

# Validation of analytical method for determination of tributyltin (TBT) in soils and bottom sediments

## Walidacja metody oznaczania tributylocyny (TBT) w gruntach i osadach dennych

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**Abstract:** Tributyltin (TBT) belongs to the group of persistent pollutants harming the marine environment. It is a danger to many aquatic organisms, especially those inhabiting seabed sediments. TBT was used mainly in production of antifouling paints applied on ship's hulls. Currently, it is prohibited to use most products containing TBT [6; 8; 16].

The objective of this study was to confirm applicability of the method for determining the levels of tributyltin compounds in soils and bottom sediments which remains in accordance with the HELCOM recommendations [11]. In Poland, there is no sufficient data on the levels of TBT concentrations in the Baltic Sea, which is why it is so important to monitor these compounds in the seabed sediments, where they tend to accumulate.

**Keywords:** tributyltin, soil, seabed sediments, gas chromatography, the Baltic Sea

**Streszczenie:** Tributylocyna (TBT) zaliczana jest do trwałych zanieczyszczeń środowiska morskiego. Zagroza wielu organizmom wodnym, zwłaszcza bytującym w osadach dennych. TBT wykorzystywana była głównie do produkcji farb przeciwporysowych używanych do malowania kadłubów statków. Obecnie stosowanie większości środków zawierających TBT zostało zabronione [6; 8; 16].

Celem przeprowadzonej walidacji było potwierdzenie możliwości stosowania metody oznaczania zawartości tributylocyny w osadach dennych i gruntach zgodnej z zaleceniami HELCOM [11]. W Polsce brakuje wystarczających danych o poziomach stężeń TBT w Bałtyku, dlatego tak ważne jest monitorowanie tych związków w osadach dennych, w których się kumulują.

**Słowa kluczowe:** tributylocyna, osad denny, grunt, chromatografia gazowa, Morze Bałtyckie

## Introduction

Tributyltin (TBT) ( $C_4H_9)_3SnX$  is an organic-metallic compound containing three Sn-C bonds, where X is an anion, e.g. Cl<sup>-</sup>. The X group has no significant influence on the compound's properties, however the alkyl chain length is of great importance when it comes to the compound's toxicity [3; 7; 9].

Tributyltin is considered to be the most toxic of all organotin compounds, and its usage in the majority of products is forbidden. It used to be employed primarily as an active substance in biocides and pesticides. It was also an ingredient of antifouling paints used on ship hulls, as well as serving as a wood pre-

servative, an additive to cotton textiles. It found application in factories producing wood pulp and paper, in tanneries and in breweries. Paints that used to be applied on the ship hulls were protecting them against getting colonized by various aquatic organisms. The ships were more streamlined, burnt less fuel, and less time had to be devoted to their maintenance in the shipyards. Unfortunately, what was not taken into account was the paints' impact on the environment [3; 6; 14; 15].

The organotin compounds are heavily accumulated in the seabed sediments. This is related to the risk of polluting the water environment, and their toxic impact on the benthic organisms. That is why the level of accumulation and the adverse effects

these compounds have on various organism in the food chain need to be examined [6; 7; 15].

TBT causes both chronic and acute poisoning of most sensitive aquatic organisms, such as zooplankton and molluscs [8]. It causes hormonal disturbances, physiological changes, and problems with reproduction of the organisms [1; 4; 12; 14].

Tributyltin degrades quite rapidly in the marine environment, but it adsorbs onto the sediment particles, where the degradation processes are much slower, and may even take many years [8]. The highest levels of the compound's concentration in the environmental samples were found in the bottom sediments taken from the shipyard basins [4; 8].

The ban on using the products containing organotin compounds was an important action from the point of view of environmental protection. However, it is necessary to continue to monitor them, track their further fate, and estimate the threats they pose [5; 8; 16].

The Member States of the European Union, including also Poland, need to prevent deterioration of the surface waters, as well as take all necessary measures to achieve and maintain a good ecological status and potential, and a good chemical status of the surface water and groundwater, which has been included in, and confirmed by the recommendations of the Water Framework Directive [20]. Transposition of the WFD rules into the Polish national legislation took place primarily due to adoption of the Water Law with its implementing acts. One such act is the Regulation of the Minister of Environment of 21 November 2013 (Journal of Laws of 2013, item 1558), the Annex 1, point 2), amending the regulation concerning forms and ways of conducting the monitoring of surface and groundwater bodies, which suggests a scope and frequency of sediments testing for the purposes of analysis of long-term trends in changes of the concentrations of the priority substances and other bioaccumulable contaminants, as well as mentioning the tributyltin compounds (tributyltin-cation) as an indicator that should be tested once every three years.

The HELCOM report (HELCOM 2009) classifies tributyltin at the top of the list of chemical compounds that should be monitored in the Baltic Sea, as well as placing an emphasis on the lack of data concerning these compounds [8; 11; 16]. In 2015, HELCOM approved changes concerning the obligation to perform research on the level of TBT content in material dredged from the area of the Baltic Sea [2].

The present work presents only a technical report about the validation of confirming applicability of the method for determining the levels of tributyltin compounds in sediments and soils, which remains in accordance with the HELCOM recommendations [11].

## The research material

In the validation process, sand samples from the sea and fine sediments were used. The sand samples were free from tribu-

tyltin impurities, while in case of the fine sediments, the expected TBT concentration was the one defined by the Ramboll Analytics accredited laboratory (Lahti, Finland). As an accredited test laboratory, Ramboll Analytics designates the organotin compounds adhering to the ISO 23161 standard.

In order to verify the concordance of the obtained results, a certified reference material PACS-3 of the National Research Council Canada was used.

## The research methods

The chosen research method was the procedure following the Polish PN-EN ISO 23161 standard. Tributyltin-cation (TBT) was determined in the samples subjected to analysis with use of the internal standard: the tripropyl-tin chloride.

The operating range for the method of determining tributyltin in the sediments was set at: 0,01 – 6,0 mg·kg<sup>-1</sup> s.m.

The final determination of the analyte in the sample was performed via the gas chromatography technology with mass spectroscopy detection (GC-MS), subsequent to derivatization of the analyzed substance. Concentration of the analyte in the sample was determined with application of the calibration curve technique of the external standard calibration.

In order to determine the organotin compounds, two alternative methods of extraction may be employed: the acid extraction, and that with a methanolic potassium hydroxide solution.

### Acid extraction

To the pre-weighted 1g of dry samples of the seabed sediment there was added the internal standard, and the extraction mixture (acetic acid:methanol:water – 1:1:1). Serving as the internal standard for the tributyltin was the tripropyl-tin chloride (TPT). This mixture underwent sonification in ultrasonic bath. The samples were centrifuged in order for the phases to get separated. The liquid phase was subjected to another extraction. To the mixed extracts there was added the NaOH solution, and the pH was adjusted to the level of 4.5. In order for the analyte to be derivatized, sodium tetraethylborate in tetrahydrofuran and hexane were added. The solution was being stirred at room temperature.

### Extraction with the simultaneous alkylation and derivatization

To the pre-weighted 1g of dry samples of the sediment there was added the internal standard and water, thus producing a slurry, 20% of which was solid material. Next, methanolic potassium hydroxide solution and hexane were added. The sample was either heated at temp. of 70 °C for 1 hour, or subjected to sonification in the ultrasonic bath, and shaken out, or was left for 12 hours at room temperature. Then, the samples were

acidified until the pH was equal to 4.5. Acetate buffer was then added. The derivatizing reagent (sodium tetraethylborate in tetrahydrofuran) was introduced into the thus prepared sample, which was then shaken for 2 hours. The derivatization procedure was repeated. The phases were separated by means of centrifugation.

From this point on, both extraction techniques - the acid one, and the one with alkylation and derivatization - follow the same path.

The hexane phase was dried and enhanced. The hexane extract obtained in this manner was then purified on columns filled with silica gel and anhydrous sodium sulfate. Elution was carried out with use of a hexane:acetone mixture (95:5). The enhanced eluate was next subjected to chromatographic analysis (GC-MS).

For each series of the analyses, there should be performed simultaneously a blank test adhering to the selected extraction procedure, and with use of the same amounts of all the reagents.

### Validation parameters

The calibration curves were prepared based on an analysis of the calibration standard solutions, encompassing the operating range, and demonstrating a linearity in this regard. Linearity for both analytical methods was checked by 7-gradual external calibration. However, to better illustrate the linearity, the operating range was divided into two sub-ranges:

- ◆ 0.01–0.20 mg·kg<sup>-1</sup> s.m.
- ◆ 0.20–6.00 mg·kg<sup>-1</sup> s.m.

In the following figures (Fig. 1–4/Rys. 1–4) there are presented the obtained calibration curves for both of the extraction procedures across the entire operating range.

Captions:  $A_i$  – analyte peak's area;  $A_1$  – area of the internal standard's peak;  $m_i$  - weight of the organotin cation (TBT);  $m_1$  – weight of the organotin cation (TPT).

## Results

### Comparison of the extraction processes

While performing the validation process for the method in question, the equivalence between the extraction processes selected for use was investigated.

The validation compounds were obtained in 4 analytical series of 7 samples for both sand and sediment respectively.

In Table I/Tabela I there the results obtained for both of the chosen extraction processes are tabulated. After comparing the results obtained via the two extraction procedures, the possibility of using them interchangeably was confirmed.

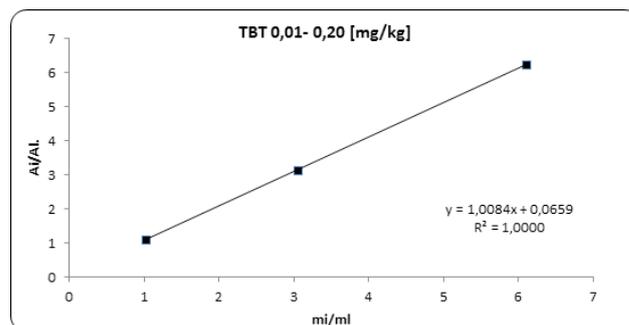


Fig. 1. Calibration curve as with use of acid extraction, and derivatization: 0.01-0.20 mg·kg<sup>-1</sup>.

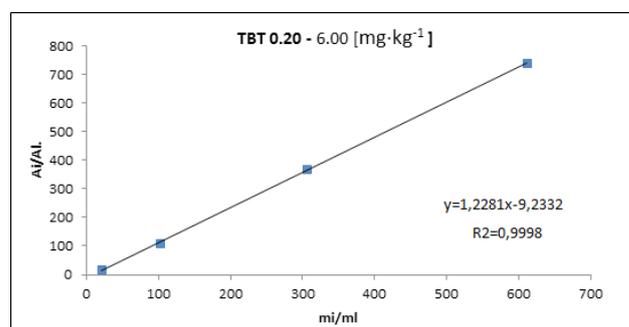


Fig. 2. Calibration curve as with use of acid extraction and derivatization: 0.20–6.00 mg·kg<sup>-1</sup>.

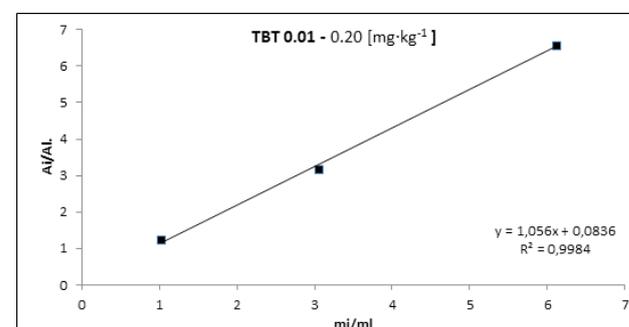


Fig. 3. Calibration curve as with use of simultaneous alkylation and derivatization: 0.01–0.20 mg·kg<sup>-1</sup>.

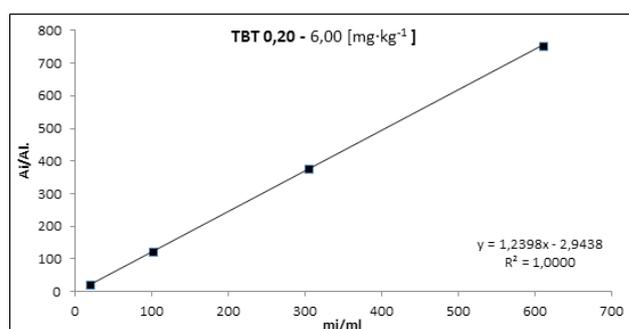


Fig. 4. Calibration curve as with use of simultaneous alkylation and derivatization: 0.20–6.00 mg·kg<sup>-1</sup>.

Tab. I. Comparison of the results obtained via the acid extraction and derivatization method, and via the simultaneous alkylation and derivatization method.

NO.	ANALYTE PEAK AREA	MATRIX	METHOD	CONCENTRATION WHILE USING THE ACID EXTRACTION AND DERIVATIZATION METHOD [ $\mu\text{G KG}^{-1}$ ]	CONCENTRATION WHILE USING THE SIMULTANEOUS ALKYLATION AND DERIVATIZATION [ $\mu\text{G KG}^{-1}$ ]	DIFFERENCE	SD
1	706.9	seabed sediment	acid extraction procedure	0.696	0.741	0.045	0.032
2	1171.1	sand	acid extraction procedure	0.861	0.915	0.054	0.038
3	1816.7	seabed sediment	simultaneous alkylation procedure	0.921	0.980	0.059	0.042
4	529.3	seabed sediment	simultaneous alkylation procedure	0.389	0.413	0.024	0.017

### recision and repeatability of the extraction processes

In order to assess the precision, a spread of the obtained determination results around the mean value, there was carried out a series of sand ( $n=21$ ) and sediment ( $n=28$ ) analyses with addition of the standard, repeated with different concentrations including the operating range. Repeatability in analyzed samples was assessed on the basis of the standard deviation values of a series of measurements performed by a given analyst, in a given laboratory, and with use of a given measuring device [10; 13].

Analysis of sands and seabed sediments was performed for a weighted amount of 1g.

Tables II, and III/Tabela II i III presented below illustrate the values of repeatability and precision of the method as obtained with the use of different environmental matrices (sand, seabed sediment).

### Correctness and accuracy of the analytical method

Accuracy, as a convergence between the value of a quantity as measured by an analyst, and the value of the true quantity [10], was assessed with use of a certified reference material, and presented in a table form with use of the deviation from the true value, and the measurement error. Correctness, i.e. the degree to which a determination result is consistent with the expected value [10], was assessed based on a series of measurements of the certified reference material, and presented in Table IV/Tabela IV with application of deviation from the true value for the average value of all measurements in a series, and the error for the average value of all measurements in the series. The value presented below was obtained via the calculations that also took into account the recovery.

The results of the TBT determination fall within the specified range of the certified reference material PACS-3 of the National Research Council Canada. However, the obtained values are below the expected value that may result from analytical procedure, which is extremely complicated and gives acceptable,

Tab. II. Compilation of statistical parameters of the method for determining TBT in sands with addition of the tributyltin chloride standard.

RANGE	REPEATABILITY [%]	PRECISION WITHIN THE REPEATABILITY LIMITS [%]
0.01-0.20 mg·kg <sup>-1</sup>	19.97	7.55
0.20-6.00 mg·kg <sup>-1</sup>	2.03	2.78

Tab. III. Compilation of statistical parameters of the method for determining TBT in seabed sediments from the Port of Gdynia.

RANGE	REPEATABILITY [%]	PRECISION WITHIN THE REPEATABILITY LIMITS [%]
0.01-0.20 mg·kg <sup>-1</sup>	5.24	8.43
0.20-6.00 mg·kg <sup>-1</sup>	5.05	6.81

but low recovery. This tendency is confirmed by other laboratories [17]. The value of the method's accuracy is 10.31% on average, and it is satisfactory with applying such complicated extraction procedures.

### Selectivity of the examination procedure

Selectivity is the possibility to determine accurately and precisely the analyte content in a sample with other components present. The bigger the amount of the expected product and less of a byproduct, the more selective a reaction is. Selectivity of a method is assessed on a basis of identification of the substances disturbing the analysis, and the ways of eliminating their impact [13]. The substances that disrupt the analysis, which may have an influence on the result of the organotin compounds determination via the chromatographic method are the boraxanes, which are removed on the columns filled with silica gel.

### The recovery

The recovery value tells us whether a test method and the examined matrix have any influence on the test's results (whether they cause any losses of the analyzed object) [13].

In Table V/Tabela V there are presented parameters of statistical processing of the recovery values obtained for various matrices and concentrations of the examined analyte.

Tab. IV. Correctness and accuracy as determined with use of a certified reference material.

NO.	1	2	3	4	5
The expected value [mg kg <sup>-1</sup> ]	0.41 +/- 0.04				
The obtained value [mg kg <sup>-1</sup> ]	0.37	0.37	0.37	0.37	0.37
Deviation from the true value [mg kg <sup>-1</sup> ]	-0.044	-0.043	-0.044	-0.041	-0.039
Single measurement error [%]	-10.66	-10.57	-10.82	-9.92	-9.61
Accuracy (single measurement absolute error) [%]	10.66	10.57	10.82	9.92	9.61
Deviation from the true value for the measurement series' average value [mg kg <sup>-1</sup> ]	0.042				
Correctness (error for the measurement series' average value) [%]	10.31				

The tD value is compared with the critical value of the bilateral distribution  $t_{crit}$  for n-1 degrees of freedom, and the level of confidence equal 95% (n – is the number of results used in estimating the recovery). If the t value is lower than the critical value, the recovery can be passed over. If the t value is higher than the critical value t, then it is necessary to apply the adjustment factor (1/recovery).

$$T_A = 10.91 > t_{crit} = 2.086$$

$$T_B = 8.13 > t_{crit} = 2.086$$

$$T_A = 16.11 > t_{crit} = 2.086$$

$$T_B = 12.29 > t_{crit} = 2.086$$

In case of analysis of tributyltin in sediments and sands, it is necessary to use the adjustment factor (1/recovery).

## Discussion

The two extraction processes are interchangeable. The differences in the levels of concentration obtained by means of the acid extraction do not differ from the ones obtained via the simultaneous alkylation and derivatization method in any statistically relevant way.

Repeatability was determined on the basis of values of standard deviations calculated for four series of samples of sands and sediments. Each of the series consisted of seven separately prepared samples for two different ranges of concentrations. In case of the sediment, repeatability amounted to approx. 5%, while in case of the material used in the comparative studies described in the PN-EN ISO 23161 standard it was equal to approx. 9.7%. This is an evidence that the laboratories determining tributyltin allow for a similar margin of error. The obtained precision was also good - dispersion of the results was not significant.

The accuracy and trueness were calculated on the basis of an analysis of TBT content in samples of the reference material.

Tab. V. Compilation of the statistical parameters.

	SAND A	SEDIMENT B
	0.003	0.037
Concentration of the analyte [mg/kg]	0.3	0.15
	3	0.88
		1.10
Average recovery [%]	84.98	79.13
Standard deviation	7.36	9.9
Precision	0.09	0.13
Number of measurements n	21	28
Significance of recovery factor tD	7.94	8.83

Taking into consideration how complicated the extraction procedures are, the value of accuracy equal to about 10% is satisfactory.

The method is selective, and it does not produce an interference of other components; the signal is generated solely by the analyte.

The applied examination procedure is laborious and time-consuming. Most of the analytical procedures of determining tributyltin presented in the publications are similar to one another, and also time-consuming. They differ in terms of extraction type, and solvent used for this purpose, as well as the derivatising reagent [3; 7]. During development and validation of the different methods described in many articles [18; 19] and International Standard [17], it was found that laboratories need to gain experience in dealing with the complexity of the compounds and the determination method.

## Conclusions

The obtained data confirm the correctness of the carried-out validation. Thus, suitability of the method for determining tributyltin in soils and seabed sediments has been documented in accordance with the binding legal regulations.

The above-described method may be successfully applied in a laboratory. The Laboratory of the Environment Protection is the only one in Poland that has been granted accreditation to

carry out the analyses of the TBT's presence in soils and seabed sediments, which was received from the Polish Centre of Accreditation in May 2015.

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