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Speciation of butyltins by dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry

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Dispersive liquid-liquid microextraction in combination with an in situ derivatization is suggested for butyltin compounds sampling and preconcentration from water solutions. The derivatization was carried out with sodium tetraethylborate at pH 4.5. The effects of extraction and disperser solvents type, volume, extraction time and ionic strength of the solution on the extraction efficiency were investigated. Tetrachloromethane containing *n*-hexadecane as an internal standard was used as an extracting solvent and methanol was used as a disperser solvent. The calibration graphs were linear from 2.8, 4.2 and 9.8 ng L^{-1} up to 10 μg L^{-1} for monobutyltin, dibutyltin and tributyltin, respectively, correlation coefficients were 0.996–0.999, detection limits were 1.7, 2.5 and 5.9 ng L^{-1} for monobutyltin, dibutyltin and tributyltin, respectively. Repeatabilities of the results were acceptable with relative standard deviations up to 17%. A possibility to apply the proposed method for butyltin compounds determination in water samples was demonstrated.

Key words: dispersive liquid-liquid microextraction, gas chromatography, butyltin compounds, water samples

INTRODUCTION

Butyltin compounds have a broad range of applications such as in polyvinylchloride as stabilizers, industrial catalysts, insecticides, fungicides, bactericides, wood preservatives, they have been also used as additives in antifouling paints for ship hulls [1]. Because of their widespread use, butyltin compounds can be found in different ecosystems [2]. Butyltin compounds are among the most toxic anthropogenic compounds introduced into the environment [3]. 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) [4]. International Maritime Organization prohibited to use organotins in anti-fouling paints used on ships since 2008 [5].

Toxicity of butyltin compounds is strongly dependent on the species. Tributyltin is very toxic and biologically active even when it is present in the environment in ultra-trace concentrations. Dibutyltin and monobutyltin are less toxic and are mostly present as degradation products of tributyltin. They are useful as indicators to degradation studies [6].

The development of accurate and sensitive analytical methods for butyltin determination is of special importance. Because of the necessity to determine different organotin species at low concentrations, gas chromatography with different detectors, such as a flame ionisation detector [7], a flame photometric detector [8, 9], an atomic emission spectrometric detector [10, 11], a mass spectrometric detector [11–13], an inductively coupled plasma mass spectrometric detector [14–16], is widely applied for the analysis.

As mono-, di- and tributyltin compounds present in the environment are in the ionized form, they need to be derivatized before gas chromatographic analysis to obtain their

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volatile and thermostabile forms. In the literature, several derivatization strategies are described. The most commonly used derivatization reactions are hydride generation with sodium borohydride, ethylation with sodium tetraethylborate ($NaBEt_4$) and alkylation with Grignard reagents [17, 18].

Since environmental concentrations of butyltins are low, as a rule, prior to the gas chromatographic determination a preconcentration is performed. In recent years, a preconcentration using microextraction techniques is gaining a growing interest. For butyltins extraction, a miniaturized version of solid phase extraction – solid phase microextraction is quite popular [7, 11, 19–22]. As a rule, derivatized butyltins are extracted from headspace. Only few articles deal with butyltins liquid phase microextraction: in [23, 24] single drop microextraction is followed by gas chromatographic analysis, in [25] organotins are extracted into a single drop of ionic liquid and analyzed by HPLC.

Recently introduced dispersive liquid-liquid microextraction (DLLME) [26] is based on the ternary solvent system. A mixture of water-immiscible extraction solvent which is dissolved in a water-miscible disperser solvent is injected rapidly into the aqueous phase. A cloudy solution is formed. It consists of fine droplets of extraction solvent that are dispersed into an aqueous phase. Due to the considerably large surface area of the finely dispersed extraction solvent, the extraction of the analytes is achieved rapidly. The extraction solvent containing the analytes is separated by centrifugation and analysed by an appropriate method.

Till now only one article has been published for butyltin compounds determination using dispersive liquid-liquid microextraction [8]. The extracted analytes were determined by gas chromatography-flame photometric detection.

This paper reports the results of the optimization of dispersive liquid-liquid microextraction and gas chromatographic-mass spectrometric determination for the speciation analysis of butyltin compounds in aqueous solutions.

EXPERIMENTAL

Reagents

Monobutyltin trichloride (MBT) (95%), dibutyltin dichloride (DBT) (96%), tributyltin chloride (TBT) (96%), sodium tetraethylborate ($NaBEt_4$) (97%), acetone (99.9%), *n*-hexane (98.5%), *n*-hexadecane (99%), methanol (99.95%), tetrachloromethane (99.5%), chlorobenzene (99%) were purchased from Sigma-Aldrich (Germany). NaCl (analytical grade) was purchased from Reachim (Ukraine).

Individual standard stock solutions each containing 10 mg mL^{-1} of MBT, DBT and TBT were prepared in methanol. Combined standard solution containing 100 μg mL^{-1} of all the three butyltins (MBT, DBT and TBT) was prepared in methanol from individual standard stock solutions. The solutions were stored at +4 °C in the dark. Working standard solutions were prepared daily by diluting the combined standard solution with distilled water.

The buffer solution was prepared by dissolving the necessary amount of sodium acetate in distilled water to get 0.1 M concentration and then adding acetic acid to adjust the pH to 4.5.

Instrumentation

The chromatographic analysis was performed on a Perkin-Elmer Clarus 580 series gas chromatograph coupled to a PerkinElmer Clarus 560 S mass spectrometer (PerkinElmer, Shelton, USA). The GC system was equipped with an 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).

GC-MS conditions

Helium was employed as a carrier gas with a constant flow of 1 mL min^{-1}. The injector temperature was held at 250 °C. Injection was performed in the pulsed splitless mode (pulsed to 4 mL min^{-1} until 1.5 min, split (50:1) open at 1.55 min).

The oven temperature was programmed as follows: 80 °C for 3 min, from 80 to 210 °C at 25 °C min^{-1}, from 210 to 250 °C at 40 °C min^{-1} and held at 250 °C for 3 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 as follows: electron energy 70 eV and temperature 180 °C.

GC-MS in the full scan mode was used for the optimization of the DLLME method. The analyses were carried out with a filament multiplier delay of 5 min and the acquisition was performed in the range of m/z 50–500. In order to improve sensitivity and reduce interferences, the selected ion monitoring (SIM) mode was used for the quantitative analysis. The ions with the high abundance which was different to the ions of fragments of the column bleed were chosen. The quantification ions (m/z values) were the following: 179 and 235 for MBT, 179, 235 and 263 for DBT, 235, 263 and 291 for TBT and 226 for internal standard *n*-hexadecane.

Derivatization and DLLME procedure

Optimized derivatization and DLLME procedure was the following: to a 10 mL centrifuge tube with a conic bottom 8 mL of butyltin compounds aqueous solution adjusted to pH 4.5 and 80 μL of 2% of $NaBEt_4$ (derivatization reagent) were placed. The solution was left for 5 min for derivatization of butyltin compounds. Then 800 μL of the mixture containing 780 μL of methanol (as a disperser solvent) and 20 μL of tetrachloromethane (as a extraction solvent) containing *n*-hexadecane as an internal standard (1 μg mL^{-1}) were rapidly injected to the solution using a 1 mL syringe. A cloudy solution formed was centrifuged for 3 min at 5 000 rpm. The carbon tetrachloride phase with the analytes was sedimented in the bottom of the tube. One μL of the extraction phase was injected into GC-MS.

RESULTS AND DISCUSSION

Derivatization conditions

Derivatization is one of the key factors in butyltin analysis. 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: to 25 mL of 10 μg L^{-1} aqueous butyltin solution, 100 μL of 10% $NaBEt_4$ solution was added (resulting in 0.04% $NaBEt_4$ concentration in the solution of butyltins) and after 15 min the solution was vigorously extracted with 1 mL of *n*-hexane for 2 min. The extract was transferred into the sampling vial and automatically injected into the GC injection port.

The pH value is a critical parameter in aqueous phase derivatization. The organotins act as weak acids that favour the reaction with $NaBEt_4$. Thus, pH values should be as low as possible. However, at pH ≤ 2, $NaBEt_4$ is rapidly decomposed to BEt_3 and ethane [18].

In this work, derivatization efficiency was studied in the pH range 4–6 using acetate buffer solutions. The maximum sensitivity was obtained at pH 4.5.

The derivatization time was studied between 1 and 30 min. The results obtained showed that the peak areas of the analytes increased up to 5–10 min (Fig. 1). Thus, 5 min derivatization time was chosen for further work.

DLLME conditions

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. Tetrachloromethane, chlorobenzene and bromobenzene were compared in the extraction of derivatized butyltins. To investigate the effect of extraction solvent, 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 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 in comparison with chlorobenzene and bromobenzene. 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.

The main requirement for the disperser solvent is its miscibility with the extraction solvent and aqueous phase. Only few solvents, namely acetone, acetonitrile, methanol and ethanol, fulfil this requirement. In this work, 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.

In order to alleviate the injected extract volume error, *n*-hexadecane (1 μg mL^{-1}) was added to the extraction solvent as an internal standard.

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 the extraction solvent volume, peak areas initially increased and reached the maximum at 20 μL.

Probably, because of a partial sedimentation of tetrachloromethane on the centrifuge tube walls, in the case of 15 μL of CCl_4, its volume in the bottom of the centrifuge tube was too small and some water phase instead of the extraction phase was withdrawn into a microsyringe. On the other hand, when the extraction solvent volume exceeded 20 μL because of the bigger dilution of the analytes, peak areas of the analytes decreased. Thus, 20 μL of extracting solvent CCl_4 was selected.

To investigate the effect of the disperser solvent volume, different methanol volumes (0.1–1.0 mL) and 20 μL of the 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

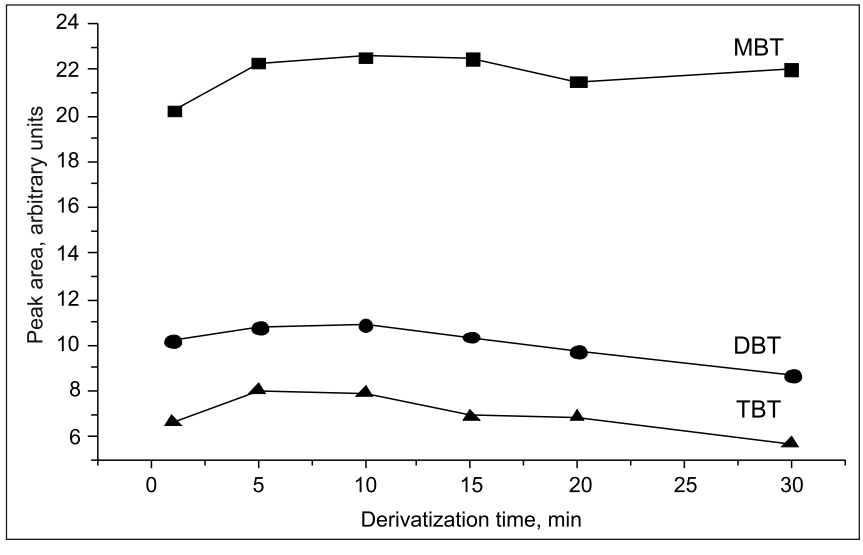

Fig. 1. Effect of the detivatization time on the derivatization efficiency

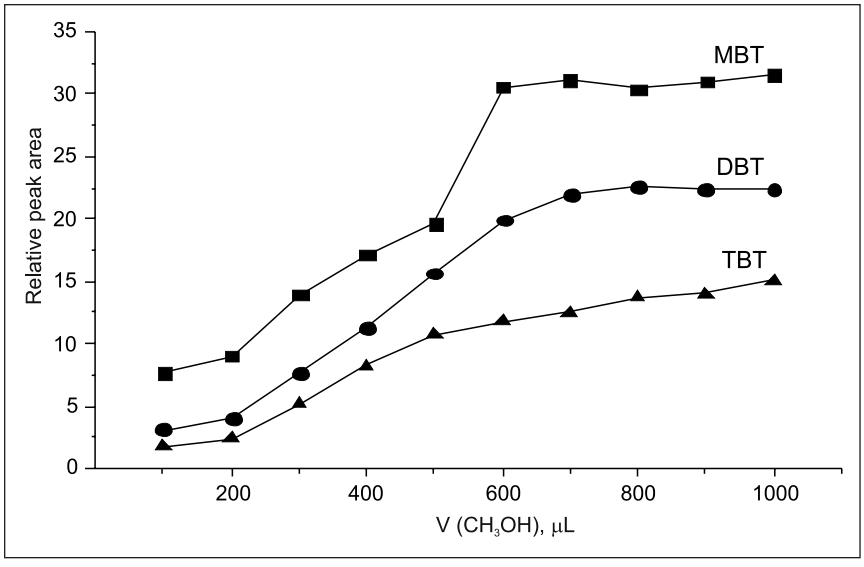

Fig. 2. Effect of the disperser solvent (methanol) volume on the DLLME efficiency. Sample volume 8 mL, concentration of butyltins 10 μg L^{-1}, CCl_4 volume 20 μL, internal standard *n*-hexadecane (1 μg mL^{-1} in CCl_4)

0.6 mL, the changes in extraction efficiency were insignificant (Fig. 2). Thus, the 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.

DLLME time was defined as the time between the injection of the mixture of the disperser solvent and the extraction solvent, and the 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 the 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.

As it was mentioned above, for preliminary studies, concentration of the derivatization reagent $NaBEt_4$ in the solution of butyltins was 0.04%. At selected DLLME conditions, a concentration of the derivatization reagent was additionally assayed in the range 0.005–0.09%.

For all the butyltins, relative peak areas increased with the increase of $NaBEt_4$ concentration (Fig. 3a). This indicates that the derivatization efficiency increases with the derivatization reagent concentration. On the other hand, absolute peak areas of the analytes at high $NaBEt_4$ concentrations decreased (Fig. 3b). This indicates that with the increase in derivatization reagent concentration the efficiency of DLLME decreases. Probably it can be explained by the fact that high $NaBEt_4$ concentration promotes bubble formation when the mixture

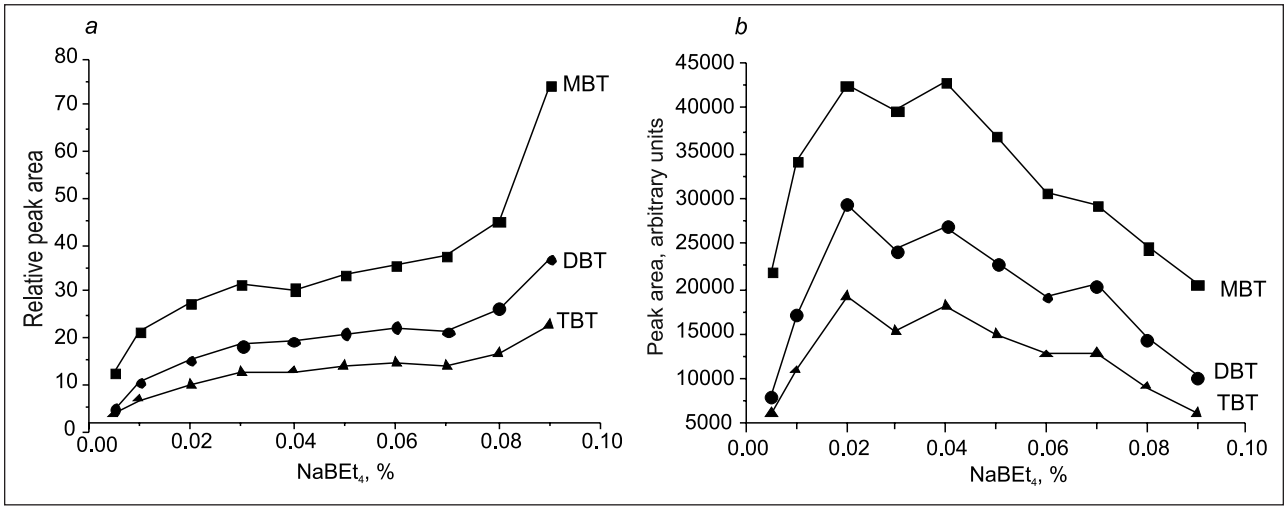

Fig. 3. Effect of the derivatization reagent concentration on the relative peak areas (*a*) and absolute peak areas (*b*) of butyltins. Sample volume 8 mL, concentration of butyltins 10 μg L^{-1}, methanol volume 780vμL, CCl_4 volume 20 μL, internal standard *n*-hexadecane (1 μg mL^{-1} in CCl_4), derivatization time 5 min

of methanol and CCl_4 is injected. Thus the extraction phase is surrounded by the gas bubbles, the interface area between the extraction phase and the aqueous phase decreases and the extraction efficiency also decreases. In addition, high percentage of the derivatization reagent caused the enhancement of the mass chromatogram baseline. Based on the results, 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 are presented in Fig. 4.

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 in SIM node was used.

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 L^{-1} up to 10 μg L^{-1} 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 1. These data show that repeatability of the method is satisfactory.

Detection limits defined as three times of base-line noise are presented in Table 1.

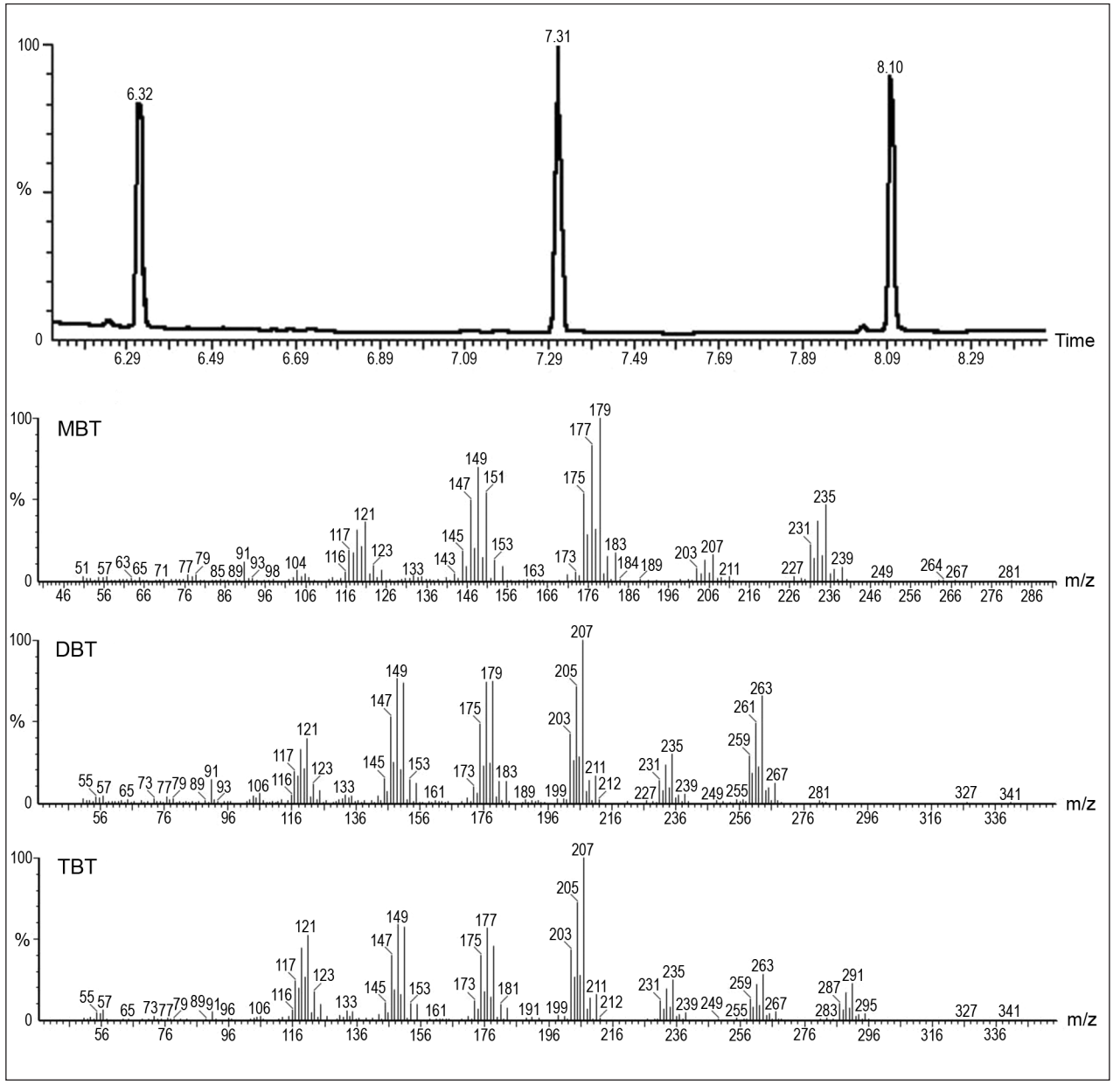

Fig. 4. Total ion GC-MS chromatogram and mass spectra of the ethylated standard mixture of MBT, DBT and TBT. For GC-MS conditions, see Experimental

Table 1. **Repeatabilities and detection limits**

Analyte	Detection limit, ng L^{-1}	RSD, % (n = 5)	
		0.1 µg L^{-1}	10 µg L^{-1}
MBT	1.7	17.0	13.0
DBT	2.5	15.1	4.8
TBT	5.9	9.0	7.5

Table 2. **Relative recoveries and RSDs of butyltin compounds spiked river water (n = 3)**

Analyte	0.1 µg L^{-1} spiked water, relative recovery, % (RSD, %)			1 µg L^{-1} spiked water, relative recovery, % (RSD, %)		
	Nemunas	Neris	Šventoji	Nemunas	Neris	Šventoji
MBT	98.3 (14.2)	94.7 (9.9)	90.9 (10.8)	96.6 (9.3)	92.0 (9.0)	99.5 (8.4)
DBT	94.0 (11.8)	97.6 (10.4)	89.6 (13.2)	97.4 (10.9)	107.6 (8.8)	108.2 (10.1)
TBT	93.9 (15.1)	105.1 (8.8)	109.3 (12.3)	93.3 (11.2)	99.5 (6.7)	94.6 (10.6)

Application

The proposed method was applied for the determination of butyltins in river water samples. Samples from three rivers, namely Nemunas near Druskininkai, Neris near Paneriai, and Šventoji in the estuary, were taken for the analysis. The derivatization, extraction and GC-MS analysis procedures were as described above. In all the three samples the studied butyltin compounds were not detected. In order to assess the matrix effect, the standard addition method was applied for the determination of butyltins. The water samples were spiked with 0.1 and 1 µg L^{-1} of the studied butyltin compounds. The obtained results were compared with those obtained from spiked distilled water samples. The resulted relative recoveries are between 89.6 and 109.3% (Table 2). This indicates that the river water matrix had little effect on the extraction efficiency.

CONCLUSIONS

Dispersive liquid-liquid microextraction and gas chromatographic-mass spectrometric determination for the speciation analysis of butyltin compounds in aqueous solutions has been developed and optimized. The proposed technique is fast, reliable and environment-friendly as it consumes only 20 µL of extraction solvent. The real sample investigations demonstrated that the proposed method can be applied for river water analysis. Fortunately, the water samples from three rivers in Lithuania were free from the studied butyltin compounds.

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BUTILALAVO JUNGINIŲ NUSTATYMAS PANAUDOJANT DISPERSINĘ SKYSČIŲ–SKYSČIŲ MIKROEKSTRAKCIJĄ IR DUJŲ CHROMATOGRAFIJĄ–MASIŲ SPEKTROMETRIJĄ

S a n t r a u k a

Butilalavo junginių ekstrakcijai ir sukoncentravimui iš vandeninių tirpalų pasiūlytas derivatizacijos ir dispersinės skysčių–skysčių mikroekstrakcijos metodas. Derivatizacija buvo atliekama natrio tetraetilboratu, terpės pH 4,5. Ištirta ekstrahuojančio ir disperguojančio tirpiklių prigimties ir tūrio, ekstrakcijos trukmės ir tirpalo joninės jėgos įtaka ekstrakcijos efektyvumui. Ekstrahentu pasirinktas tetrachlormetanas, disperguojančiuoju tirpikliu – metanolis, vidiniu standartu – *n*-heksadekanas.

Kalibracinės kreivės tiesinės nuo 2,8 ng l^{-1} (monobutilalavo), 4,2 ng l^{-1} (dibutilalavo) ir 9,8 ng l^{-1} (tributilalavo) iki 10 mg ml^{-1} (visų analičių) koncentracijos, koreliacijos koeficientai 0,996–0,999, aptikimo ribos 1,7 ng l^{-1} (monobutilalavo), 2,5 ng l^{-1} (dibutilalavo) ir 5,9 ng l^{-1} (tributilalavo). Santykiniai standartiniai nuokrypiai neviršija 17 %. Parodyta galimybė pritaikyti paruoštą metodą butilalavo junginių nustatymui vandens mėginiuose.