Scientific paper

Critical Evaluation of Different Extraction Procedures for Determination of Organotin Compounds in Mussels

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Abstract

The efficiency of different extraction procedures for the simultaneous determination of organotin compounds in mussels *Mytilus galloprovincialis* by gas chromatography – mass spectrometry was critically evaluated. Three different solvents, hydrochloric acid (0.1 mol L^{-1}, 0.5 mol L^{-1} and 1 mol L^{-1}) in methanol, acetic acid (0.5 mol L^{-1}, 5 mol L^{-1} and 13 mol L^{-1}) in methanol and a 25% aqueous solution of tetramethylammonium hydroxide, and three different extraction approaches of mechanical shaking, ultrasonic and closed vessel microwave-assisted extraction were used for the extraction of organotin compounds from ERM-CE477 certified mussel tissue reference material. Before determination by GC-MS, extracted organotin species were derivatised with sodium tetraethyl borate and extracted into iso-octane. The results of analyses of ERM-CE477 reference material obtained after the different extraction procedures indicated that 1 h of ultrasonic extraction at 50 °C with 0.1 mol L^{-1} hydrochloric acid in methanol as extraction solvent provided satisfactory recoveries for all organotin compounds certified in this reference material.

The analytical method developed was successfully applied to determination of organotin compounds in mussels *Mytilus galloprovincialis* from the Slovenian costal area. Butyltins were found in mussels from all locations investigated. Among them, the highest concentration was of tributyltin, reaching 1100 ng Sn g^{-1} (dry mass).

Keywords: organotin compounds, mussels, extraction procedures, GC-MS

1. Introduction

The widespread use of organotin compounds (OTC) and their subsequent release into the environment started in 1950s when the fungicidal and biocidal activities of triorganotins were recognised. OTC consist of a central tin atom covalently bound to one or more organic substituents, i.e. methyl, ethyl, butyl, propyl, phenyl. The basic chemical formula for OTC is expressed as R_nSnX_{4-n}, in which R is an alkyl or aryl group and X is an inorganic substituent.[1,2] Trisubstituted OTC are mainly used as agricultural biocides, as wood preservatives and as marine antifoulants, disubstituted OTC as stabilisers for polyvinyl chloride (PVC), and monosubstituted OTC as synergists for PVC stabilisation and in glass coating.[3–5] Trisubstituted OTC, especially tributyltin (TBT) and triphenyltin (TPhT) are among the most hazardous pollutants encountered so far in aquatic systems.[1,6,7] TBT is present in the marine environment as a result of its use in antifouling paints that prevent setting of barnacles, seaweeds and tubeworms on immersed surfaces.[4] In the 1970s the negative effects of TBT on non-target organisms, mainly bivalves and gastropods, were discovered.[8] The European Commission banned the use of TBT-containing antifouling paints on the hulls of boats of less than twenty-five metres and vessels of any length used predominantly on inland waters.[9] TBT were included in the list of priority pollutants in the field of water policy in the EU Water Framework Directive – integrated river basin management for Europe.[10] From January 1st 2008, any OTC should be either removed from the surfaces of ships, or efficient sealing should be performed to prevent OTC leaching into the water.[11] Because of the persistence of OTC in the environment they will represent a risk long after they will have been banned, remaining a matter of major concern and requiring constant monitoring in years to come.[7] In aqueous media TBT undergoes slow stepwise microbial and UV degradation through less toxic dibutyltin (DBT) and monobutyltin (MBT) to non-toxic inorganic tin. The

degradation can be considered as a mechanism of detoxification.[12]

The high but different toxicity of OTC stimulated development of numerous analytical methods for determination of OTC at trace level. For separation and detection of OTC, gas (GC) or liquid chromatography (LC) coupled with a sensitive and element or molecule selective detection method, such as atomic absorption spectrometry,[13] mass spectrometry,[14,15] inductively coupled mass spectrometry[16,17] or pulsed flame photometric detection[18–20] have been commonly used. GC separation has the high resolution which is needed for simultaneous determination of all OTC present in different samples.[21] Prior to GC analysis extracted OTC are converted into volatile hydrides with $NaBH_4$, or more usually, alkylated with a Grignard reagent or sodium tetraetylborate ($NaBEt_4$).[22]

Extraction of OTC from solid samples, such as sediment, soil, sewage sludge and biological samples is the most difficult step in OTC determination, due to the limited stability of the analyte and the strong interactions between the analyte and matrices.[21] For extraction of OTC from biological matrices acidic extractants,[21,23] basic extractants (tetramethylammonium hydroxide – TMAH),[21,24] and enzymatic hydrolysis[21] have been used in order to enhance the solubility of ionic OTC. Extraction has been performed by mechanical shaking,[25] ultrasonic extraction,[21,25] microwave extraction,[21,23,26,27] supercritical fluid extraction[28] and solid phase microextraction as an alternative method to liquid-liquid extraction.[29,30]

The real efficiency of different extraction and derivatisation steps of analytical methods for OTC determination are generally unknown and hence techniques and results are difficult to compare. There are some publications comparing extractions,[21,23] derivatisations[21,22] or analytical methods[21,31,32] for determination of OTC in sediments,[33] water[34] and biological samples.[25]

Extraction and derivatisation are the major sources of error in the determination of OTC in environmental and biological samples.[3] Therefore, the aim of this work was to optimize and critically evaluate different extraction procedures for the determination of OTC in mussels by gas chromatography – mass spectrometry (GC-MS). For this purpose three different solvents, namely hydrochloric acid (HCl, 0.1 mol L^{-1}, 0.5 mol L^{-1}, and 1 mol L^{-1}) in methanol, acetic acid (CH_3COOH, 0.5 mol L^{-1}, 5 mol L^{-1}, and 13 mol L^{-1}) in methanol, and 25% aqueous solution of TMAH, and three different modes of extraction i.e. mechanical shaking, ultrasonic and closed vessel microwave-assisted extraction were compared. An accurate and reliable analytical method developed in the present work on the certified mussel tissue reference material ERM-CE477 was then applied for the analyses of mussels *Mytilus galloprovincialis* collected from six sampling sites in the Slovenian costal area of the Northern Adriatic Sea.

2. Experimental

2.1. Apparatus

In the extraction procedures applied, a mechanical shaker (Vibromax 40, Tehtnica Železniki, Slovenia), an ultrasonic bath (VWR, Model 550D, VWR International, West Chester, PA, USA) and a microwave digestion system (MARS X, CEM Corporation, Mathews, NC, USA) were used. The determination of OTC was carried out on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with an HP6890 Series automatic injector and connected to an HP5972A MSD. The injection port, transfer line and detector temperatures were maintained at 240, 280 and 180 °C, respectively. For the separation of OTC with a HP-MS5 capillary column (30 m × 0.25 mm × 0.25 μm) the following temperature programme was applied: for the first minute the column temperature was held at 90 °C, increased to 170 °C at a heating rate 10 °C min^{-1}, held for 2 min, increased to 220 °C at a heating rate 20 °C min^{-1}, held for 1 min, increased to 270 °C at a heating rate 30 °C min^{-1} and held at the final temperature for 6 min. The injection volume in the splitless injection mode was 1 μL. As the carrier gas, helium at the rate 1 mL min^{-1} was used. For MSD electron impact (70 eV) ionisation was used. The MSD was operated in the selected ion monitoring (SIM) mode where the three most abundant tin isotopic peaks of the first fragment ion were applied.[35] The selected ions for individual OTC are presented in Table 1.

Table 1. Selected ions for monitoring of OTC by GC-MS.

Compound	Starting time (min)	*m/z*
MBT	4.0	231, 233, 235
TPrT	6.0	245, 247, 249
DBT	7.1	259, 261, 263
MPhT	8.3	251, 253, 255
TBT	9.3	287, 289, 291
MOcT	10.1	287, 289, 291
DPhT	13.3	299, 301, 303
DOcT	15.0	371, 373, 375
TPhT	16.5	347, 349, 351
TOcT	18.0	371, 373, 375

2.2. Standards and Reagents

Monobutyltin trichloride ($MBTCl_3$, 95%), monophenyltin trichloride ($MPhTCl_3$, 98%) and diphenyltin dichloride ($DPhTCl_2$, 96%) were purchased from Aldrich (Milwaukee, WI, USA). Dibutyltin dichloride ($DBTCl_2$, 97%), tributyltin chloride (TBTCl, 96%), triphenyltin chloride (TPhTCl, 95%) and tripropyltin chloride (TPrTCl, 98%) were obtained from Merck (Darmstadt, Germany). Monooctyltin trichloride ($MOcTCl_3$, 98%) and dioctyltin dichloride ($DOcTCl_2$, 98%) were purchased

from LGC Promochem (Wesel, Germany) and trioctyltin chloride (TOcTCl, 95%) from Fluka (Buchs, Switzerland). OTC standard stock solutions containing 1 g (expressed as Sn) OTC L^{-1} were prepared in methanol. Fresh standard stock solutions were made every 6 months. Working OTC standard solutions were prepared weekly (10 mg (Sn) L^{-1}) or daily (100 μg (Sn) L^{-1} and lower). All the standards were stored in the dark at 4 °C.

CH_3COOH, HCl, nitric acid, iso-octane, methanol, sodium acetate trihydrate, and ammonia were obtained from Merck (Darmstadt, Germany). Sodium hydroxide was purchased from Carlo Erba (Milan, Italy), 25% TMAH solution in water from Fluka (Buchs, Switzerland), and $NaBEt_4$ from Galab products (Geesthacht, Germany). The water used was of Milli-Q water (18.2 MΩ) quality (Milipore, Bedford, MA, USA).

Acetate buffer (0.4 mol L^{-1}) was prepared weekly and an aqueous solution of $NaBEt_4$ (2% w/v) daily.

2.3. Cleaning Procedure

Laboratory ware was rinsed throughly with tap water, put into a polyethylene container with 10% nitric acid and left for 48 h to avoid contamination and analyte adsorption on surfaces. It was then rinsed three times with Milli-Q water.

2.4. Reference Material

For evaluation of the various extraction procedures for the determination of OTC in mussel samples by GC-MS, the certified reference material ERM-CE477, mussel tissue from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) was used. ERM-CE477 is certified for MBT, DBT and TBT content.

2.5. Sampling and Sample Preparation

Mussel (*Mytilus galloprovincialis*) samples were collected from the Slovenian part of the Adriatic Sea. *Mytilus galloprovincialis* is a widely distributed mussel species in the Northern Adriatic Sea and suitable for biomonitoring of toxic chemical compounds in the marine environment.[36] Samples were collected at 6 sampling points in July 2006: the camp site at Debeli rtič (DR), the area near the shipbuilding yard at Izola (SI), the area near Lucija marina (ML), the beach at Portorož (PO), and from two mussel farms at Strunjan (ST) and Sečovlje (SE). Sampling sites are shown on Figure 1.

From each sampling site 25–40 mussels were taken ranging 45–74 mm in length. Their biometric parameters are shown in Table 2. After scrubbing the mussels clean they were placed in dark containers, chilled on ice and transported to the laboratory within 10 h. In the laboratory mussels were rinsed with fresh water and methanol to prevent extraneous contamination.[15] After removing the shells the whole tissues were homogenized in a blender, lyophilized and stored at –20 °C.

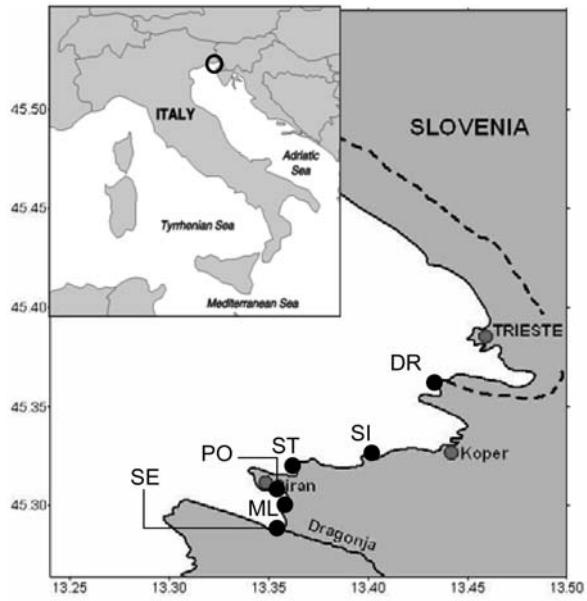


Figure 1. Sampling sites of the area investigated: Debeli Rtič (DR), Izola shipbuilding yard (SI), Strunjan mussel farm (ST), Portorož beach (PO), Lucija marina (ML) and Sečovlje mussel farm (SE).

Table 2. Relevant biometric parameters of the mussel *(Mytilus galloprovincialis)* samples.

Sampling site	Number of mussels	Length (mm)	Width (mm)	Water content, %
ML	25	53.2 ± 2,5	28.5 ± 1.4	82.5
PO	20	58.0 ± 5.2	32.1 ± 3.4	86.7
SE	25	71.5 ± 5.2	36.1 ± 2.8	84.9
ST	40	73.8 ± 4.1	35.8 ± 2.0	83.1
SI	30	45.5 ± 2.0	24.0 ± 1.3	87.2
DR	25	51.4 ± 3.4	27.5 ± 2.0	85.0

2.6. Analytical Method

The analytical method for the determination of OTC in mussels by GC-MS can be divided into four steps: extraction, derivatisation, separation and detection. In our work different extraction procedures consisting of the use of three different extraction solvents and modes of extraction were compared and critically evaluated. For this purpose the certified reference material ERM-CE477 was used.

Approximately 0.5 to 1 g of lyophilised mussel tissue was weighed into a 50 mL polypropylene centrifuge tube (Nalgen International, Rochester, NY, USA). To the sample 10 mL of selected extraction solvent and TPrT

(for ERM-CE477 500 ng of TPrT as Sn and for other samples from 300 to 500 ng of TPrT as Sn) as an internal standard were added. After the different extraction procedures described below, samples were centrifuged for 5 min at 4000 rpm (Centrifuge LC-320, Tehtnica, Železniki, Slovenia) and derivatised. The derivatisation step was adopted from the literature.[37] 1 mL of extract was added to a glass flask containing 100 mL of 0.4 mol L^{-1} acetate buffer. The pH was adjusted to 4.8 ± 0.2 with glacial acetic acid or a 25% aqueous solution of NH_3. For quantification by the standard addition method, which was carried out at 3 different OTC levels[38], appropriate amounts of diluted OTC stock solutions were added. For derivatisation 0.5 mL of 2% $NaBEt_4$ was added to the extract followed by the addition of 1 mL of iso-octane. The sample was then shaken for 45 min on a mechanical shaker at 300 rpm. The iso-octane extract was centrifuged for 5 min at 5000 rpm and directly injected into the GC-MS. The concentration of OTC in mussel samples was calculated on a peak area basis. All samples were analysed in three parallel determinations.

2.6.1. Extraction Procedure

To optimise and evaluate the extraction step in the determination of OTC in mussels, different extraction solvents and modes of extraction were compared. The extraction solvents were HCl (0.1, 0.5 and 1.0 mol L^{-1}) in methanol, CH_3COOH (0.5, 5 and 13 mol L^{-1}) in methanol and a 25% aqueous solution of TMAH, while extraction was performed either ultrasonically, by mechanical shaking or by closed vessel microwave-assisted extraction. Ultrasonic extractions were carried out at 25, 50, and 70 °C for 0.5, 1, and 3 h. Mechanical shaking was performed at room temperature for 8 and 16 h. Microwave-assisted extractions were carried out at 50 and 70 °C for 3 and 10 min. The temperature in the microwave extraction rose to the final value in 1 min. The different extraction procedures compared for the determination of OTC in ERM-CE477 mussel tissue certified reference material are schematically presented in Table 3.

3. Results and Discussion

3.1. Analytical Performance

The repeatability of determinations was evaluated by the relative standard deviation (RSD) of six consecutive analyses of a mussel sample with a concentration similar to that of ERM-CE477 reference material. It was found to be better than 3% for TBT and MBT and 9% for DBT. The reproducibility of determination was checked from a set of 12 analyses of the same sample over a period of 30 days. The RSD for MBT, DBT and TBT was found to be better than 9% and for TPrT 6%, respectively.

Table 3. Scheme of different extraction procedures applied for the determination of OTC in ERM-CE477 mussel tissue certified reference material by GC-MS.

Mode of extraction	Solvent	Extraction variables
Ultrasonic extraction (700 W)	HCl in methanol	conc: 0.1, 0.5, 1 mol L^{-1} t: 0.5, 1, 3 h T: 25, 50, 70 °C
	CH_3COOH in methanol	conc: 0.5, 5, 13 mol L^{-1} t: 1 h T: 50 °C
	25% aqueous solution of TMAH	t: 0.5, 1, 3 h T: 50 °C
Mechanical shaking (20 °C, 300 rpm)	HCl in methanol	conc: 0.1 mol L^{-1} t: 8, 16 h
	CH_3COOH in methanol	conc.: 13 mol L^{-1} t: 8, 16 h
	25% aqueous solution of TMAH	t: 16 h
Microwave assisted extraction (1200 W, ramp to temperature 1 min, hold 2 or 9 min)	HCl in methanol	conc.: 0.1 mol L^{-1} t: 3, 10 min T: 50, 90 °C
	CH_3COOH in methanol	conc.: 13 mol L^{-1} t: 3, 10 min T: 50, 90 °C
	25% aqueous solution of TMAH	t: 3, 10 min T: 50, 90 °C

Linearity of determination was obtained over a concentration range from 0.5 to 500 ng Sn mL^{-1} for all OTC. The correlation coefficients were better than 0.998.

The limits of detection (LOD) calculated on a 3*s* basis (three times the standard deviation of the blank) are presented in Table 4.

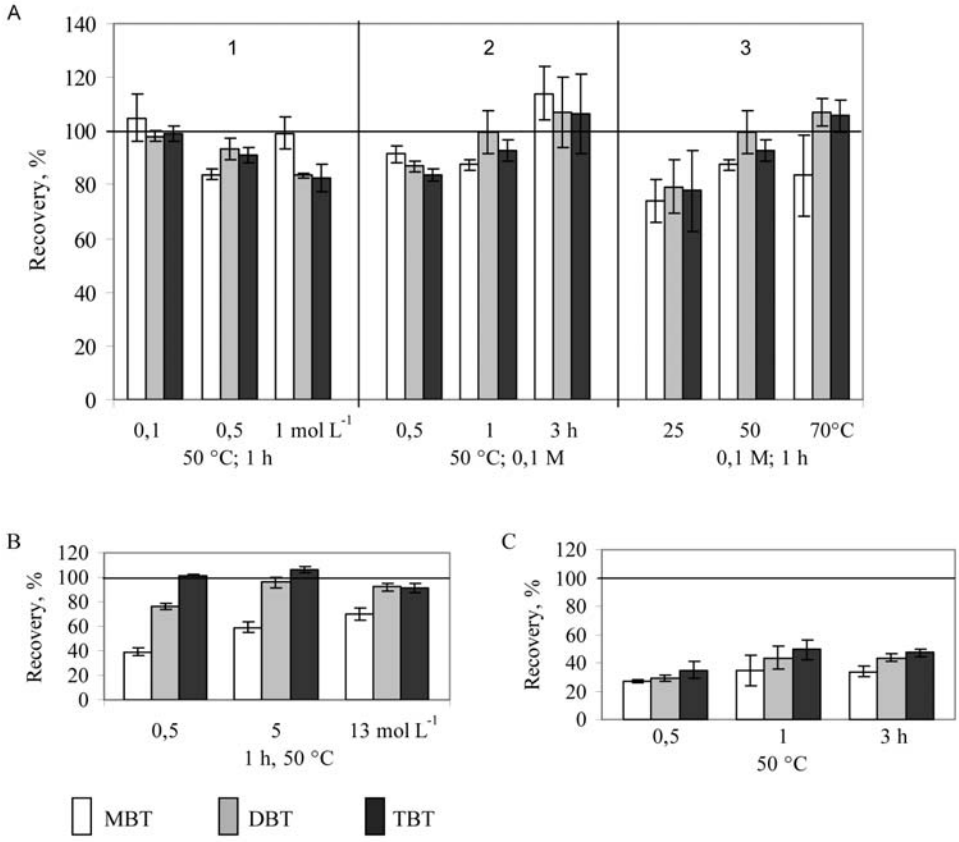
Table 4. LOD for OTC in mussel samples.

	MBT	DBT	MPhT	TBT	MOcT	DPhT	DOcT	TPhT	TOcT
LOD (ng Sn g^{-1})	11	3	7	5	5	4	8	10	20

3.2. Evaluation of the Extraction Efficiencies

The efficiencies of different extraction procedures for the determination of OTC in mussel samples by GC-MS were evaluated by analyses of the certified reference material ERM-CE477. The use of a reference material ensured that differences between results are not caused by poor homogeneity of the analysed sample.[25] Certified values for TBT, DBT and MBT in ERM-CE477 reference material are 900 ± 78, 785 ± 61 and 1012 ± 189 ng Sn g^{-1}, respectively. Recoveries were calculated from the results of analyses of ERM-CE477 obtained with different extraction procedures (see 2.6.1). They represent the ratio of the analyte content found to the certified value.[38] The differences between recoveries depended only on the differences in extraction procedure, as the post-extraction steps in the analytical method remained the same.

Extraction recoveries for ultrasonic extraction are presented in Figure 2.



Legend:
A1: Extraction recoveries after applying 0.1, 0.5 or 0.5 mol L^{-1} HCl in methanol; temperature (50 °C) and time (1 h) were constant.
A2: Extraction recoveries after applying 0.1 mol L^{-1} HCl in methanol for 0.5, 1 or 3 h; temperature (50 °C) was constant.
A3: Extraction recoveries after applying 0.1 mol L^{-1} HCl in methanol for 1 h at 25, 50 or 75 °C.
B: Extraction recoveries after applying 0.5, 5 and 13 mol L^{-1} CH_3COOH in methanol; temperature (50 °C) and time (1 h) were constant.
C: Extraction recoveries after applying 25% aqueous solution of TMAH for 0.5, 1 or 3 h; temperature (50 °C) was constant.

Figure 2. Extraction recoveries for ultrasound-assisted extraction.

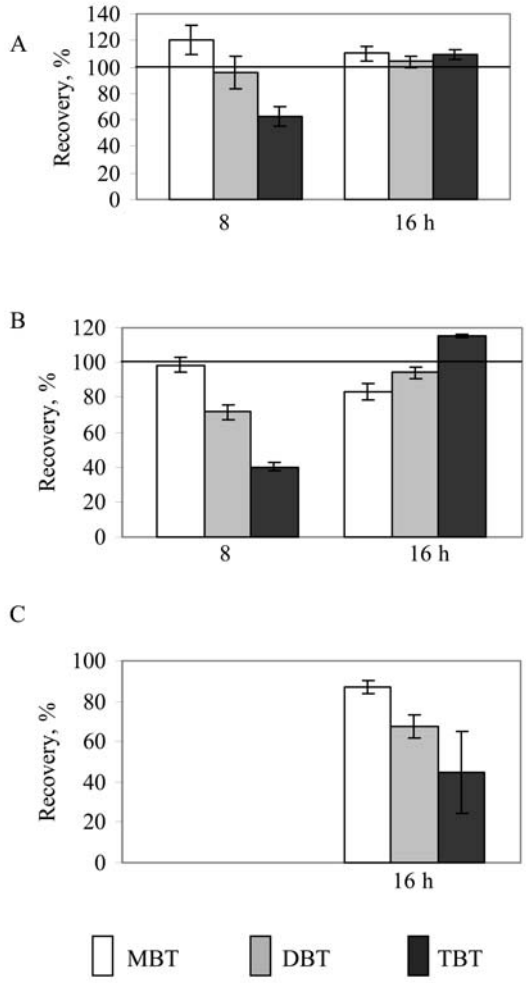
For three extraction solvents, the extraction variables concentration, time and temperature were evaluated (see Table 3). The influence of HCl concentration in methanol on recoveries was checked first at 50 ºC for 1 h (Figure 2A1). It was experimentally found that optimal recoveries were obtained when 0.1 mol L^{-1} HCl in methanol was used. The extraction time (0.5, 1 and 3 h) and temperature (25, 50 and 70 ºC) were optimised subsequently (Figures 2A2 and 2A3). From the results presented in Figure 2A1 to 2A3 can be seen that satisfactory recoveries (on average 94 ± 5% for MBT, 99 ± 6% for DBT and 95 ± 4% for TBT) for all OTC certified in ERM-CE477 were obtained for 1 h ultrasonic extraction at 50 °C using 0.1 mol L^{-1} HCl in methanol.

On the basis of the previous data on ultrasonic extraction with HCl in methanol, extraction conditions for CH_3COOH in methanol and 25% aqueous solution of TMAH were optimised (Figures 2B and 2C). It is evident from Figure. 2B that ultrasonic extraction was the most efficient when 13 mol L^{-1} CH_3COOH in methanol at 50 °C for 1 h was applied (recoveries 71± 4% for MBT, 93 ± 3% for DBT and 92 ± 3% for TBT). Results from Figure 2C indicated that 25% aqueous solution of TMAH was not an appropriate extracting solvent for ultrasonic extraction of OTC from mussels (recoveries below 50%).

Mechanical shaking was performed at room temperature. Extraction recoveries are presented in Figure 3.

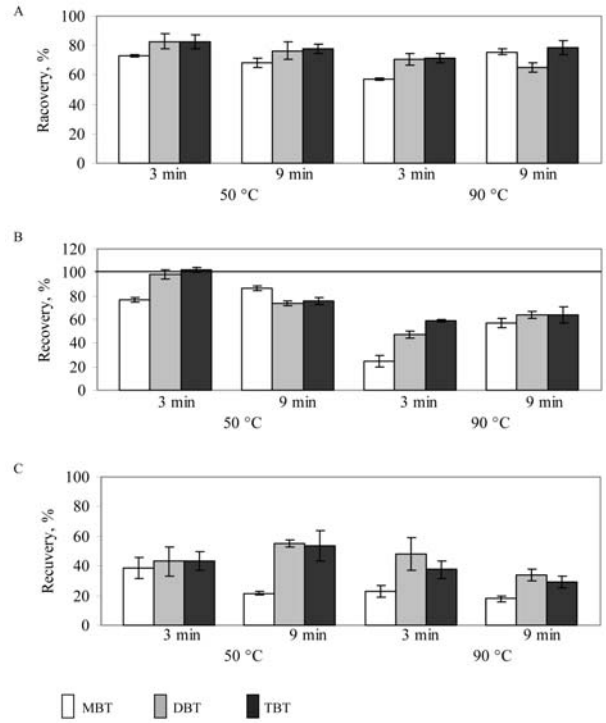
It can be seen that for the efficient extraction of OTC with 0.1 mol L^{-1} HCl in methanol, 16 h were needed (recoveries 110 ± 5% for MBT, 104 ± 3% for DBT, and 109 ± 4% for TBT, respectively). 16 h extraction with 13 mol L^{-1} CH_3COOH in methanol (Figure 3B) did not provide satisfactory recoveries for MBT and TBT (recoveries 83 ± 5% and 115 ± 2%, respectively), while a 25% aqueous solution of TMAH (Figure 3C) was not an efficient extracting solvent (recoveries 87 ± 3% for MBT, 67 ± 6% for DBT, and 45 ± 20% for TBT, respectively).

Results for the extraction efficiency of microwave-assisted extraction are presented in Figure 4.



Legend:
A: Extraction recoveries after applying 0.1 mol L^{-1} HCl in methanol for 8 or 16 h; temperature (20 °C) was constant.
B: Extraction recoveries after applying 13 mol L^{-1} CH_3COOH in methanol for 8 or 16 h; temperature (20 °C) was constant.
C: Extraction recoveries after applying 25% aqueous solution of TMAH for 16 h; temperature (20 °C) was constant.

Figure 3. Extraction recoveries for mechanical shaking.



Legend:
A: Extraction recoveries after applying 0.1 mol L^{-1} HCl in methanol for 3 or 9 min; temperature 50 °C or 90 °C.
B: Extraction recoveries after applying 13 mol L^{-1} CH_3COOH in methanol for 3 or 9 min; temperature 50 °C or 90 °C.
C: Extraction recoveries after applying 25% aqueous solution of TMAH for 3 or 9 min; temperature 50 °C or 90 °C.

Figure 4. Extraction recoveries for microwave-assisted extraction.

The extraction efficiency at different time (3 and 9 min) and temperature (50 and 90 °C) was checked. Higher temperature (90 °C) and prolongation of time (9 min) of microwave extraction in general worsened the extraction efficiency. Microwave-assisted extraction with 0.1 mol L^{-1} HCl in methanol (Figure 4A) did not efficiently extract OTC from mussel tissue (recoveries below 83% for all OTC analysed). Extraction with 13 mol L^{-1} CH_3COOH in methanol at 50 °C for 3 min (Figure 4B) was effective for DBT and TBT (recoveries 98 ± 4% and 102 ± 2%, respectively), while it was less efficient for MBT (recovery 77 ± 2%).

The results from Figure 4C indicated that a 25% aqueous solution of TMAH is not an appropriate extracting solvent for microwave-assisted extraction of OTC from mussels (recoveries below 50%).

From the results presented in Figs. 2–4 it can be concluded that with the respect to extraction efficiency 1-h ultrasonic extraction at 50 °C with 0.1 mol L^{-1} HCl in methanol is the extraction approach of choice. This extraction approach had the additional advantages of shortening the duration of extraction in comparison to mechanical shaking and of sample handling in comparison to microwave-assisted extraction.

In Figure 5, GC-MS (SIM) chromatogram of ethylated OTC in the extract of ERM-CE477 certified reference material is presented.

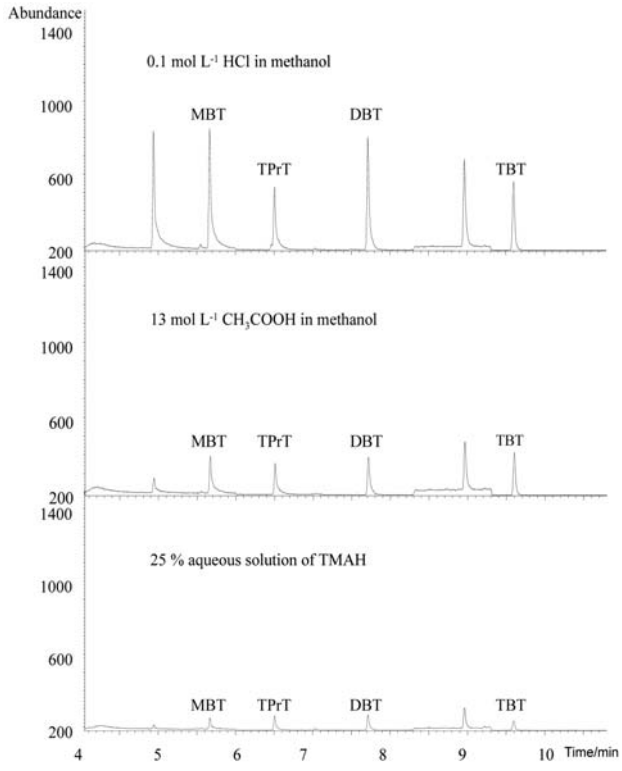


Figure 5. GC-MS (SIM) chromatograms of ethylated OTC in extract of ERM-CE477 certified reference material.

OTC were extracted under optimal conditions for a given extractant (0.1 mol L^{-1} HCl in methanol, 1 h, ultrasonic extraction at 50 °C; 13 mol L^{-1} CH_3COOH in methanol, 3 min, microwave-assisted extraction at 50 °C; 25% aqueous solution of TMAH, 16 h, mechanical shaking). These data indicated that 0.1 mol L^{-1} HCl in methanol also provided the highest sensitivity of measurement of OTC by GC-MS. No interfering peaks were observed in GC-MS chromatograms of ethylated OTC in mussel extracts.

3.3. The Determination of OTC in Mussels

The level of pollution with OTC was assessed from the results of OTC determination in mussels collected in July 2006 at six representative sampling sites of the Slovenian part of the Northern Adriatic Sea. The optimised extraction procedure was used for isolation of OTC from mussels. The results are presented in Table 5.

Table 5. Organotin concentrations (ng Sn g^{-1}) in mussels determined by GC-MS.

Location	MBT	DBT	TBT	MPhT	DPhT	TPhT
ST	< 11	15 ± 1	36 ± 11	< 7	< 4	< 10
SE	< 11	20 ± 3	57 ± 13	< 7	< 4	< 10
DR	< 11	19 ± 7	61 ± 26	< 7	< 4	< 10
PO	20 ± 9	66 ± 7	107 ± 25	< 7	12 ± 2	13 ± 3
ML	102 ± 1	565 ± 5	1090 ± 2	< 7	< 4	57 ± 3
SI	29 ± 16	178 ± 45	293 ± 27	< 7	< 4	13 ± 3

In Figure 6 a typical GC-MS chromatogram of OTC compounds in the extract of a mussel sample is shown.

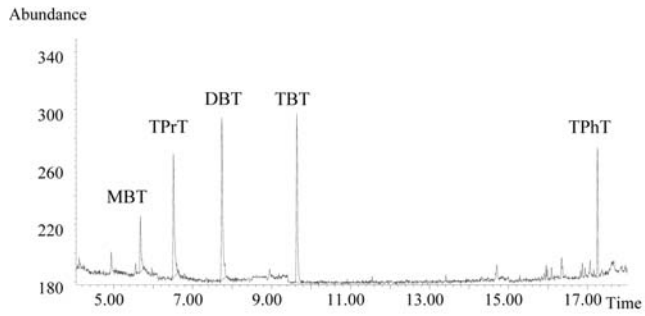


Figure 6. GC-MS (SIM) chromatogram of ethylated OTC in extract of representative mussel sample.

The data in Table 5 indicate that butyltins were present in mussels from all sampling sites. Concentrations of TBT were in general much higher than those of its degradation products DBT and MBT, suggesting a recent input

of TBT. Similar findings were reported by Bortoli et al. for the Lagoon of Venice.[39] The highest concentrations of butyltins were found in the vicinity of the Izola shipbuilding yard (SI): 1090, 565 and 102 ng Sn g^{-1} for TBT, DBT and MBT respectively. Concentrations of all butyltins at Portorož (PO) and Lucija marina (ML) were approximately 10 times lower, while concentrations of butyltins for other sites investigated were below 60 ng Sn g^{-1}. These values are in agreement with results from less contaminated sites in Italy[40] and Corsica.[41]

The level of phenyltins for the mussel farms (ST, SE) and Debeli rtič (DR) was below 10 ng Sn g^{-1}. At PO and SI the concentration of TPhT was 13 ng Sn g^{-1} and at ML 57 ng Sn g^{-1}. These results are comparable to previous data for the Bay of Piran[36] and the Lagoon of Venice.[42]

Mussels from none of the sampling sites investigated contained measurable concentrations of octyltin compounds.

4. Conclusion

A study was performed to optimise the extraction for isolation of OTC from mussels. For this purpose ERM-CE477 certified reference material was used. It was found that 1 h ultrasonic extraction at 50 °C using 0.1 mol L^{-1} HCl in methanol as extraction solvent can be recommended for extraction of OTC from mussels. Ultrasonic extraction provides quantitative recoveries for all butyltin species certified in ERM-CE477. This extraction approach has the advantage of shortening the duration of extraction in comparison to mechanical shaking, and simplifies sample handling and manipulation in comparison to microwave-assisted extraction. No interfering peaks were observed in the GC-MS chromatograms of ethylated OTC in mussel extracts.

The optimised extraction procedure was used for isolation of OTC from mussels from the Slovenian part of the Northern Adriatic Sea. From the results, it can be concluded that the sampling area is contaminated with butyltin compounds. Contamination is more pronounced at locations such as marinas and shipbuilding yards. Higher concentrations of TBT than those of its degradation products indicate that TBT is still being introduced into the marine environment. Concentrations of phenyltin compounds in mussels were in general low, while octyltin compounds were not detected in mussels.

5. Acknowledgements

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Povzetek

Kritično smo ovrednotili učinkovitost različnih ekstrakcijskih postopkov za izolacijo butilkositrovih spojin iz školjk *Mytilus galloprovincialis* s plinsko kromatografijo v povezavi z masnospektrometričnim detektorjem (GC-MS). Uporabili smo tri različna ekstrakcijska topila: klorovodikovo kislino (0.1 mol L^{-1}, 0.5 mol L^{-1} in 1 mol L^{-1}) v metanolu, ocetno kislino (0.5 mol L^{-1}, 5 mol L^{-1} in 13 mol L^{-1}) v metanolu in 25% vodno raztopino tetrametilamonijevega hidroksida ter tri različne načine ekstrakcije: mehansko stresanje, ultrazvočno in mikrovalovno ekstrakcijo. Učinkovitost posamezne ekstarkcije smo ocenili z analizo certificiranega referenčnega materiala školjk ERM-CE477. Pred določitvijo z GC-MS smo organokositrove spojine derivatizirali z natrijevim tetraetil boratom in ekstrahirali v izo-oktan. Rezultati analiz referenčnega materiala so pokazali, da je za ekstarkcijo organokositrovih spojin iz školjk najučinkovitejša enourna ultrazvočna ekstrakcija pri 50 °C z uporabo 0.1 mol L^{-1} HCl v metanolu.
Optimiziran ekstrakcijski postopek smo uporabili za določitev OTC z GC-MS v školjkah *Mytilus galloprovincialis*, ki smo jih vzorčili v slovenskem morju. V vseh vzorcih so bile prisotne butil kositrove spojine. Najvišja izmerjena je bila koncentracija tributil kositra (TBT) in sicer 1100 ng Sn g^{-1} (suhe teže).