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,[23,24] 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.

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

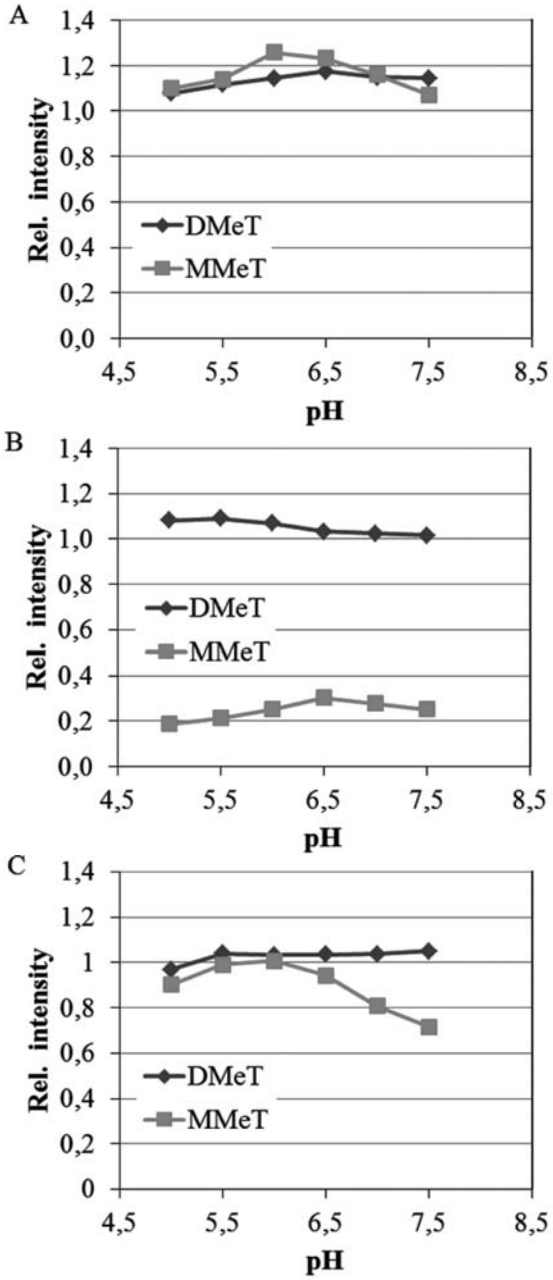


Figure 1. Effect of pH on the relative analytical signal intensities of spiked methyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

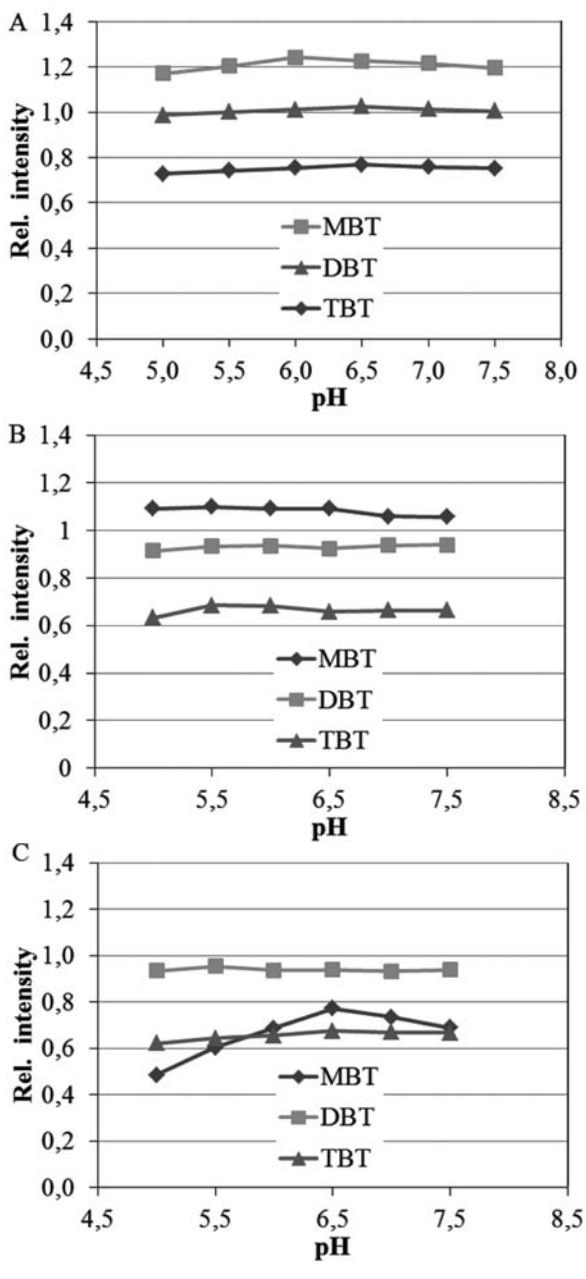


Figure 2. Effect of pH on the relative analytical signal intensities of spiked butyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

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 butyl-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

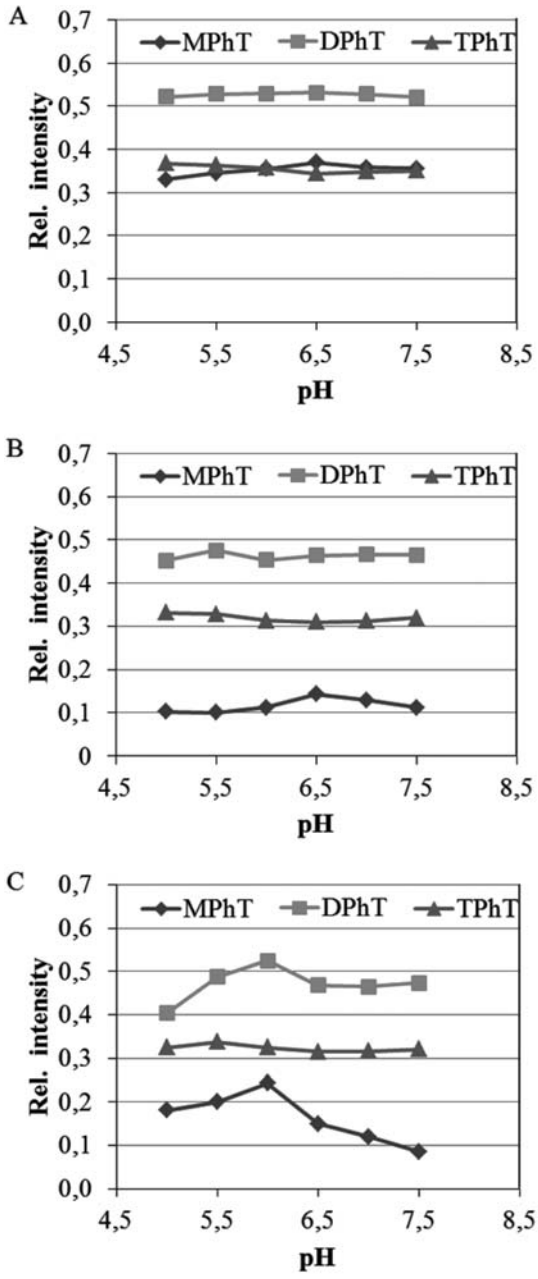


Figure 3. Effect of pH on the relative analytical signal intensities of spiked phenyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

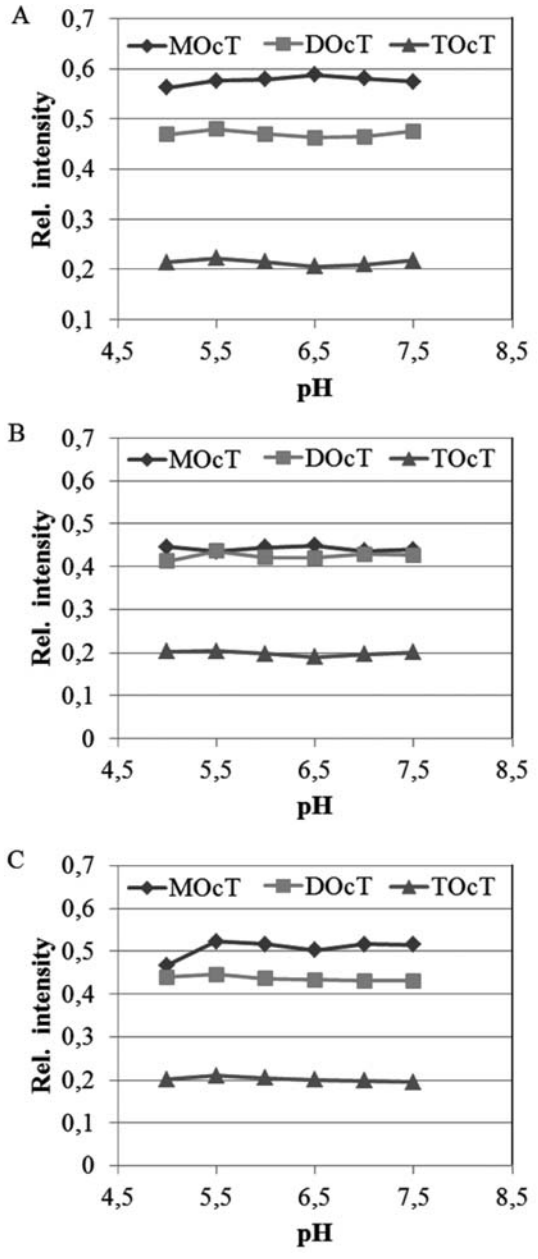


Figure 4. Effect of pH on the relative analytical signal intensities of spiked octyl-tin species (33.3 ng Sn L^{-1}) in Milli-Q water sample when different buffers were used for pH adjustment (A: carbonate, B: phosphate, C: Tris-citrate buffer). For separation 15 m GC column was applied.

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.

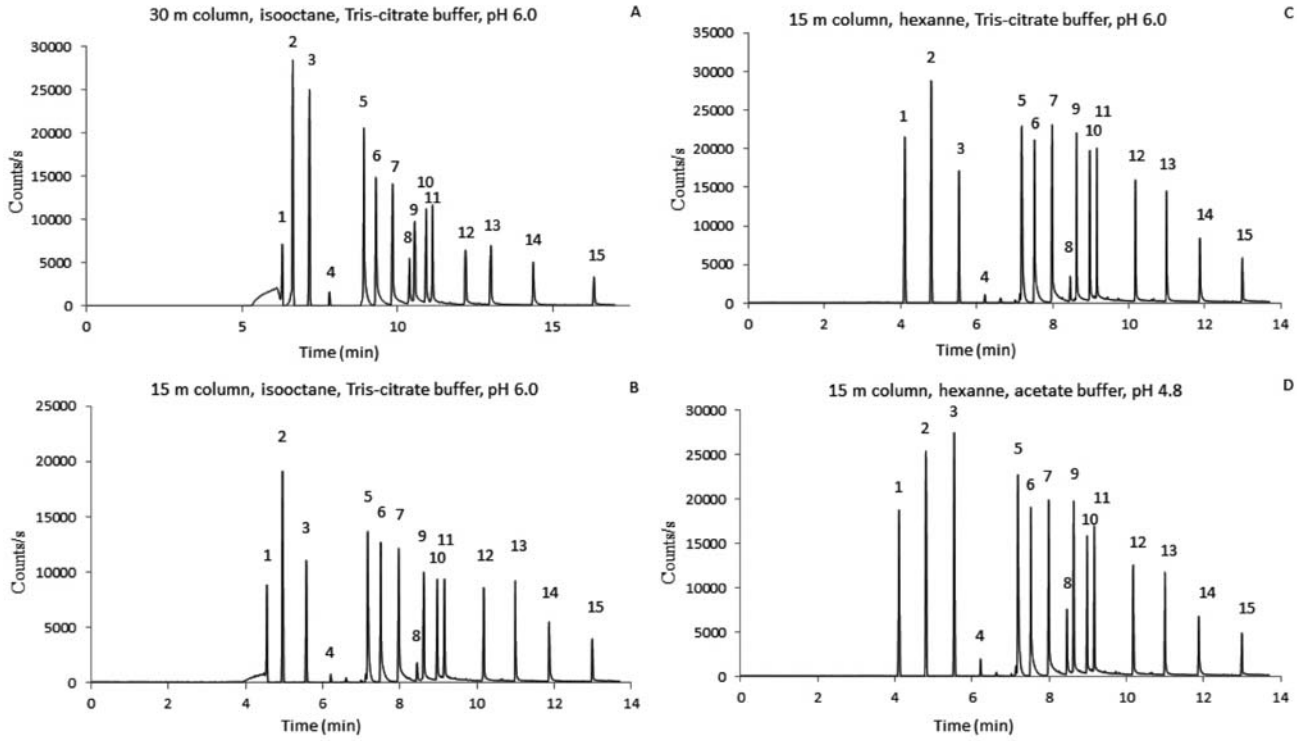
Results of optimisation of pH for ethylation of octyl-tin compounds are presented in Figure 4. In carbonate buffer (Figure 4A) ethylation of all tested octyl-tin compounds was optimal between pH 6.0 and 7.0. Similar were results when phosphate (Figure 4B) or Tris-citrate (Figure 4C) buffer was applied.

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, TeBuT 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

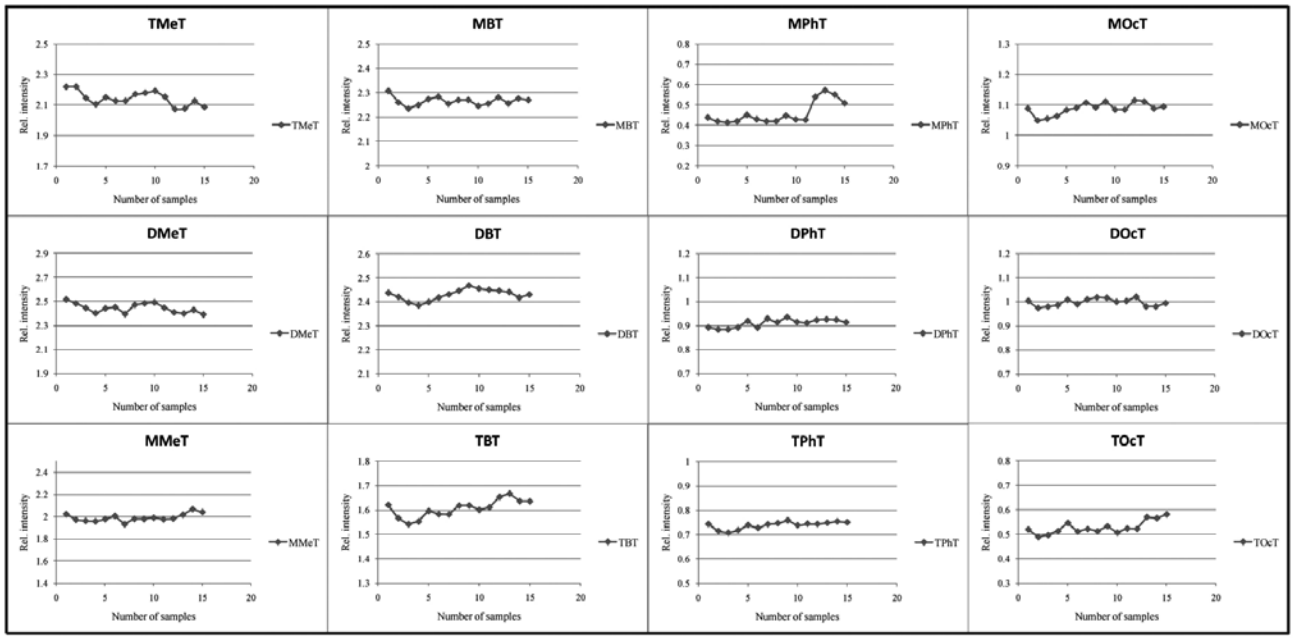


Figure 6. Stability of analytical signal (hexane used for extraction of ethylated OTC into organic phase, 15 m GC column, concentrations of OTC 33.3 ng Sn L^{-1}).

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.

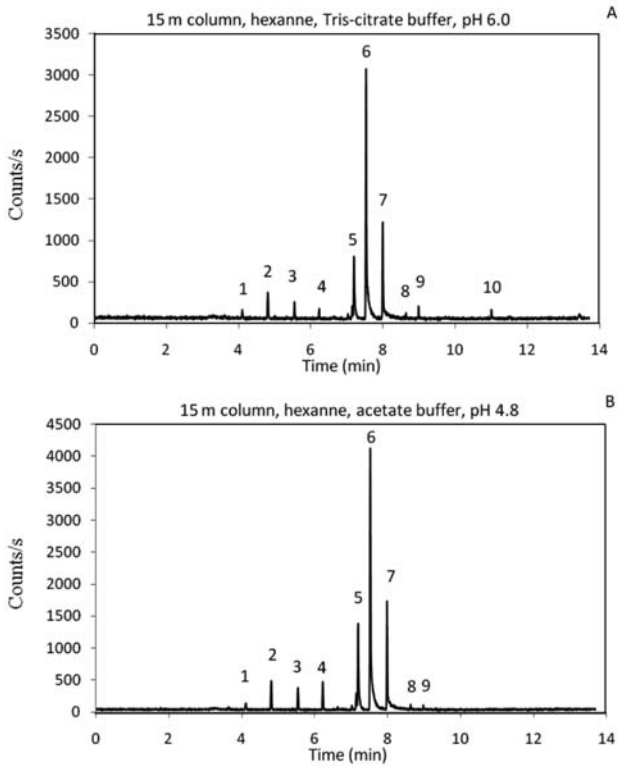
Table 3. Concentration ranges of the commonly present OTC in water samples from the Northern Adriatic Sea determined by GC-ICP-MS.

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 the 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.

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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.