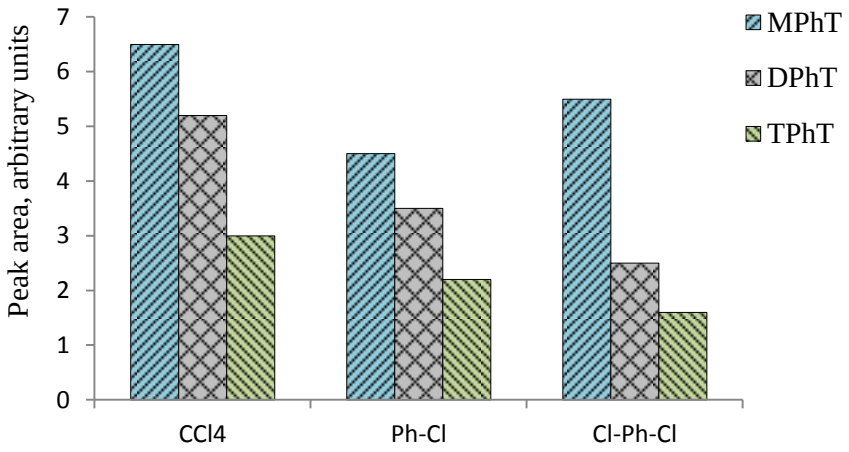

Fig. 13. Effect of the extraction solvent type on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 5 µg/l, derivatization with 0.04% $NaBEt_4$ for 15 min, centrifugation for 3 min at 5000 rpm.

Acetone, acetonitrile, methanol and ethanol, were studied as disperser solvents. The mixture, containing 500 µl of the disperser solvent and 50 µl of CCl_4, was used. Ethanol was selected as a disperser solvent because the extraction efficiency using ethanol was higher than using acetone, methanol or acetonitrile.

To investigate the effect of the extraction solvent volume, a solution containing 500 µl of ethanol and 15–50 µl of CCl_4 was used. With the increase in the extraction solvent volume, the peak areas initially increased and reached the maximum at 20 µl (Fig. 14). Thus, for the further work 20 µl of CCl_4 were used.

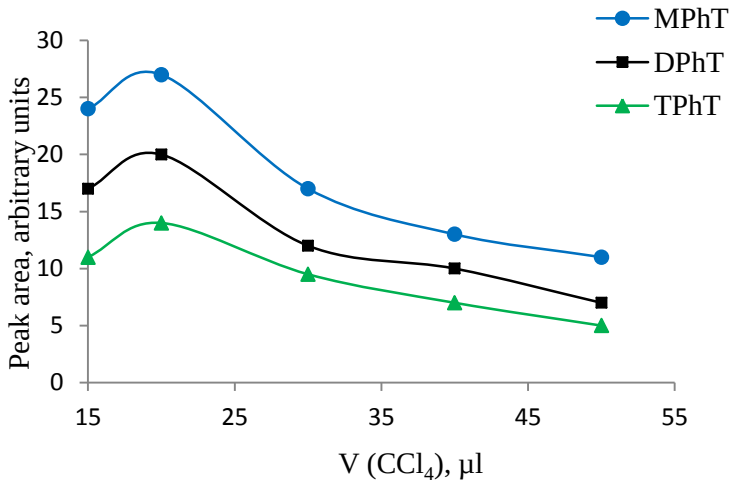

Fig. 14. Effect of the extraction solvent (CCl_4) volume on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 5 µg/l, derivatization with 0.04% $NaBEt_4$ for 15 min, disperser solvent ethanol (500 µl), centrifugation for 3 min at 5 000 rpm.

To investigate the effect of the disperser solvent volume, different ethanol volumes (0.1–0.7 ml) and 20 µl of the extracting solvent were used. As it is demonstrated in Fig. 15, the highest extraction efficiency was achieved using 0.4–0.6 ml of ethanol. For the further work, in order to have a convenient ethanol–tetrachloromethane mixture volume for the injection (0.5 ml) and considering that the optimum CCl_4 volume is 20 µl, 0.48 ml of ethanol was selected. At the optimized extraction conditions, a concentration of the $NaBEt_4$ was additionally assayed in the range 0.0025–0.05% and 0.04% concentration of $NaBEt_4$ was selected (Fig. 16).

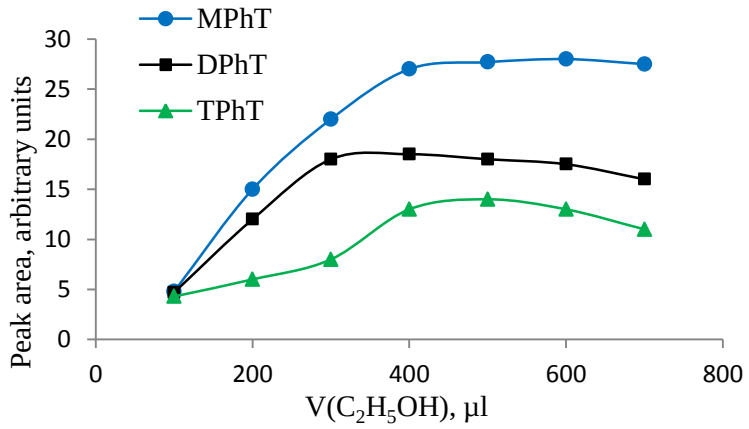

Fig. 15. Effect of the disperser solvent (ethanol) volume on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 5 µg/l, derivatization with 0.04% $NaBEt_4$ for 15 min, extraction solvent CCl_4 (20 µl), centrifugation for 3 min at 5000 rpm.

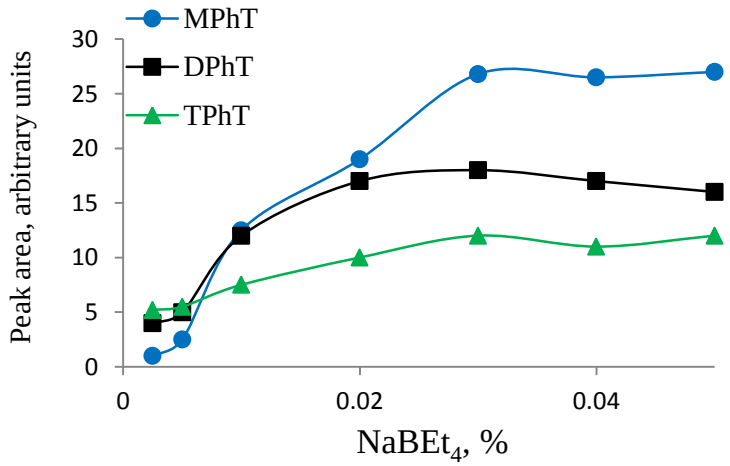

Fig. 16. Effect of $NaBEt_4$ quantity on the derivatization efficiency. Sample volume 8 ml, concentration of phenyltins 5 µg/l, derivatization with 0.04% $NaBEt_4$ for 15 min, extraction solvent CCl_4 (20 µl), disperser solvent ethanol (480 µl), centrifugation for 3 min at 5000 rpm.

Validation of the method

The quality parameters of the suggested method such as linearity, limits of detection and repeatabilities were determined under the optimized extraction conditions. In order to alleviate the injected extract volume error, hexachloroethane (1 µg/ml) was added to the extraction solvent as an internal standard. The calibration curves were drawn with 8 calibration points with three replicate injections of the extracts obtained after applying a DLLME procedure. The linear ranges were from 46, 191 and 152 ng(Sn)/l for MPhT, DPhT and TPhT, respectively, up to 1 mg(Sn)/l for all the analytes. The correlation coefficients were 0.996–0.999. The repeatabilities were determined by five repetitions analysis for 1 and 10 µg(Sn)/l of phenyltin compounds. The relative standard deviations were 4.6–17.3%. The limits of detection were defined as three times of base-line noise and were 14–58 ng(Sn)/l (Table 7).

Table 7. Phenyltins detection limits and result repeatabilities after DLLME

Analyte	Detection limit, µg(Sn)/l	RSD, % (n=5)	
		1 µg(Sn)/l	10 µg(Sn)/l
MPhT	0.014	10.1	4.6
DPhT	0.058	17.3	11.2
TPhT	0.046	13.5	10.8

3.3.2. Dispersive liquid-liquid microextraction based on the solidification of a floating organic drop of phenyltin compounds

Optimization of extraction conditions

Extraction solvents used for DLLME-SFO should demonstrate a good extraction capability of the compounds of interest; their solubility in water should be low and their melting point should be near room temperature (10 – 30 °C). In this work, seven organic solvents, namely cyclohexanol, 1-chlorooctadecane, *n*-hexadecane, *n*-heptadecane, 1-undecanol, 1-dodecanol and 2-decanol, were tested and their extraction efficiency were studied. Due to the highest analyte peak areas obtained, *n*-Hexadecane was selected as extraction solvent. Acetone, acetonitrile, methanol and ethanol, were studied as disperser solvents and ethanol was selected as the best.

To investigate the effect of the extraction solvent volume, a solution containing 500 µl of ethanol and 15–50 µl of *n*-hexadecane was used. With the increase in the extraction solvent volume, the peak areas initially increased and reached the maximum at 15 µl. Thus, for the further work 15 µl of *n*-hexadecane were used.

To investigate the effect of the disperser solvent volume, different ethanol volumes (0.1–0.7 ml) and 15 µl of the extracting solvent were used. The highest extraction efficiency was achieved using 0.4 ml of ethanol. For the further work, in order to have a convenient ethanol– *n*-hexadecane mixture volume for the injection (0.4 ml) and considering that the optimum *n*-hexadecane volume is 15 µl, 0,385 ml of ethanol was selected.

Validation of the method

The quality parameters of the suggested method such as linearity, limits of detection and repeatabilities were determined under the optimized extraction conditions. In order to alleviate the injected extract volume error, chlorohexadecane (1 µg/ml) was added to the extraction solvent as an internal standard. The calibration curves were drawn with 8 calibration points with three replicate injections of the extracts obtained after applying a DLLME-SFO procedure. The linear ranges were from 248, 198 and 228 ng(Sn)/l for MPhT, DPhT and TPhT, respectively, up to 10 µg(Sn)/l for all the analytes. The correlation coefficients were 0.993–0.997. The repeatabilities were determined by five repetitions analysis for 1 and 10 ng(Sn)/l of phenyltin compounds. The relative standard deviations were 18.4-32.8%. The limits of detection are presented in Table 8.

Table 8. Phenyltins detection limits and result repeatabilities after DLLME-SFO

Analyte	Detection limit, µg(Sn)/l	RSD, % (n=5)	
		1 µg(Sn)/l	10 µg(Sn)/l
MPhT	0.075	23.1	22.6
DPhT	0.060	24.1	18.4
TPhT	0.069	32.8	26.9

3.3.3. Application

Comparison of analytical characteristics of DLLME and DLLME-SFO techniques demonstrated that DLLME is more suitable technique for the determination of phenyltin compounds, thus it was applied for the determination of phenyltins in river water samples. Samples from three rivers in Lithuania, namely Nemunas near Kaunas, Venta near Kuršėnai, and Akmena in the estuary, were taken for the analysis. In all the three samples the studied phenyltin compounds were not detected. In order to assess the matrix effect, the water samples were spiked with 1.0 and 10.0 µg(Sn)/l of the studied phenyltin compounds. The obtained results were compared with those obtained from spiked distilled water samples. Relative recoveries were determined as the ratio of the concentrations found in real and distilled water samples spiked at the same analyte concentration and were between 91 and 108% indicating that the river water matrix has little effect on the extraction efficiency (Table 9).

Table 9. Relative recoveries and RSDs of phenyltin compounds spiked river water (n = 3)

Analyte	**1,0 µg(Sn)/l** spiked water, relative recovery, % (RSD, %)			**10,0 µg(Sn)/l** spiked water, relative recovery, % (RSD, %)		
	Nemunas	Venta	Akemena	Nemunas	Venta	Akmena
MPhT	94 (13.4)	100 (16.0)	95 (12.6)	99 (15.5)	108 (18.0)	104 (14.8)
DPhT	97 (15.2)	98 (18.4)	102 (21.2)	95 (16.9)	102 (20.0)	93 (20.5)
TPhT	91 (17.9)	101 (19.6)	104 (22.0)	97 (19.3)	92 (21.1)	96 (21.0)

CONCLUSIONS

1. Derivatization conditions of organotin compounds have been examined and optimized: derivatization reaction pH= 3 for methyltins and pH= 4.5 for butyl- and phenyltins, derivatization time is 10 min for methyltins, 10 min for butyltins and 15 min for phenyltins. Gas chromatographic-mass spectrometric conditions for derivatized organotin compounds have been optimized.

2. A possibility to apply dispersive liquid-liquid microextraction for the extraction of organotin compounds has been investigated. Extraction and disperser solvents were selected: 1,2-dichlorobenzene and ethanol for methyltins, tetrachloromethane and methanol for butyltins and tetrachloromethane and ethanol for phenyltins, respectively. It was determined that for all the OTC 20 µl extraction solvent volume was sufficient. Analytical characteristics of the prepared techniques have been determined: limits of detection were 0.05 – 0.13 ng(Sn)/l for MTC, 1.7 – 5.9 ng(Sn)/l for BTC and 14 – 58 ng(Sn)/l for PhTC, relative standard deviations were 6.9 – 12.1 % for MTC, 9.0 – 17.0 % for BTC and 4.6 – 17.3 % for PhTC.

3. A possibility to apply microextraction based on the solidification of a floating organic droplet for the extraction of methyl- and butyltin compounds has been investigated. For methyl- and butyltin compounds, n-hexadecane and 1-undecanol have been selected as extraction solvents, respectively. Analytical characteristics of the prepared techniques have been determined: limits of detection were 28 – 158 ng(Sn)/l for MTC and 300 – 500 ng(Sn)/l for BTC, relative standard deviations were 18.1 – 23.7% for MTC and 15.6 – 31.0 % for BTC.

4. A possibility to apply dispersive liquid– liquid microextraction based on solidification of a floating organic droplet for the extraction of phenyltin compounds has been investigated. n-Hexadecane has been selected as an extraction solvent and ethanol has been selected as a disperser solvent. Analytical

characteristics of the prepared technique have been determined: limits of detection were 60 – 75 ng(Sn)/l, relative standard deviations were 18.4 – 32.8%.

5. Comparison of the prepared microextraction techniques demonstrated that dispersive liquid-liquid microextraction is the most suitable technique for real samples analysis. This technique has been applied for the analysis of Lithuania rivers water and it was determined that water samples from the rivers Nemunas, Skirvytė, Šventoji, Akmena, Venta and Neris did not contain organotin compounds or that the concentrations of the compounds were below the limits of detection. A standard addition method has been applied and it was determined that the river water matrix had little effect on the extraction efficiency.

THE LIST OF ORIGINAL PUBLICATIONS BY THE AUTHOR

Articles in journals

1. **Vaida Šmitienė**, Inga Baškirova, Vida Vičkačkaitė. Speciation of butyltins by dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry. *Chemija* **24**(3) (2013) 210-216.
2. **V. Smitiene**, I. Semasko, V. Vickackaite. Speciation of methyltins by dispersive liquid-liquid microextraction and gas chromatography with mass spectrometry. *J. Sep. Sci*. **37** (2014) 1989-1995.
3. **Vaida Šmitienė**, Birutė Bugelytė, Vida Vičkačkaitė. Phenyltin compounds: dispersive liquid-liquid microextraction and gas chromatographic-mass spectrometric determination. *Chemija* **26** (2015) 32-37.

Published contributions to academic conferences

1. **Vaida Šmitienė**, Vida Vičkačkaitė. Determination of organotin compounds in water using GC-MS with selected ion recording. Konferencijos „10th International conference of Lithuanian chemists „Chemistry 2011““ tezės, Vilnius, Lietuva (2011) 101.

2. **V. Šmitienė**, I. Semaško, I. Baškirova, V. Vičkačkaitė. Dispersive liquid-liquid microextraction of methyltin compounds. Konferencijos „18th International Scientific Conference „EcoBalt 2013““ tezės, Vilnius, Lietuva, (2013) 33.

3. **V. Smitiene**, I. Semasko, V. Vickackaite. Dispersive Liquid-Liquid Microextraction of Methylstannanes. Konferencijos "16th International Symposium on Advances in Extraction Technologies, ExTech 2014" tezės, Chania-Kreta, Graikija, (2014) 145.

CURRICULUM VITAE

Name, surname Vaida Šmitienė

Birth date July 29, 1985

Education:

2010 - 2015 Vilnius University, Faculty of Chemistry, The Department of Analytical and Environmental Chemistry, PhD studies.

2008 - 2010 Vilnius University, Faculty of Chemistry, The Department of Analytical and Environmental Chemistry, Master‘s degree.

2004 - 2008 Vilnius University, Faculty of Chemistry, The Department of Organic Chemistry, Bachelor‘s degree.

ORGANINIŲ ALAVO JUNGINIŲ MIKROEKSTRAKCIJA IR DUJŲ CHROMATOGRAFINIS NUSTATYMAS

SANTRAUKA

Šioje daktaro disertacijoje apibendrintų mokslinių tyrimų tikslas - ištirti skysčių-skysčių mikroekstrakcijos metodų galimybes greitai ir efektyviai organinių alavo junginių (OAJ) ekstrakcijai bei paruošti ir pritaikyti tinkamiausią metodiką vandens mėginių analizei.

OAJ realiuose mėginiuose egzistuoja katijono forma, yra mažai lakūs ir poliniai, todėl prieš atliekant šių junginių nustatymą dujų chromatografijos metodu, jie derivatizuojami. Derivatizacijai naudotas natrio tetraetilboratas. Ištirtos OAJ derivatizavimo sąlygos (terpės pH, derivatizacijos trukmė, derivatizacijos reagento koncentracija), optimizuotos derivatizuotų OAJ dujų chromatografinio-masių spektrometrinio nustatymo sąlygos. Ištirtos trijų skysčių-skysčių mikroekstrakcijos metodų - dispersinės skysčių-skysčių mikroekstrakcijos, mikroekstrakcijos užšaldomu tirpiklio lašu ir dispersinės skysčių-skysčių mikroekstrakcijos panaudojant užšaldomą tirpiklio lašą – galimybės ekstrahuoti OAJ iš vandeninių tirpalų. Optimizavus tirtų ekstrakcijos metodų sąlygas, nustatytos pagrindinės analizinės charakteristikos: aptikimo ribos, tiesiniai koncentracijų intervalai, rezultatų pasikartojamumas. Nustatyta, kad tinkamiausia metodika realių mėginių analizei yra dispersinė skysčių-skysčių mikroekstrakcija. Šis metodas buvo pritaikytas upių vandens analizei. Nustatyta, kad šešių Lietuvos upių vandens mėginiuose organinių alavo junginių nėra arba jų koncentracijos mažesnės už aptikimo ribas.