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Validation of phosphorus compounds in seawater

Walidacja związków fosforu w wodzie morskiej

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Abstract: Phosphorus and phosphates, which determine biological development of organisms, are very important components of seawater. Their concentration in the coastal waters of the Baltic Sea is very low, which means that either an analysis of the samples should be performed within a 2 hour limit, or, they must be frozen immediately for further investigation [4; 6; 7]. If it is not possible to perform the analysis of the samples immediately, they should be frozen and shielded from solar radiation in order to halt the biological and biochemical processes. Such a procedure of using the samples, calls for a research method characterized by a low level of the lower limit of quantification, as well as adequate precision, repeatability, and accuracy.
The aim of the carried out validation was to confirm the applicability of the method for determination of phosphorus compounds (total phosphorus, phosphates) in seawater for commercial purposes, in accordance with the recommendations of HELCOM [1; 6]. Within the scope of this work, the lower limit of quantification was determined, and the precision, repeatability, accuracy, and reproducibility were identified. The studies confirm that the requirements for validation of the research methods were met.

Keywords: phosphorus, phosphates, nutrients, Baltic Sea, validation

Streszczenie: Fosfor oraz fosforany są bardzo ważnym składnikiem wody morskiej warunkującym biologiczny rozwój organizmów. Ich stężenia w wodach przybrzeżnych Morza Bałtyckiego są bardzo małe, dlatego należy wykonać analizę próbek najpóźniej do 2 godzin po pobraniu lub natychmiast zamrozić próbki w celu dokonania późniejszych oznaczeń [4; 6; 7]. Jeśli nie jest możliwe natychmiastowe wykonanie analizy próbek należy je zamrozić oraz zabezpieczyć przed światłem słonecznym w celu zatrzymania procesów biologicznych i biochemicznych. Taka procedura postępowania z próbką wymaga metody badawczej o niskiej dolnej granicy oznaczalności oraz odpowiedniej precyzji, powtarzalności i dokładności.
Celem przeprowadzonej walidacji było potwierdzenie możliwości stosowania metody oznaczania zawartości związków fosforu (fosfor ogólny, fosforany) w wodzie morskiej do celów komercyjnych zgodnie z zaleceniami HELCOM [1; 6]. W ramach niniejszej pracy wyznaczono dolną granicę oznaczalności, określono precyzję, powtarzalność, dokładność oraz odtwarzalność. Przeprowadzone badania potwierdzają spełnienie wymagań dotyczących walidacji metod badawczych.

Słowa kluczowe: fosfor, fosforany, biogeny, substancje odżywcze, Morze Bałtyckie, walidacja

Introduction

Biogenic elements are transferred into the Baltic Sea primarily with water flowing from the land, as well as being created in the sea as decomposition and metabolic products of organisms [4]. As a result, a water body becomes richer in nutritional elements, and finally, its eutrophication occurs there. The most significant effect of eutrophication is a decrease in the level of oxygen in the near-bottom waters, which in turn leads to the formation of azoic zones, and the demise of organisms. Under the Convention for the protection of the marine environment of the Baltic Sea region, also known as the Helsinki Convention, the Baltic states conduct a monitoring of the surface waters in order to assess the size of the main pollutant sources. The Baltic states' mission is to gradually reduce the inflow of nutrients into the marine environment from the onshore sources [1]. As far as Poland is concerned, it was a right course of action to introduce the national wastewater treatment program, which has significantly limited the quantity of nutrients getting into the seawater from the onshore point sources [8].

The aim of the performed validation was to confirm applicability of the method for determination of phosphorus compounds content (total phosphorus, phosphates) in seawater in accordance with the HELCOM recommendations [1; 6]. The method, which was based on the recommendations of the Helsinki Commission, is now employed by laboratories conducting monitoring studies of nutrients concentrations in the waters of the Baltic Sea. The carried out research confirms that the requirements concerning the validation of the research methods were fulfilled.

The research material

The material to be used in the analyses for phosphorus and phosphates content consisted of seawater, samples of which had been taken in accordance with the PN-ISO 5667-9:2005 standard - into glass bottles, and into PET bottles in case of samples allotted for freezing. Until being subjected to the analysis, the samples were stored at +4°C temperature. Pursuant to the recommendations, the analysis was conducted within two hours from the moment of sampling [4; 6]. In cases where there was no possibility of doing it this way, the samples had to be frozen. Before initiating the determination procedure, the samples were taken out of the freezer and allowed to thaw and slowly reach the ambient temperature. Every effort was made in order for the analysis to be conducted instantly after the samples had been taken, or after they defrosted. It was a necessity as, in the coastal waters of the Baltic Sea, there are very little nutrients, and after the samples are taken, a rapid decomposition of the compounds to be studied occurs [4; 6; 7].

In order to confirm the correctness of the obtained measurement results, certified reference material QC SW3.2B, Coastal Water by the VKI company no. VKI-32-4-0910 was used.

Research methods

Both of the employed research methods are based on the spectrophotometric technique developed in 1974 by Koroleff, and described in literature by Grasshoff in 1976 [4; 6; 7]. The optical instrumental techniques often find application in chemical oceanography. They make it possible to determine the examined components which occur in very small quantities.

Determination of the total phosphorus

To determine total phosphorus, the selected method was where the very stable organic bonds are broken in an acidic medium by means of oxidation by potassium peroxodisulphate [4; 6; 7]. To achieve this aim, digestion of the samples with the addition of an oxidation mixture was carried out in Teflon vessels, boiled in an autoclave under constant pressure of 800 hPa. The conditions were sustained for a period of 1 hour. Next, 35 cm^3 of well cooled sample was taken, and 1 cm^3 of ascorbic acid was added for the purposes of the reduction of free chlorine. Further analysis consisted of producing phosphomolybdate complex by adding 1 cm^3 of reactive reagent (solution of: antimony potassium tartrate, ammonium molybdate, sulphuric acid). After 15 minutes, the sum of phosphate ions in the created solution was determined via the spectrophotometric method at a wavelength of 820 nm. The possibility of applying the wavelength of 820 nm was confirmed in the course of the interlaboratory comparison.

Determination of phosphates

The determination of dissolved phosphates in marine waters was based on the reaction of phosphate ions with molybdate reagent in an acidic medium in the presence of antimony ions. The created phosphomolybdate complex was next reduced by means of using 1 cm^3 of ascorbic acid, resulting in the production of a compound of blue color. After 5–10 minutes, the sum of phosphate ions in the solution produced this way was determined via the spectrophotometric method at a wavelength of 820 nm.

Accuracy of the method is dependent on the concentration of phosphates to a great degree. Silicates and arsenates occurring in natural conditions may overstate the results of the analysis, what with their make and also their blue complex with molybdate. The pace of these reactions is, however, not identical. In the first few hours the intensity of the silicomolybdate complex's color increases in an almost linear fashion, which is why the interferences of silicates should be eliminated by determining the phosphates, not later than 10 minutes after the ascorbic acid is added [4; 6].

Validation of the analytical methods

The limit of quantification; the measuring range

The limit of quantification (that is – the lowest possible concentration of a substance, that may be determined with use of a particular analytical method with a previously assumed accuracy and precision) was assessed. Moreover, the measuring range was set, which is a range between the lowest and the highest concentrations that may be determined with use of this analytical method with previously assumed precision, repeatability and linearity [9]. The values were defined on the basis of multiple measurements of the blank sample, and of artificial seawater and seawater with an addition of the standard.

Total phosphorus

In Table I/*Tabela I* there results of the lower limit of quantification calculations for the total phosphorus in the marine waters are presented.

On the basis of measurement data, it was established that the 0.3 to 10.0 µmol·dm-3 range of concentrations that maintains linearity is a satisfactory operating range for the method of determining total phosphorus in the marine waters, which is illustrated in Figure 1/*Rysunek 1*.

Tab. I. Compilation of results of the lower limit of quantification calculations for total phosphorus.

NO.	MATRIX	CONCENTRATION [µMOL·DM^{-3}]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]
1	distilled water	0.30	4.58	92.16	7.84
2	artificial seawater	0.30	10.41	100.19	8.39
3	seawater	0.30	5.02	91.09	8.38

Tab. II. Compilation of results of the lower limit of quantification calculation for phosphates.

NO.	MATRIX	CONCENTRATION [µMOL·DM^{-3}]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]
1	distilled water	0.30	3.89	138.37	38.38
2	artificial seawater	0.30	3.39	139.63	39.63
3	seawater	0.30	7.88	79.68	20.32

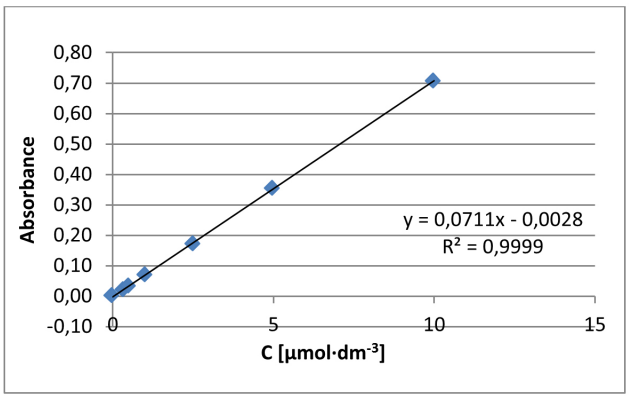

Fig. 1. Total phosphorus calibration curve.

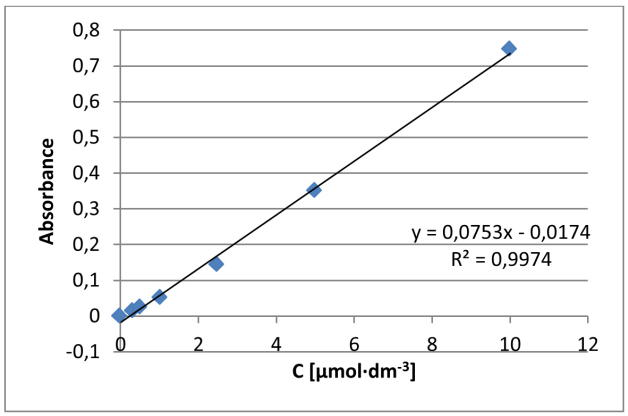

Fig. 2. Phosphates calibration curve.

Phosphates

In Table II/*Tabela II* the calculation results of the lower limit of quantification of phosphates in the marine waters are gathered.

On the basis of measurement data, it was established that the 0.3 to 10.0 µmol·dm^{-3} range of concentrations that maintains linearity is a satisfactory operating range for the method of determining phosphates in the marine waters, which is illustrated in the Figure 2/*Rysunek* 2.

Precision, repeatability, accuracy

Assessment of the method's precision consisted of performing multiple, repetitive measurements for a given material. A series of independent analyses of the actual samples, and the reference materials were carried out. To check whether the analytical methods are appropriate, seven repetitions for the three levels of concentration were performed in respect to each method. While conducting the tests, the QC SW3.2B Coastal Water certified reference material of the VKI company no. VKI-32-4-0910 was used. By means of carrying out the analysis of the reference material and the actual samples under within-laboratory repeatability and reproducibility conditions, the measures of precision were established for the method's measuring range.

Repeatability conditions are provided when independent results of tests of the same samples are obtained via the same method, in the same laboratory, by the same analyst, and with use of the same equipment [9; 11]. By reproducibility we understand obtaining the same results by different laboratories, by different analysts, and with use of different measuring devices [9; 11]. Accuracy is the degree of consistency between the real value, and the value that is a result of an analysis not free from systematic and random errors [2; 3].

Total phosphorus

In Table III/*Tabela III* there are tabulated results of the validation of total phosphorus in the marine waters via spectrophotometric method. The obtained average repeatability of the method was equal to 20.56% in the 0.3 – 0.5 µmol·dm^{-3} range of concentrations; and 2.40% in the 0.6 – 10.0 µmol·dm^{-3} concentration range. The average precision within the repeatability limits was 5.94% in the 0.3 – 0.5 µmol·dm^{-3} range of concentrations; and 0.69% in the 0.6 – 10.0 µmol·dm^{-3} range.

Phosphates

In the Table IV/*Tabela IV* you will find tabulated results of the validation of phosphates in the marine waters via spectrophotometric method. The obtained average repeatability of the method as equal 19.29% in the 0.3 – 0.5 µmol·dm^{-3} range of concentrations; and 2.86% in the 0.6 – 10.0 µmol·dm^{-3} concentration range. The average precision within the repeatability limits was 5.57% in the 0.3 – 0.5 µmol·dm^{-3} range of concentrations; and 083% in the 0.6 – 10.0 µmol·dm^{-3} range.

Tab. III. Precision in the repeatability limits for the method of determining total phosphorus.

NO.	MATRIX	CONCENTRATION [µMOL·DM^{-3}]	PRECISION [%]	REPEATABILITY [%]
1	distilled water	0.30	4.58	15.85
2	artificial seawater	0.30	10.41	36.02
3	seawater	0.30	5.02	17.37
4	distilled water	0.50	2.35	8.13
5	artificial seawater	0.50	4.86	16.,82
6	seawater	0.50	8.43	29.17
7	distilled water	2.50	0.61	2.11
8	artificial seawater	2.50	0.79	2.73
9	seawater	2.50	1.03	3.56
10	distilled water	10.00	0.7	2.42
11	artificial seawater	10.00	0.6	2.08
12	seawater	10.00	0.43	1.49

Tab. IV. Precision in the repeatability limits for the method of determining phosphates.

NO.	MATRIX	CONCENTRATION [µMOL·DM^{-3}]	PRECISION [%]	REPEATABILITY [%]
1	distilled water	0.30	3.89	13.46
2	artificial seawater	0.30	3.39	11.73
3	seawater	0.30	7.88	27.27
4	distilled water	0.50	2.78	9.62
5	artificial seawater	0.50	3.02	10.45
6	seawater	0.50	12.48	43.19
7	distilled water	2.50	2.24	7.75
8	artificial seawater	2.50	0.76	2.63
9	seawater	2.50	1.06	3.67
10	distilled water	10.00	0.26	0.9
11	artificial seawater	10.00	0.56	1.94
12	seawater	10.00	0.072	0.25

Results of the comparative tests

As part of the confirmation of the obtained validation results, the QC SW3.2B Coastal Water reference material of the VKI company No. VKI-32-4-0910, and a sample of seawater taken from the Gulf of Gdansk underwent an assessment. They were tested by the Laboratory of Environment Protection Department of the Marine Institute in Gdansk, as well as by the Division of Marine Chemistry and Environment Protection of the Gdansk University's Institute of Oceanography. Results obtained from the carried out proficiency testing are considered satisfactory. The results are within the range indicated for the certified reference material (Table V/*Tabela V*).

While determining the phosphorus compounds in a sample of seawater, the value measured by the Division of Marine Chemistry and Environment Protection of the Gdansk University's Institute of Oceanography was assumed to be "true", due to the Division's many years experience in this field. In the case of determining the total phosphorus, the percentage of error is significantly lower than in the case of determining the phosphates, the obtained results of the phosphorus compounds are, nonetheless, satisfactory (Table VI /*Tabela VI*).

Discussion

The aim of this work was to confirm the possibility of applying the newly introduced research method. By means of conducting a validation of the method in question proposed by the HELCOM, the method's applicability in an accredited testing laboratory for both scientific and commercial use was proven. The method may be successfully used in assessment of port basins water purity, or of surface water quality. Accuracy of the determinations was ensured by performing the analytical method validation process on various matrices (artificial seawater, deionized water, and the certified reference material) [10].

Tab. V. Results of the comparative tests for phosphorus content in the QC SW3.2B Coastal Water certified reference material by the VKI company no. VKI-32-4-0910.

	RESULTS	
substance	phosphates	total phosphorus
unit	µmol·dm-3	
analytical method	spectrophotometric method	
expected value*	2.11	2.19
determined value (Univ. of Gdansk)	1.99	1.99
deviation from the true value	-0.12	-0.20
% of the error	-5.70	-9.10
determined value (Dept. of EP, MI)	2.06	2.21
deviation from the true value	-0.05	0.02
% of the error	-2.37	0.91

*Expected value i.e. the average obtained for the certified reference material, phosphates concentration range of which is 1.99–2.38 µmol·dm^{-3}, and total phosphorus concentration range is 2.01-2.20 µmol·dm^{-}3.

As a result of the carried out tests, there occurred less errors of the analyses while determining the higher concentrations. The method's validation was verified by undergoing an interlaboratory test, complying with the principles of good laboratory practice [2; 3]. Error for the certified reference material at the 95% confidence level cannot exceed 20%, that is, the recovery must be equal 80 – 120% [3; 5]. The errors obtained as an outcome of the comparative tests were assessed as a satisfying result. For the method of determining phosphates, a level 2.4% of error was reached with the concentration of analyte equaling 2.11 µmol·dm^{-3}, while with the concentration of total phosphorus equaling 2.19 µmol·dm^{-3}, the error was 0.9%. The results are in line with results of the international ICES/SCOR intercalibration experiment, which proved that relative standard error for accuracy of determination of phosphates is equal (0,9 µmol·dm^{-3}) ±5% at the medium level, and with high levels of concentration it is (2,8–9 µmol·dm^{-3}) ±2% [7].

Tab. VI. Results of the comparative tests for phosphates content in a sample of seawater.

		RESULTS			
substance		phosphates	phosphates	total phosphorus	total phosphorus
unit		µmol·dm-3			
Metoda analityczna		metoda spektrofotometryczna			
the real "true" value	(Univ. of Gdansk)	0,71	0,87	1,13	1,44
expanded uncertainty	k=2	0,001	0,004	0,003	0,002
determined value	(Dept. of EP, MI)	0,78	0,95	1,16	1,45
deviation from the true value		0,07	1,16	0,03	0,01
% of the error		9,86	1,45	2,65	0,69

Conclusions

The obtained data confirms the correctness of the carried out validation. Thus, the suitability of the method for determining the compounds in question in the marine waters has been documented in concordance with the binding legal regulations. Due to the low levels of concentrations of phosphorus forms in the Baltic Sea, they are very hard to determine. All precautions should be taken while collecting and analyzing the samples, in order to prevent contamination of the samples by the phosphates leaching out the from the skin. In 2014, the Laboratory of the Environment Protection Department was granted, by the Polish Centre of Accreditation, an accreditation to carry out analyses of the total phosphorus content in the marine waters. The method described above may be used successfully for routine laboratory tests.

Special thanks

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