APPLICATION NOTE 71395

Automated total oxidized nitrogen method using vanadium as reductant with correlation to cadmium and hydrazine reductant methods in sea, natural, and waste waters

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Keywords

Discrete Analyzer, Aquakem 250, Gallery, Gallery Plus

Goal

Correlation of a Nitrate method (TON Vanadium) to TON Cadmium and TON Hydrazine methods using automated discrete analysis

Introduction

Total oxidized nitrogen (TON) or the sum of nitrate and nitrite in water samples can be determined using a variety of techniques. Hydrazine is often used in automated analysis as the reducing agent, converting nitrate to nitrite.[1] Unfortunately the hydrazine method cannot be used to analyze a seawater sample limiting its use as a general purpose method.[2] The Cadmium (Cd) Reduction method, therefore, is still the reference method used in many countries. The Cd Reduction method has several drawbacks which include the personal risks to users when handling the Cd-column and the column itself may be easily damaged by using an oily sample matrix.[3] Enzymatic reduction is a very good alternate method;[4,5] however it is not widely known to most laboratories, despite recently having been described as an alternate method by the United States Environmental Protection Agency (US EPA).

In this application note an automated, two reagent method using vanadium chloride as a reducing agent was studied and compared to both the hydrazine and Cd reduction methods. Vanadium chloride as reductant has routinely been used in nitrate analysis. In one application, the reduction step in the method is performed at a temperature greater than 80 °C and nitrous oxide (NO) is measured by chemiluminescent detection.[6,7] When the temperature of the reduction step is lowered to room temperature or 37 °C, a nitrite (NO_2) ion is formed and it can be analyzed photometrically.[8] Based on the literature, a method with vanadium chloride reduction followed by the Griess reaction[9] has been used to determine TON in water samples.[10,11]

The principle of the TON Vanadium method is to reduce the sample nitrate (NO_3) with vanadium chloride to NO_2[6-8] which then reacts to form a pink colored diazodye by Griess reagents, sulphanilamide, and N-(1-naphthyl)-ethylenediamine.[9] The NO_2 originally present in the sample will also be determined. The intensity of the color is measured photometrically at 540 nm and the concentration of $NO_3 + NO_2$ in the sample is calculated using a calibration curve. (TON or the sum of nitrate and nitrite is calculated as N or NO_3-N+NO_2-N. All results presented in this study are reported as TON.)

Multiple types of water samples were tested: waste, natural, low natural, brackish, household, and swimming pool water. Natural water or low natural waters are defined as surface water from lakes, rivers, or trenches with TON from 0–500 µg/L. Household water includes both well water and municipal drinking water. Brackish water contains a higher level of salinity than fresh water but not at the level of seawater. Seawater samples from the Indian and Andaman Oceans were also analyzed to understand the performance of this method using water with a higher salt concentration than local Finnish brackish seawaters.

The correlation study was performed in the Finnish food, water, and environmental laboratory, MetropoliLab, accredited by FINAS, a national accreditation body in Finland. The laboratory has a long history of analyzing various types of water samples; one of the main nutrients analyzed for TON.

Experimental

Materials and methods

Instruments

- Thermo Scientific™ Aquakem™ 250, a discrete analyzer (Thermo Fisher Scientific, Vantaa, Finland)
- FOSS Tecator FIAstar™ 5000 Systems analyzer (FOSS, Hillerod, Denmark) with Prepacked FOSS Cd-column (no: 5000 3139)
- Thermo Scientific™ Gallery™, a discrete analyzer (Thermo Fisher Scientific, Vantaa, Finland)

Applications

The application for TON Cd reduction (FOSS FIAstar™) is a FINAS accredited method for nitrate nitrogen using the sum of nitrite nitrogen and nitrate nitrogen analysis. This method is based on SFS-EN ISO 13395:1997.

The application for TON Hydrazine reduction (Aquakem) is a FINAS accredited method for nitrite nitrogen analysis. This in-house method is based on SFS 3029:1976.

Table 1 contains a list of the sample types analyzed and the specific methods used and Table 2 shows specific parameters, such as reagent and sample volumes used in the TON Vanadium application test flows.

Table 1. Methods and sample types for the correlation study

Sample Type	Hydrazine Application (Aquakem 250)	Cadmium Application (FIAstar™ 5000 Systems)	Vanadium Application (Aquakem 250)
Waste Water	x	—	TON-V 2 mg TON-V 5 mg
Natural Water	x	x	TON-V 2 mg TON-V 5 mg
Low Concentration Natural Water	x	x	NO32-VV200
Finnish Brackish Water	—	x	NO32-VV200
Household Water	x	—	TON-V 2 mg TON-V 5 mg
Chlorinated Swimming Pool Water	x	—	TON-V 5 mg
Ocean Water	—	—	TON-V SW*

*Ocean water samples were studied with a Gallery discrete analyzer in the Thermo Scientific Vantaa R&D laboratory.

Table 2. TON Vanadium applications

Application	Test Flow							
	V (R1) (µL)	V (R2) (µL)	Inc. 1 (s)	Blank	V (Sample) (µL)	Inc. 2 (s)	Mix	End Point
NO32-VV200	80	40	300	x	40 + 80 water	1200	x	540 nm
TON-V 2 mg	84	42	300	x	10 + 64 water	1200	x	540 nm
TON-V 5 mg	84	42	300	x	10 + 64 water	1200	x	540 nm
TON-V SW	120, Combination reagent R1 + R2 (1:1)		18	x	120	1200	—	540 nm

Reagents and calibrator

Thermo Scientific™ system reagents for TON Hydrazine and TON Vanadium were used.

The standard solution stock used was NO_3-N 200 mg/L N prepared by dissolving 1.444 g KNO_3 in one liter of distilled water.

A solution of 200 mg/L N, prepared by dissolving 0.6068 g $NaNO_3$ in one liter of distilled water was used.

For determination of reduction efficiency (% of sample nitrate to nitrite reduced), a commercial standard stock solution, N as 100 mg/L N was used.

Samples and controls

Sample types

Tested samples were waste water, local seawater, natural water samples from lakes and rivers, swimming pool water, and household water. The local seawater is brackish water with lower salinity levels than typical ocean water.

Four high saline samples of ocean water were also tested by the Thermo Fisher Scientific Vantaa R&D laboratory using the TON Vanadium seawater application. Samples shown in Table 3 were spiked to test linearity and recovery.

Number of samples and sample TON concentrations

Ninety-three brackish water samples with a concentration range between 2.2 and 157 µg/L were analyzed. Two replicates were analyzed using the TON Vanadium method and single samples using the TON Cd method.

The total number of natural water samples analyzed was 128. Twenty-four with a concentration range from 110 to 4100 µg/L were analyzed using the TON Vanadium 5 mg application in replicate and compared to the TON Hydrazine method. One hundred four of the natural water samples with a concentration range from 0.5 to 320 µg/L were tested in replicate with the TON Vanadium method (NO32-VV200). Ninety-nine of these samples were analyzed as single samples with TON Cd with the remaining 37 as replicates with the TON Hydrazine method.

Eleven household water samples ranging from 0.022 to 4.11 mg/L and 20 waste water samples ranging from 1.2 to 13.7 mg/L were tested. In addition, water from two swimming pools at a concentration level of 1.5 mg/L was tested. All samples were analyzed in replicate using both the TON Vanadium and TON Hydrazine methods.

Controls

Water based control samples were routinely run (0.01, 0.1, 0.4, 1, and 4 mg/L) with the appropriate application. In addition a certified reference standard VKI RW1 with a concentration of 100 µg/L was analyzed.

Table 3. Seawater samples

Sample	Details
Similan 1	Andaman Ocean near Similan, Koh Lak, Thailand (from sea/reef 26 m deep)
Similan 2	Andaman Ocean near Similan, Koh Lak, Thailand (from sea/reef 26 m deep)
Similan 3	Andaman Ocean near Similan, Koh Lak, Thailand (from sea/reef surface)
GOA	Indian Ocean, Goa (from beach)

Results and discussion

Linear calibration

Figure 1 shows a typical calibration curve from the TON Vanadium method, application TON-V 5 mg. Figure 2 shows a calibration curve with the application of NO32-VV200. Both calibrations were performed with the Aquakem 250 analyzer which includes an automated calibrator dilution option.

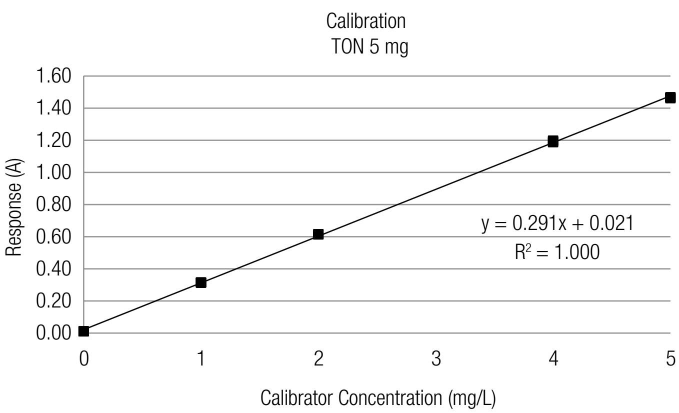

Figure 1. Calibration curve for TON-V 5 mg application

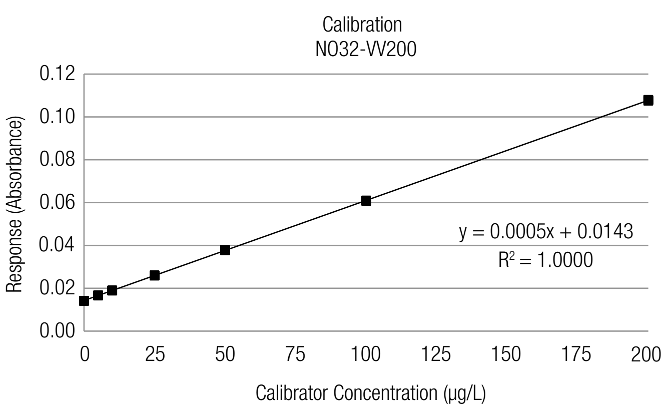

Figure 2. Calibration curve for NO32-VV200 application

Comparison study results and discussion

Samples were analyzed side by side with the Thermo Scientific Aquakem discrete analyzer using vanadium chloride reduction or hydrazine reduction or with FOSS Tecator FIAstar™ 5000 Systems analyzer using Cd reduction.

Depending on the sample type, some of the correlation graphs (Figures 3–8) show the TON Vanadium method correlated to TON Hydrazine or to TON Cd. Measurement units in the figures can be expressed in either µg/L or mg/L depending upon sample concentrations.

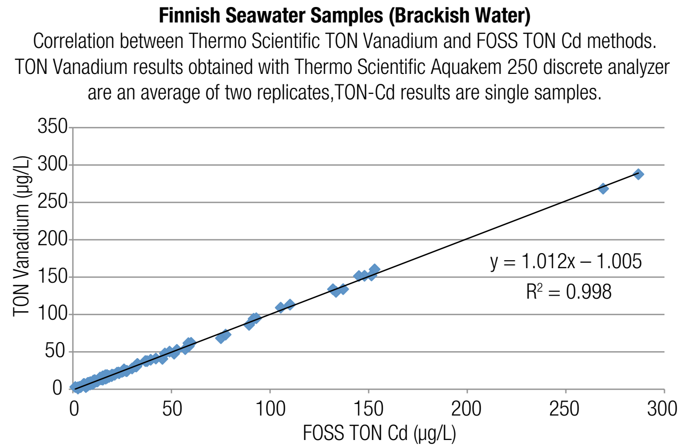

Figure 3. Brackish water sample correlation between TON Vanadium and TON Cd (93 samples, concentration range 2.2–157 µg/L)

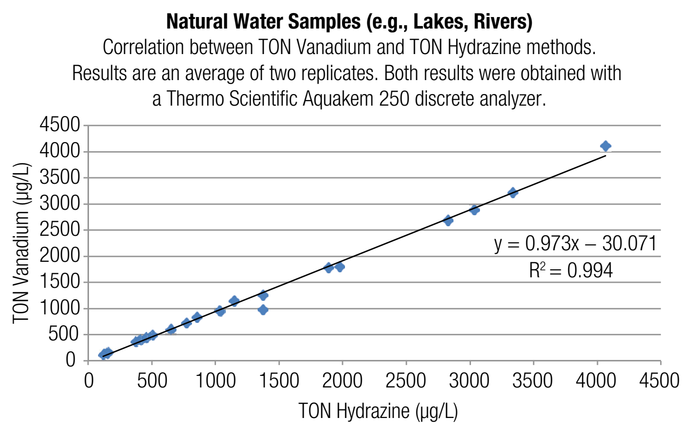

Figure 4. Natural water sample correlation between TON Vanadium and TON Hydrazine (24 samples, concentration range 119–4064 µg/L)

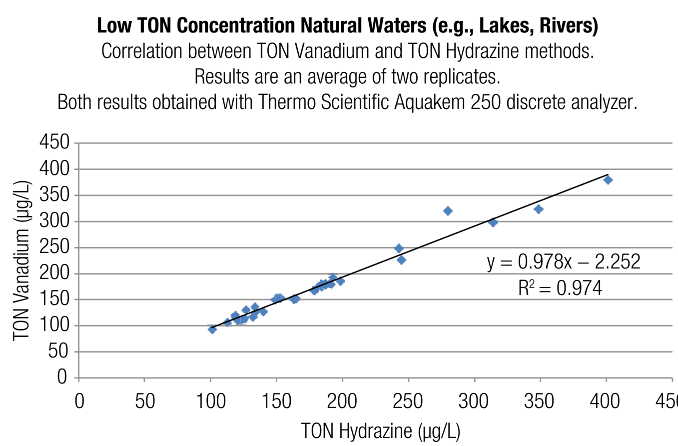

Figure 5. Low concentration natural water sample correlation between TON Vanadium and TON Hydrazine (37 samples, concentration range 101–401 µg/L)

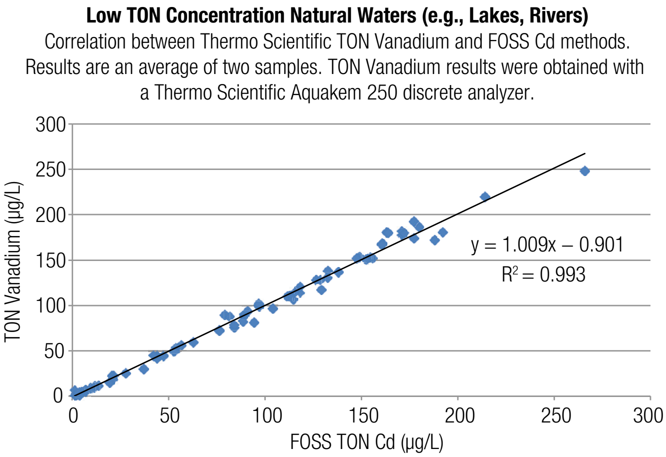

Figure 6. Low concentration natural water sample correlation between TON Vanadium and TON Cd (99 samples, concentration range 0.5–320 µg/L)

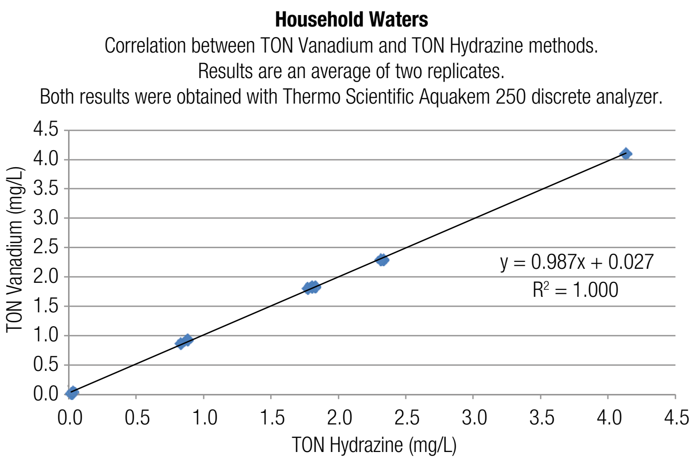

Figure 7. Household water sample correlation between TON Vanadium and TON Hydrazine (12 samples, concentration range 0.022–4.11 mg/L)

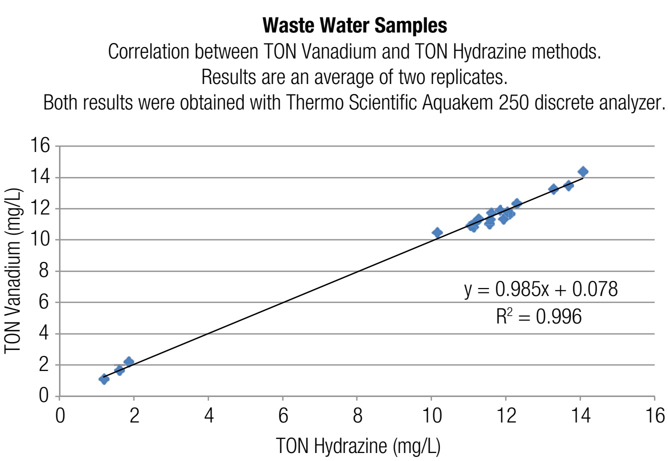

Figure 8. Waste water sample correlation between TON Vanadium and TON Hydrazine (20 samples, concentration range 1.2–13.7 mg/L)

Brackish water samples

Brackish water samples were analyzed with TON Vanadium and TON Cd methods. Based on the results shown in Figure 3, we conclude these two methods show very good correlation. Recoveries (expressed as %) were between 62 and 122%, well within the limits of 2.2 to 157 µg/L.

Natural water samples

Natural water samples were analyzed with TON Vanadium, TON Hydrazine and TON Cd methods. Based on Figures 4 and 5, we conclude that natural water samples in a wide concentration range show very good correlation between TON Vanadium and TON Hydrazine methods. The correlation between TON Vanadium and TON Cd from low concentration natural water is also very good. See Figure 6 for details. Recoveries (%) vary from 71 to 115% when comparing the TON Vanadium method to the TON Hydrazine method, and from 73 to 121% when compared to the TON Cd method. These recoveries were considered acceptable at the low concentrations studied.

Household water samples

Household water samples were analyzed using the TON Vanadium and TON Hydrazine methods. Based on results from the correlation study (Figure 7), we conclude that household water samples show very good correlation between the TON Vanadium and TON Hydrazine methods. Sample recoveries (%) are from 98 to 141%. The exceptionally high recoveries were from samples that have a very low concentration (3 samples, 0.023–0.043 mg/L) of TON for the application used. Otherwise the highest recovery (%) from household water samples was 105%. We recommend that low concentration samples are analyzed using an application specially designed for a high sample volume to improve recovery.

Waste water samples

Waste water samples were analyzed using both TON Vanadium and TON Hydrazine methods. As seen in Figure 8, waste water sample correlation between TON Vanadium and TON Hydrazine shows very good correlation in the concentration range where the samples were analyzed (1–2 and 11–15 mg/L). Recovery (%) of samples varies from 94 to 118%. It is very likely that the concentration between 2–11 mg/L shows similar correlation. In this correlation study both methods were analyzed using the same Aquakem discrete analyzer and the results shown are a true correlation of the chemistries rather than the technologies.

Swimming pool samples

Water samples from two swimming pools were analyzed with the TON Vanadium and TON Hydrazine methods. As seen in Table 4, a fairly good correlation was obtained from this pair of samples. Recovery was 91% with TON Vanadium when compared to TON Hydrazine.

Seawater sample spikes

The recovery of TON in high saline sea water samples was also studied by spiking the sea water samples with 0, 40, and 80 µg/L nitrate-N. These seawater samples were analyzed at Thermo Scientific Vantaa R&D laboratory.

As seen in Table 5, the TON Vanadium seawater application showed good spike recovery (%) to all analyzed seawater samples (92–103%). Also the reduction efficiency (100 µg/L NO3-N vs. 100 µg/L NO2-N) was good (105%).

The TON Cd method can be used for saline samples but samples containing oil or grease should be avoided. Interference is caused by residual chlorine, iron, copper, and other metals. The TON Hydrazine method is recommended for potable and surface waters and domestic or industrial waste, but not for samples containing saline. The TON Vanadium method uses fresh reagents without the requirement of a column.

Table 5. Spiked seawater samples with TON Vanadium (4 samples, spike 0, 40 or 80 µg/L NO_3-N or NO_2-N)

Sample or Sample + Spike	Result (µg/L)	Recovery (%)
Similan 1	0	
Similan 1 + 40 µg/L NO^3-N	41	103
Similan 1 + 80 µg/L NO_3-N	81	102
Similan 1 + 40 µg/L NO_2-N	37	93
Similan 1 + 80 µg/L NO_2-N	80	99
Similan 2	0	
Similan 2 + 40 µg/L NO_3-N	39	98
Similan 2 + 80 µg/L NO_3-N	82	102
Similan 2 + 40 µg/L NO_2-N	38	95
Similan 2 + 80 µg/L NO_2-N	80	99
Similan 3	0	
Similan 3 + 40 µg/L NO_3-N	37	98
Similan 3 + 80 µg/L NO_3-N	79	102
Similan 3 + 40 µg/L NO_2-N	37	99
Similan 3 + 80 µg/L NO_2-N	78	101
GOA	0	
GOA + 40 µg/L NO_3-N	34	92
GOA + 80 µg/L NO_3-N	78	101
GOA + 40 µg/L NO_2-N	34	92
GOA + 80 µg/L NO_2-N	72	94
st NO_3-N 100 µg/L	101	
st NO_2-N 100 µg/L	96	
Reduction efficiency (100 µg/L)	**105%**	
Distilled water	0	

Table 4. Swimming pool sample correlation between TON Vanadium and TON Hydrazine (2 samples, 1.4 mg/L)

	TON Vanadium			TON Hydrazine			Recovery (%)
	Result 1 (mg/L)	Result 2 (mg/L)	Average	Result 1 (mg/L)	Result 2 (mg/L)	Average	
Sample 1	1.39	1.40	**1.40**	1.54	1.52	**1.53**	**91**
Sample 2	1.36	1.39	**1.38**	1.52	1.50	**1.51**	**91**

Conclusion

Table 6 summarizes the results of the TON Vanadium method compared to the TON Hydrazine and FOSS TON Cd methods. This evaluation study shows that the TON Vanadium method used with a discrete analyzer is a suitable method for analyzing several types of water samples. Good correlation was obtained when compared to the laboratory accredited TON Hydrazine method and to the laboratory accredited FIA technique, using the Cd column for the reduction step.

The variety of sample types used in this study and results from the comparison of methods clearly demonstrate that the TON Vanadium method is a viable alternative for analysis of the most common Finnish water laboratory matrixes. In addition, it is effective for analyzing high saline sea water samples from the Indian and Andaman Oceans. This is a significant advantage considering the current TON method, which uses hydrazine reduction, is not capable of analyzing samples from seawater or other high salinity matrixes.

By using discrete analysis and the TON Vanadium method, reagent consumption can also be minimized and any Cd related toxic waste eliminated.

The TON Vanadium method presents a good multipurpose reagent system for any water laboratory using either the Thermo Scientific Aquakem or Gallery discrete analyzer.

Table 6A. Summary of the results (part 1)

	TON Vanadium Compared to TON Hydrazine Method	
	Natural Water Samples	Low Natural Water Samples
N	24	37
Concentration Range	110–4100 µg/L	101–401 µg/L
Slope	0.973	0.978
R^2	0.994	0.974
Recovery (%)	71–106	89–115
Average Recovery (%)	**95**	**96**

Table 6B. Summary of the results (part 2)

	TON Vanadium Compared to TON Hydrazine Method	
	Household Water Samples	Waste Water Samples
N	11	20
Concentration Range	0.022–4.11 mg/L	1.2–13.7 mg/L
Slope	1.009	0.985
R^2	0.993	0.996
Recovery (%)	98–141	94–118
Average Recovery (%)	**109**	**100**

Table 6C. Summary of the results (part 3)

	TON Vanadium Compared to FOSS TON Cd Method	
	Brackish Water Samples	Natural Water Samples
N	93	99
Concentration Range	2.2–157 µg/L	0.5–320 µg/L
Slope	1.012	1.009
R^2	0.998	0.993
Recovery (%)	62–122	73–121
Average Recovery (%)	**95**	**99**

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References

1. *Standard Methods for the Examination of Water and Wastewater;* Clescerl, L.S.; Greenberg, A. E.; Eaton, A. D., Eds.; Amer Public Health Assn, 1998, 20th Edition.
2. *Handbook of Water Analysis;* Nollet, Leo M. L., Ed.; Marcel Dekker, Inc.: New York, 2000.
3. *Methods for Chemical Analysis of Water and Wastes.* Method 353.3.; U.S. Environmental Protection Agency, Washington DC, 1979.
4. Patton, C.J.; Kryskalla, J.R. *Colorimetric determination of nitrate plus nitrite in water by enzymatic reduction, automated discrete analyzer methods;* U.S. Geological Survey Techniques and Methods; Denver 2011, Book 5, Ch. B8, p 34.
5. Automated Discrete Photometry. [Online] https://www.thermofisher.com/us/en/home/industrial/environmental/water-analysis/automated-discrete-photometry.html (accessed August 29, 2018).
6. Cox, R. D. Determination of Nitrate and Nitrite at the Parts per Billion Level by Chemiluminescence; *Anal. Chem.* **1980,** 52, 332–335.
7. Braman, R. S.; Hedrix, S. A. Nanogram Nitrite and Nitrite determination in Environmental and Biological Materials by Vanadium (III) Reduction with Chemiluminescense Detection; *Anal. Chem.* **1989,** *61,* 2715–2718.
8. Miranda, K. M.; Epsey, M. G.; Wink D.A. *A Rapid, Simple Spectophotometric Method for Simultaneous Detection of Nitrate and Nitrite; Nitric Oxide* 2001, 5, pp 62–71.
9. Griess P. Bemerkungen zu der Abhandlung der HH. Weselky und Benedikt Ueber einige Azoverbindungen; *Berichte der Deutschen chemischen Gesellschaft,* **1879,** *12,* 1, 426–428.
10. Doane, T.A.; Horwath, W. R. Spectrophotometric Determination of Nitrate with a Single Reagent; *Anal Lett.* **2003,** *36,* 2713–2722.
11. Nitrate via manual vanadium(III) reduction; [Online] https://www.nemi.gov/methods/method_summary/ 9171/ (accessed August 29, 2018).

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