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Validation of nitrogen compounds in marine waters

Walidacja metod oznaczania związków azotu w wodach morskich

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Abstract: Nitrogen compounds in sea water (total nitrogen, ammonia, nitrates, and nitrites) belong to the nutrients involved in biochemical changes. Nutrients are transferred into the Baltic Sea e.g. from onshore point sources, from diffuse sources, from marine sediments, or from the atmosphere, which results in a water body's becoming richer in nutrition elements, and finally in its eutrophication. Excessive eutrophication creates an imbalance in the ecosystem; among other things, it encourages massive phytoplankton blooms, increases the level of the organic matter as well as of its sedimentation, and it reduces the level of oxygen in the bottom water, which leads to the death of benthic organisms, and demersal fish. An analysis of marine waters constitutes a difficult task e.g. due to the small values of the biogenic substances (μmol/dm^3), their short period of maintenance in a water sample, as well as the high salinity, which may hinder the examinations. An analysis made on a secured sample should be performed within a period not longer than 4-6 hours after the sample's collection. In this article, you will find presented a validation of methods for determining nitrogen compounds (total nitrogen, ammonia, nitrates, and nitrites) in sea water via spectrophotometric method, in accordance with procedures recommended by the HELCOM [8,14]. In analytical chemistry, validation of methods is an important element. It is an obligatory affirmation that all the requirements of a specific intended use have been met. Due to the performed validation of the method, there were determined: limits of detection and quantification, the selectivity of the method, its linearity, precision within the repeatability and/or reproducibility limits, as well as recovery rate and accuracy of the method. Credibility and reliability of the testing procedures in question were confirmed by the positive results of certified reference materials analysis: the VKI Reference Material QC SW3.1B, and QC.SW3.2B, and of comparative (inter-laboratory) research. The methods developed in the process were incorporated into the routine analysis in the Laboratory of Environment Protection of the Maritime Institute in Gdansk, which was accredited by the Polish Centre of Accreditation in 2014 with respect to the discussed research methods.

Keywords: biogenic substances, nutrients, nitrogen forms, validation

Streszczenie: Związki azotu w wodzie morskiej (azot ogólny, amoniak, azotany, azotyny) należą do substancji odżywczych wchodzących w skład przemian biochemicznych. Sole odżywcze dostają się do Bałtyku m.in. z lądowych źródeł punktowych, ze źródeł rozproszonych, z osadów morskich czy też z atmosfery w wyniku czego następuje wzbogacanie zbiornika wodnego w substancje pokarmowe a w rezultacie jego eutrofizacja. Nadmierna eutrofizacja prowadzi do zachwiania równowagi ekosystemu, powoduje m.in. wzrost: masowych zakwitów fitoplanktonu, zawartości materii organicznej i jej sedymentacji oraz spadek zawartości tlenu w wodach przydennych co prowadzi do śmierci organizmów bentosowych i ryb przydennych. Analiza wód morskich jest trudna m.in. ze względu na małe wartości substancji biogenicznych (μmol/dm^3), ich krótki okres utrzymywania się w próbce wody, jak również wysokie zasolenie, które może utrudniać przeprowadzenie badań. Analiza w zabezpieczonej próbce powinna być wykonana w okresie nie dłuższym niż 4-6 h po pobraniu. W niniejszym artykule przedstawiono walidację metod oznaczania związków azotu (azot ogólny, amoniak, azotany, azotyny) w wodach morskich metodą spektrofotometryczną, w oparciu o procedury zalecane przez HELCOM [8,14]. Walidacja metod jest ważnym elementem w praktyce chemii analitycznej. Jest obowiązkowym potwierdzeniem, że zostały spełnione wymagania konkretnie zamierzonego zastosowania. W wyniku przeprowadzonej walidacji metody określono: granice wykrywalności i oznaczalności, selektywność metody, liniowość, precyzję w granicach powtarzalności i/lub odtwarzalności oraz odzysk i dokładność metody. Wiarygodność i rzetelność omawianych procedur badawczych potwierdzono pozytywnymi wynikami analizy certyfikowanych materiałów odniesienia: VKI Reference Material QC SW3.1B i QC.SW3.2B oraz badań porównawczych (międzylaboratoryjnych). Opracowane metody zostały wdrożone do rutynowej analizy Laboratorium ZOŚ IM, które w 2014 r. uzyskało akredytację PCA w zakresie omawianych metod badawczych.

Słowa kluczowe substancje biogeniczne, sole odżywcze, związki azotu, walidacja

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Introduction

In marine waters, 95% of nitrogen occurs in the N_2 form, and the remaining 5% is the organic nitrogen, nitrates, nitrites, and ammonia. Concentrations of nutrients in the marine water are dependent on numerous processes. Some of them are of a seasonal character – changes of the circadian cycle, primary production, or periodical dynamic phenomena taking place in the sea. Other ones, such as seabed or coastal erosion resulting from marine currents' impact, depend on wind speed and direction [7]. Diversity and complex physical, chemical, and biological nature of the processes occurring in the water column and the biological absorption in the zone euphotic affect the levels of concentration, as well as the quality of given substances' transformation [7]. Nutrients are transferred into the Baltic Sea e.g. from onshore point sources (sewage transfer collectors of the sewage treatment plants), from diffuse sources (outflow of the urbane sewage networks), an intake from the atmosphere, or from the marine sediments, the result of which is the water body's becoming richer and richer in nutrition elements, and finally, its eutrophication. The threats eutrophication poses are first of all: an intensive growth and massive phytoplankton blooms, increase the level of organic matter in the water, a rise in sedimentation of organic matter, reduction of oxygen level in the bottom water, which in turn leads to the death of benthic organisms and demersal fish.

A basic process of eliminating inorganic nitrogen from the seawater is its assimilationby phytoplankton. The total amount of elements removed from seawater this way is relatively small. The degree of inorganic nitrogen's accumulation in deep waters is controlled by the biological production occurring in surface water by means of regenerative processes in the environment ratio, vertical mixing, diffusion, lateral movements of deep waters, and diffusion from the sediments [2].

The content of biogenic compounds in the marine environment is also dependent on what season of the year it is. The lowest concentrations of biogens in water occur in the summer. The maximum level of nitrogen forms' intake is observed in the winter period [13,13a].

The most common form of dissolved inorganic nitrogen occurring in the Baltic Sea is the nitrates. In the spring–summer period, concentrations of nitrogen compounds may decrease to the level of trace amounts due to their being more intensively absorbed by phytoplankton and phytobenthos, while in the winter period there occurs a rise in the nutrients content [3,13a]. Nitrates reach their highest levels right after the winter, and then a decrease of their levels is observed. Nitrites and ammonia, on the other hand, reach their maximum levels in August and September. At great depths, there is observed a high concentration of nitrates, and in the anaerobic areas, bacteria may transform nitrates into free nitrogen as a result of denitrification [9,10,11,12].

In 2007, in Cracow, there was created within the framework of the Helsinki Convention, a programme called the HELCOM Baltic Sea Action Plan. "Its basic aim is to obtain good ecological status by the waters of the Baltic through gradual reduction of the discharge of nutrients, that is loads of nitrogen and phosphorus from land sources, getting to it through the catchment area or as a result of wet and dry atmospheric deposition" (Manual for Marine Monitoring in the COMBINE Programme of HELCOM, 17th of Jan. 2014 [8, 14, 15]).

Test material

What we call validation, is an affirmation through examining and presenting an objective proof of that all the requirements of a specific intended use have been met [5]. A validation should be performed to the extent necessary for the intended use. Thanks to data gathered through validation, there may be determined the actual extent - limits of detection, precision and accuracy of the measurement, repeatability, and there also may be estimated the measurement uncertainty [4].

The material designated for testing and determination consisted of seawater from the coastal regions of the South Baltic Sea. The water samples were taken by trained employees of the Operational Oceanography Department and the Laboratory of Environment Protection of the Maritime Institute in Gdansk. Taken with a rosette sampler at selected points distributed over the vertical section, put into the plastic bottles, and the samples were immediately frozen in order to be transported to the laboratory. The tests were also performed with use of standard solutions, and artificial seawater.

To determine the correctness – accuracy, there were used the certified reference materials VKI QC SW3.1B, and QC.SW3.2B.

The reproducibility was determined in the course of interlaboratory research [5]. The repeatability and reproducibility were assessed with use of deionized water, and artificial seawater (deionized water mixed with a little of 5.77g/dm^3 NaCl) with the addition of the KNO$_3$ MERCK standard no. A0256461 – nitrates, with the addition of the NH4Cl MERCK standard no. HC258088 – ammonia, with the addition of the NaNO$_2$ MERCK standard no. HC 258100 - nitrites.

Test methods

Determination of ammonia – NH_3 and NH_4^+

Ammonia was determined with use of the spectrophotometric method [6, 8]. The analysis consists of:

- staining a 50 cm^3 sample: 2 cm^3 of phenolic reagent, 1 cm^3 of citrate buffer, and 2 cm^3 of sodium hypochlorite,
- keeping the sample at a temperature of 37 ± 1°C in a thermostat for 1 hour,
- performing the measurement on the spectrophotometer at a wavelength of λ =620 nm against a blank after the samples are cooled to ambient temperature.

Determination of nitrites

The nitrites were determined with use of the spectrophotometric method [6, 8]. The analysis consists of:

- staining a 50 cm^3 sample: 1 cm^3 sulfanilamide and 1 cm^3 of N-(1-naphthyl)-ethylenediamine with dihydrochloride,
- performing the measurement on the spectrophotometer at a wavelength of λ =550 nm against a blank after 20 minutes.

Determination of nitrates and total nitrogen (TN)

Analysis of nitrates and total nitrogen requires a proper preparation of cadmium reduction columns, which are used for reducing nitrates to nitrites. Granules of cadmium are washed with 2M HCl in order to remove the oxide film, after which they are washed thoroughly with distilled water on an oxygen-free basis. Next, 100 cm^3 of 1% solution of copper sulfate is added and stirred vigorously for 3 minutes, so that the granules get covered with a copper film. Thereafter, cadmium needs to be thoroughly cleaned with distilled water until the water is clean and transparent.

A cadmium column should be filled in such a way, so that the granules are evenly distributed [Figure 1/*Rysunek* 1]. In order for the reduction to be performed, cadmium has to be activated by means of washing it subsequently with 250 cm^3 ammonium buffer with the addition of 2.5 cm^3 of the KNO_3 standard at a concentration of 10 µmol/cm^3; 1000 cm^3 of deionized water; and 500 cm^3 of ammonium buffer. Each of the determinations (TN, and NO_3) is performed with use of separately prepared columns, and calibration curves.

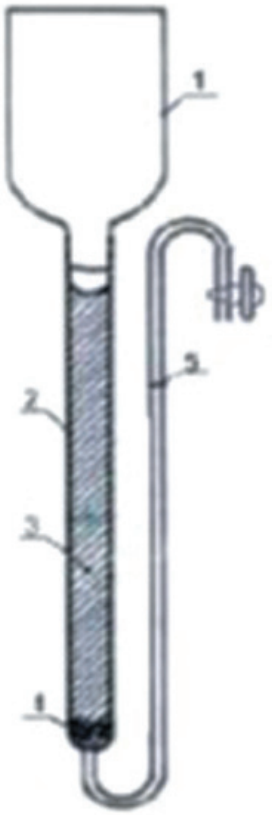

Fig 1. Model of a cadmium reduction column [1].
1-a 110 mm high container with an internal diameter of 50 mm; 2-a 300 mm long pipe with an internal diameter of 10 mm; 3-filler, amalgamated cadmium (Cd/Cu); 4-glass wool; 5-a 400 mm long capillary tube with an internal diameter of 2 mm.

Total nitrogen

The analysis involves determining the total nitrogen using the colorimetric method with oxidation of the samples, and reduction to nitrites [6, 8]. Mineralization of the samples takes place in Teflon containers with an oxidation mixture inside a pressure cooker under the constant pressure of 0.79 atm (800 hPa) for a period of 1 hour.

For the purposes of reduction performed on cadmium columns, a 5 cm^3 of the mineralized cooled sample is taken, and diluted with 45 cm^3 of deionized water, and 1 cm^3 of concentrated ammonium buffer with a 7.4 pH is next added. After the reduction, 20 cm^3 of the filtrate is removed, and another 25 cm^3 of it is taken for the staining. The column flow rate - the reduction should take 6-8 ml / min. Next, 0.5 cm^3 of sulfanilamide, and 0.5 cm^3 of the N-(1-naphthyl)-ethylenediamine of dihydrochloride solution is added. The absorbance of the solutions is determined after 20 minutes on the spectrophotometer at a wavelength of λ =550 nm against a blank, which consists of mineralized oxidation mixture run through the cadmium columns after being diluted in the relation of 0.65 cm^3 of the oxidation mixture to 49.35 cm^3 of deionized water.

Nitrates

Nitrates were determined using the spectrophotometric method after reduction to nitrites [6, 8]. An amount of 50 cm^3 of the sample and next 50 cm^3 of the ammonium buffer with an 8.5 pH should be measured with a graduated cylinder and mixed together. Samples prepared this way are next poured onto the reduction columns. The first 40 cm^3 of the filtrate is discarded, and the following 50 cm^3 is collected for the purposes of further analysis. The column flow rate - the reduction should take 6-8 ml / min. Nitrite ions are determined by adding 1 cm^3 of sulfanilamide and 1 cm^3 of the N-(1-naphthyl)-ethylenediamine of dihydrochloride solution. The samples are then stirred and left for staining for 30 minutes in the dark. The absorbance of the solutions is determined after 30 minutes on the spectrophotometer at a wavelength of λ =550 nm against a blank. The concentration of nitrites performed parallel on the very same sample is then deduced from the obtained measurement value.

Validation of the methods

The limit of quantification

The first validation activity to be taken was a determination of the quantification limits of the particular analyses, and next, setting the working range.

Tab. I. Ammonia: summary of results of the lower limit of quantification assessment.

INDICATED VALUE [µMOL/DM³]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
0.2	9.83	99.3	7.86	deionized water
0.2	11.09	96.4	9.29	artificial seawater
0.2	12.07	88.6	11.43	seawater with the addition of standard NH4Cl [6]

Tab. II. Nitrites: summary of results of the lower limit of quantification assessment.

INDICATED VALUE [µMOL/DM3]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
0.2	2.9	97.2	3.45	distilled water
0.2	3.5	109.6	9.57	artificial seawater
0.2	1.7	93.3	6.74	seawater with the addition of standard $NaNO_2$ [6]

Tab. III. Nitrates: summary of results of the lower limit of quantification assessment.

INDICATED VALUE [µMOL/DM³]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
0.2	11.63	102.8	9.42	distilled water
0.2	21.12	97.8	17.19	distilled water
0.2	22.41	85.8	18.90	distilled water
0.2	14.94	81.8	19.75	artificial seawater
0.2	19.71	69.8	30.17	artificial seawater
0.2	20.83	107.4	15.28	artificial seawater

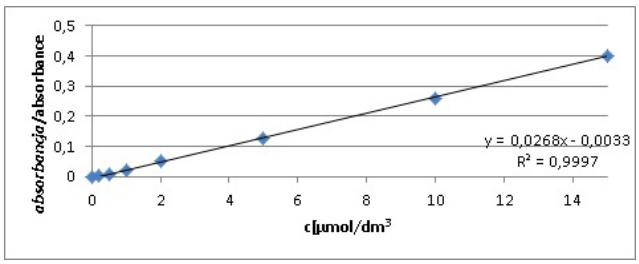

Fig 2. Ammonia calibration curve, λ= 620nm, k=1cm.

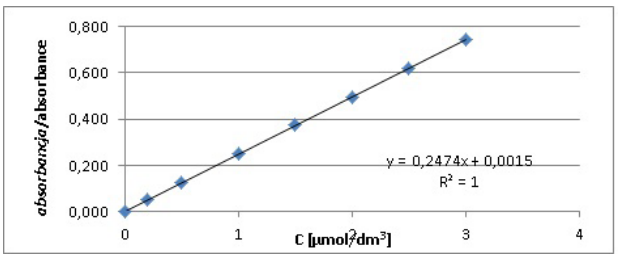

Fig 3. Nitrites calibration curve, λ= 550nm, k=5cm.

Limit of quantification is the lowest possible concentration of an analyte that can be determined on an acceptable level of repeatability, precision, and correctness. Working range is a span between the minimum and maximum level of concentration obtained via a given research method keeping an acceptable level of repeatability, precision, and correctness [5].

Ammonia

In Table I /*Tabela I*, there are tabulated results of the lower limit of quantification assessment as performed for the ammonia in the marine waters.

The working range set for the method of determining ammonia in the marine waters via the spectrophotometric method is from 0.2 to 15 µmol/dm^3 [see Graph 1/*Wykres 1*, Table I/*Table I*].

Nitrites

In Table II/*Tabela II*, there are tabulated results of the lower limit of quantification assessment as performed for the nitrites in the marine waters.

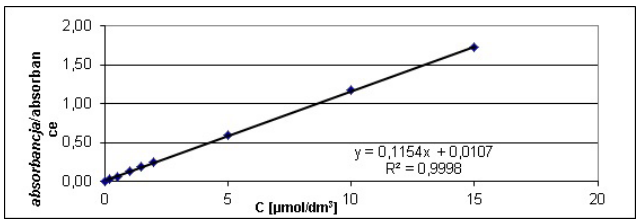

Fig 4. Nitrates calibration curve per column reductive, λ= 550nm, k=5cm.

The working range set for the method of determining nitrites in the marine waters via the spectrophotometric method is from 0.2 to 3.0 µmol/dm^3 [see Graph 2/*Wykres 2*, Table II/*Table II*].

Nitrates

In Table III/*Tabela III*, there are tabulated results of the lower limit of quantification assessment as performed for the nitrates in the marine waters.

The working range set for the method of determining nitrates in the marine waters via the spectrophotometric method is from 0.2 to 15.0 µmol/dm^3 [see Graph 3/*Wykres 3*, Table III/*Table III*].

Tab. IV. Total nitrogen: summary of results of the lower limit of quantification assessment.

INDICATED VALUE [µMOL/DM³]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
5	5.03	94.7	5.97	distilled water
5	9.79	100.3	7.86	distilled water
5	8.41	99.1	6.89	distilled water
5	10.20	102.3	9.17	artificial seawater
5	7.29	100.0	5.94	artificial seawater
5	9.57	101.7	8.57	artificial seawater
5	19.14	107.0	18.66	seawater with the addition of standard KNO_3 [6]
5	14.73	101.6	12.14	seawater with the addition of standard KNO3 [6]

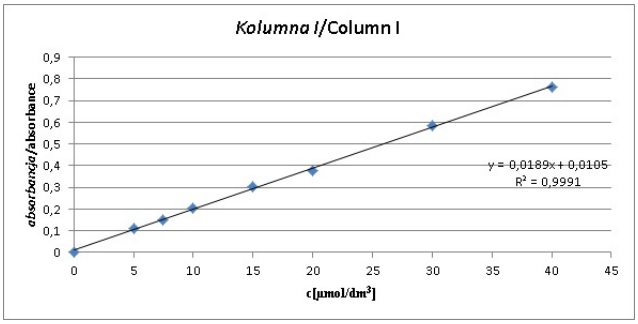

Fig 5. Total nitrogen calibration curve λ= 550nm, k=5cm.

Total nitrogen

In Table IV/*Tabela IV*, there are tabulated results of the lower limit of quantification assessment as performed for the total nitrogen in the marine waters.

The working range set for the method of determining total nitrogen in the marine waters via the spectrophotometric method is from 6 to 60 µmol/dm^3 [see Graph 4/*Wykres 4*, Table IV/*Table IV*].

Precision, repeatability, accuracy

The accuracy of a measurement is the degree to which its result complies with a certain accepted true value of the quantity subjected to measurement [5].

Repeatability is the precision of determinations carried out by the same analyst, in the same laboratory, with the usage of the same method, and the same equipment within a short period of time [5].

Ammonia

In Table V/*Tabela V*, there are tabulated results of the validation of ammonia in the marine waters via the spectrophotometric method. The obtained repeatability of the method was equal 30% for the concentrations within the 0.2 – 10 µmol/dm^3 range, and 12.5% for the 10 – 15 µmol/dm^3 range.

In Table VI/*Tabela VI*, there are presented results obtained from analysis of certified reference material VKI Reference Material QC SW3.1B, on the basis of which the correctness – accuracy was determined.

Nitrites

In Table VII/*Tabela VII*, there are presented exemplary results of the validation of nitrites in the marine waters via the spectrophotometric method. The obtained repeatability of the method was equal 10% for the quantification limit equal 0.2 µmol/dm^3, and 5% for concentrations within the 0.3 – 3 µmol/dm^3 range.

In Table VIII/*Tabela VIII*, there are presented results obtained from analysis of certified reference material VKI Reference Ma-

Tab. V. Summary of results for ammonia.

INDICATED VALUE [µMOL/DM3]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
2	8.62	102.7	7.71	distilled water
2	11.54	98.7	9.86	artificial seawater
2	3.26	109.8	9.79	seawater with the addition of standard NH_4Cl [6]
10	9.25	101.8	8.07	distilled water
10	2.08	92.2	7.79	artificial seawater
10	6.17	116.2	16.24	seawater with the addition of standard NH_4Cl [6]
15	2.03	106.9	6.91	distilled water
15	3.38	107.2	7.17	artificial seawater
15	3.41	94.3	5.53	seawater with the addition of standard NH_4Cl [6]

Tab. VI. The VKI QC SW3.1B – VKI – 32 – 0910 reference material; permissible range (1.81 – 2.25 µmol/dm³).

REFERENCE VALUE [µMOL/DM³]	INDICATED VALUE [µMOL/DM³]	RECOVERY [%]	ERROR [%]	ABSOLUTE ERROR [%]
2.03	1.92	94.6	-5.42	5.42
2.03	1.85	91.1	-8.87	8.87
2.03	2.00	98.5	-1.48	1.48
2.03	2.04	100.5	0.49	0.49
2.03	1.96	96.5	-3.45	3.45
2.03	2.23	109.8	9.85	9.85
2.03	1.85	91.1	-8.87	8.87
average	1.98	97.5		5.49
SD	0.13			
RSD	6.66			

SD – standard deviation / odchylenie standardowe

RSD – relative standard deviation / względne odchylenie standardowe

Tab. VII. Summary of results for nitrites.

INDICATED VALUE [µMOL/DM³]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
0.5	0.67	91.2	8.82	distilled water
0.5	1.96	106.0	6.00	artificial seawater
1.5	1.16	95.4	4.55	distilled water
1.5	1.55	100.6	1.36	artificial seawater
1.5	0.69	95.4	4.57	seawater with the addition of standard $NaNO_2$ [6]
3	0.41	97.6	2.39	distilled water
3	1.55	100.6	1.36	artificial seawater
3	0.15	94.9	5.05	seawater with the addition of standard $NaNO_2$ [6]

Tab. VIII. The VKI QC SW3.1B – VKI – 32 – 0910 reference material; permissible range (0.84 – 0.89 µmol/dm³).

REFERENCE VALUE [µMOL/DM3]	INDICATED VALUE [µMOL/DM3]	RECOVERY [%]	ERROR [%]	ABSOLUTE ERROR [%]
0.86	0.87	101.2	1.16	1.16
0.86	0.86	100.0	0.00	0.00
0.86	0.87	101.2	1.16	1.16
0.86	0.86	100.0	0.00	0.00
0.86	0.87	101.2	1.16	1.16
0.86	0.86	100.0	0.00	0.00
0.86	0.87	101.2	1.16	1.16
average	0.87	100.7		0.66
SD	0.01			
RSD%	0.62			

SD – standard deviation / odchylenie standardowe

RSD – relative standard deviation / względne odchylenie standardowe

terial QC SW3.1B, on the basis of which the correctness – accuracy was determined.

Nitrates

In Table IX/*Tabela IXI*, there are presented exemplary results of the validation of nitrates in the marine waters via the spectrophotometric method. The obtained repeatability of the method was equal 32% for the quantification limit equal 0.2 µmol/dm^3, it was 21.2% within the 0.3 – 0.5 µmol/dm^3 range, and 12.5% within the 0.5 – 15 µmol/dm^3 range.

In Table X/*Tabela X*, there are presented results obtained from analysis of certified reference material VKI Reference Material QC SW3.1B, on the basis of which the correctness – accuracy was determined.

Tab. IX. Summary of results for nitrates.

INDICATED VALUE [µMOL/DM³]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
0.5	6.87	89.6	10.35	K I distilled water
0.5	6.86	95.9	6.80	K II distilled water
0.5	8.86	87.2	12.76	K III distilled water
5	1.21	101.3	1.36	K I distilled water
5	0.69	102.1	2.13	K II distilled water
5	1.22	102.5	2.49	K III distilled water
5	0.74	99.7	0.54	K I artificial seawater
5	2.11	100.6	1.59	K II artificial seawater
5	1.69	100.5	1.58	K III artificial seawater
5	8.97	101.1	7.14	K I seawater with addition of standard KNO_3 [6]
5	7.87	102.3	6.35	K II seawater with addition of standard KNO_3 [6]
5	17.84	103.1	13.75	K III seawater with addition of standard KNO_3 [6]
15	1.05	95.2	4.79	K I distilled water
15	1.17	96.1	3.88	K II distilled water
15	1.00	96.3	3.74	K III distilled water
15	0.99	96.5	3.49	K I artificial seawater
15	0.92	96.8	3.15	K II artificial seawater
15	0.94	96.5	3.47	K III artificial seawater
15	5.97	85.9	14.11	K II seawater with addition of standard KNO_3 [6]
15	0.54	92.0	7.96	K seawater with addition of standard KNO_3 [6]

K I – cadmium reduction column no. 1 / kolumna kadmowa 1
K II – cadmium reduction column no.2 / kolumna kadmowa 2
K III – cadmium reduction column no. 3 / kolumna kadmowa 3

Tab. X. The VKI QC SW3.1B – VKI – 32 – 0910 reference material – column no. 2; permissible range (11.2 – 11.8 µmol/dm³).

REFERENCE VALUE [µMOL/DM³]	INDICATED VALUE [µMOL/DM³]	RECOVERY [%]	ERROR [%]	ABSOLUTE ERROR [%]
11.5	11.309	98.3	-1.66	1.66
11.5	11.387	99.0	-0.99	0.99
11.5	11.361	98.8	-1.21	1.21
11.5	11.343	98.6	-1.36	1.36
11.5	11.413	99.2	-0.76	0.76
11.5	11.343	98.6	-1.36	1.36
11.5	11.361	98.8	-1.21	1.21
average	11.36	98.8		1.22
SD	0.03			
RSD%	0.29			

SD – standard deviation / odchylenie standardowe

RSD – relative standard deviation / względne odchylenie standardowe

Total nitrogen

In Table XI/*Tabela XI*, there are presented exemplary results of the validation of total nitrogen in the marine waters via the spectrophotometric method. The obtained repeatability of the method was equal 25% within the 5 – 15 µmol/dm^3 range, and 17.5% within the 15 – 60 µmol/dm^3 range.

In Table XII/*Tabela XII*, there are presented results obtained from analysis of certified reference material VKI Reference Material QC SW3.1B, on the basis of which the correctness – accuracy was determined.

Comparative tests results

Validation of the research of the discussed biogenic substances in marine waters involved the participation of the Laboratory of

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Tab. XI. Summary of results for total nitrogen.

INDICATED VALUE [µMOL/DM³]	PRECISION [%]	RECOVERY [%]	ABSOLUTE ERROR [%]	MATRIX
15	9.07	100.9	7.48	K I deionized water
15	8.81	97.1	7.54	K II deionized water
15	8.02	102.3	6.92	K III deionized water
15	8.26	93.1	8.60	K I artificial seawater
15	5.34	99.2	4.20	K II artificial seawater
15	5.48	101.2	4.81	K III artificial seawater
15	5.21	82.8	17.19	KI seawater with addition of standard KNO3 [6]
15	9.63	83.7	16.74	K III seawater with addition of standard KNO3 [6]
40	4.48	100.3	4.03	K I deionized water
40	7.19	102.9	6.88	K II deionized water
40	5.55	101.1	4.84	K III deionized water
40	6.99	96.1	5.69	K I artificial seawater
40	3.09	100.2	2.52	K II artificial seawater
40	1.64	103.7	3.75	K III artificial seawater
40	2.15	84.3	15.73	K I seawater with addition of standard KNO3 [6]
40	3.84	85.8	14.20	K III seawater with addition of standard KNO3 [6]
50	2.31	100.5	1.81	K I deionized water
50	1.58	90.6	9.37	K II deionized water
50	2.08	99.5	1.75	K III deionized water
60	5.19	92.7	7.43	K I deionized water
60	5.30	92.6	7.46	K II deionized water
60	4.83	99.8	3.53	K III deionized water
60	4.45	88.3	11.73	K I seawater with addition of standard KNO3 [6]
60	2.10	86.0	14.00	K III seawater with addition of standard KNO3 [6]

K I – cadmium reduction column no. 1 / kolumna kadmowa 1
K II – cadmium reduction column no.2 / kolumna kadmowa 2
K III – cadmium reduction column no. 3 / kolumna kadmowa 3

Tab. XII. The VKI QC SW3.1B – VKI – 32 – 0910 reference material – column no. 1; permissible range (13.9 – 16.0 µmol/dm³).

REFERENCE VALUE [µMOL/DM³]	INDICATED VALUE [µMOL/DM3]	RECOVERY [%]	BŁĄD [%]	ABSOLUTE ERROR [%]
14.9	15.32	102.8	2.82	2.82
14.9	14.15	95.0	-5.03	5.03
14.9	14.21	95.4	-4.63	4.63
14.9	14.68	98.5	-1.48	1.48
14.9	15.53	104.2	4.23	4.23
14.9	14.52	97.4	-2.55	2.55
14.9	15.26	102.4	2.42	2.42
average	14.81	99.4		3.31
SD	0.56			
RSD%	3.78			

SD – standard deviation / odchylenie standardowe
RSD – relative standard deviation / względne odchylenie standardowe

Environment Protection of the Maritime Institute in Gdansk in an inter-laboratory comparison with the Department of Marine Chemistry and Environmental Protection of the Gdansk University's Faculty of Oceanography. The department can boast of having a substantial experience in researching biogenic substances in the marine waters. The results obtained by the University of Gdansk were assumed to be the "true" value. A synthesis of inter-laboratory research findings can be found in Table XIII/*Tabela XIII.*

Tab. XIII. Results of the inter-laboratory research.

NAME OF THE SUBSTANCE	UNITS	ANALYTICAL METHOD	„TRUE" REAL VALUE (UG)	EXPANDED UNCERTAINTY	INDICATED VALUE (DEP, MI)	DEVIATION FROM THE TRUE VALUE	% OF ERROR
Nitrites (NO2)	µmol/dm3	PB-29, issue 2 MI, Dept. of EP Spectrophotometric method UG	0.32	0.002	0.36	0.04	12.5
Nitrates (N_{O_3})	µmol/dm3	PB-29, issue 2 MI, Dept. of EP Spectrophotometric method UG	27.97	0.036	27.57	-0.4	-1.43
Ammonia (NH_4)	µmol/dm3	PB-29, issue 2 MI, Dept. of EP Spectrophotometric method UG	0.59	0.013	0.55	-0.04	-6.78
Total nitrogen (TN)	µmol/dm3	PB-29, issue 2 MI, Dept. of EP Spectrophotometric method UG	39.98	0.013	48.54	8.56	21.4

MI, Dept. of EP – Maritime Institute, the Laboratory of Environment Protection Department / Instytut Morski w Gdańsku, laboratorium Zakładu Ochrony Środowiska (ZOŚ IM).

UG – University of Gdańsk, Department of Marine Chemistry and Environmental Protection / Uniwersytet Gdański, Zakład Chemii Morza i Ochrony Środowiska.

Tab. XIV. Summary of the averaged validation's results.

	PRECISION WITHIN THE REPEATABILITY LIMITS	REPEATABILITY	CORRECTNESS - ACCURACY	EXPANDED UNCERTAINTY
TN	5 - 15 µmol N/dm - 10.0% 15 - 60 µmol N/dm - 7.0%	5 - 15 µmol N/dm - 25.0% 15 - 60 µmol N/dm - 17.5%	9%	5 - 15 µmol N/dm - 36.0% 15 - 60 µmol N/dm - 21.0%
NO_2	0.2 µmol NO2/dm3 - 4.0% 0.3-3.0 µmol NO2/dm3 – 2.0%	0.2 µmol NO2/dm3 - 5.0% 0.3-3.0 µmol NO2/dm3 -10.0%	2%	0.2 µmol NO2/dm3 - 25.0% 0.3 - 3.0 µmol NO2/dm3 - 9.8%
NO_3	0.2 µmol NO3/dm3 - 18.5% 0.3 - 0.5 µmol NO3/dm3 - 8.5% 0.5 - 15 µmol NO3/dm3 - 5.0%	0.2 µmol NO3/dm3 - 32.0% 0.3 - 0.5 µmol NO3/dm3 - 21.2% 0.5 - 15 µmol NO3/dm3 - 12.5%	2.5%	0.2 µmol NO3/dm3 - 42.5% 0.3 - 15 µmol NO3/dm3 - 21.5%
NH_4	0.2 - 2.0 µmol NH4/dm3 - 12.0% 2.0 - 10 µmol NH4/dm3 - 9.0% 10 - 15 NH4/dm3 - 5.0%	0.2 - 10.0 µmol NH4/dm3 - 30.0% 10 - 15 NH4/dm3 - 12.5%	10%	0.2 - 2.0 µmol NH4/dm3 - 34.0% 2.0 - 10 µmol NH4/dm3 - 22.0% 10 - 15 µmol NH4/dm3 - 17.0%

Tab. XV. Results obtained for the VKI Reference Material QC SW3.1B certified reference material.

	REFERENCE VALUE [µMOL/DM³]	ACCEPTABLE RANGE	MARKED AVERAGE VALUE [µMOL/DM³]	RECOVERY [%]	ERROR [%]	ABSOLUTE ERROR [%]	SD	RSD%
TN	14.90	13.9 - 16.0	15.08	101.2	1.18	4.29	0.75	4.94
NH_4	2.03	1.81 - 2.25	1.98	97.5	- 2.54	5.49	0.13	6.66
NO_3	11.50	11.2 - 11.8	11.29	98.1	-1.86	1.87	0.06	0.54
NO_2	0.86	0.84 - 0.89	0.87	100.7	0.66	0.66	0.01	0.62

Discussion and conclusions

Due to a large number of samples validation were performed on three cadmium columns to accelerate the analysis.

As a result of the performed validation of the method, there were determined the following: the result's uncertainty, limits of detection and quantification, selectivity of the method, its linearity, precision within the repeatability and/or reproducibility limits, as well as recovery rate and accuracy of the method. In Table XIV/*Tabela XIV* below, there are tabulated the obtained values.

Credibility and reliability of the developed testing procedures were confirmed by the positive results of the VKI Reference Material QC SW3.1B certified reference material's analysis, and of comparative (inter-laboratory) research. Table XV/*Tabela XV* contains a summary of the VKI material's research results.

As an outcome of the research performed, it was noted that:

- due to small amounts of nitrogen compounds (µmol/dm³) in the marine waters, the shortness of their period of maintenance in a water sample (ability to be transformed into other forms), as well the high salinity, a thorough analysis of the substances in question as present in the marine waters is a very difficult and time-consuming task, especially for the newbie analysts.
- the obtained results of the validation of the methods in question prove they're being useful in the process of determining nitrogen compounds in the marine waters, and referring them to the applicable regulations.
- methods developed in the process were incorporated into the analysis routine in the Laboratory of Environment Protection of the Maritime Institute in Gdansk and were accredited by the Polish Centre of Accreditation in 2014.
- with respect to the time-consuming nature of the applied spectrophotometric methodologies, and the de-

gree of their complexity, the Laboratory of Environment Protection of the Maritime Institute in Gdansk has already launched preparatory actions for performing analyses of biogenic substances in marine waters via the ion chromatography method with usage of the Dionex ICS-1100 chromatograph by the Thermo Scientific company.

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