Automated Nitrate (TON) Assay Method Using Vanadium as Reductant - Correlation to Cadmium and Hydrazine Reductant Methods in Sea, Natural and Waste Waters

The most common methods for the examination of TON, total oxidised nitrogen, are reduction of nitrate to nitrite by cadmium or hydrazine followed by Griess reaction and photometric measurement. However both of these methods have limitations, cadmium being toxic and hydrazine not working with saline water samples. The purpose of this paper is to demonstrate, by means of method comparison, that there is a better alternative to the chemistries mentioned. The alternative chemistry is based on a fairly new method, where the reduction step is conducted with nontoxic vanadium chloride.

> *"This evaluation study shows that the new TON Vanadium method for discrete analyser is a suitable method for several types of water samples."*

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This study showed extremely good correlation between three methods, reduction by cadmium, hydrazine or vanadium chloride. The correlation study was performed in a Finnish food-, water- and environmental laboratory. The average recovery of TON Vanadium method to TON Hydrazine for 24 natural water samples was 95 % and for 37 low natural water samples 96 %. Eleven household water samples gave an average recovery of 109 % and 20 waste water samples 100 %. When comparing the TON Vanadium method to TON Cd method, the average recovery of 93 brackish water samples was 95 % and 99 natural water samples 99 %.

Also a low concentration application was developed and now the natural water sample TON (nitrate + nitrite) concentrations can be analysed to as low as 0.5 µg/l. Brackish water samples were successfully analysed down to 2.2 µg/l.

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

Introduction

TON, Total Oxidised Nitrogen, or sum of nitrate and nitrite can be performed in many different techniques from water samples. Hydrazine is often used in automated analysis as the reducing agent, causing the change from nitrate to nitrite. Only nitrite-ions can be analysed photometrically and that is why the reduction step is needed. Unfortunately hydrazine method has interference from seawater matrix and this feature limits its use as a general purpose method. (1). Therefore the Cadmium reduction method seems still to be the reference method in many countries. Handling the Cd-column may pose risks to the users and the column may be easily damaged by using e.g. oily sample matrix (2). Enzymatic reduction can also be used (3, 11). Enzymatic reduction is a very good method but it is not familiar to most of the laboratories and is not yet described as an alternative method by US/EPA.

In this paper automated, two reagent method using vanadium chloride as a reducing agent was studied and compared to hydrazine and cadmium reduction methods. Vanadium chloride as reductant has been routinely used in nitrate analysis before. In one application, the reduction step in the method is done as far as to NO in temperature over 80 °C and NO is detected by a chemiluminescent detection method (4, 5). When the temperature of the reduction step is lowered to room temperature or 37 °C, a nitrite ion is formed and it can be analysed also photometrically (6). Based on the literature, a method with vanadium chloride reduction followed by Griess reaction (7) has been used in examining TON from water samples also before (8, 9).

The principle of the TON Vanadium method is to reduce the sample nitrate, NO_3, with vanadium chloride to nitrite, NO_2, (4-6) which then reacts to form a pink coloured diazodye by Griess reagents, sulphanilamide and N-(1-naphtyl)-ethylenediamine (7). The nitrite originally present in the sample will also be analysed. The intensity of the colour is measured photometrically at 540 nm (2). $NO_3 + NO_2$ concentration of the sample is calculated using the calibration curve.

Tested sample types were waste water, natural waters, brackish water, household water and swimming pool water. Additionally seawater samples from Indian Ocean and Andaman Ocean were analysed to understand method performance in higher salt concentration than the local Finnish brackish seawaters.

The correlation study was performed in a Finnish food-, water- and environmental laboratory MetropoliLab which is accredited by FINAS, a national accreditation body in Finland. The laboratory has a long history in analysing various types of water samples, TON being one of the main nutrients to be analysed.

Abbreviations and Terms

TON: Total Oxidised Nitrogen, or sum of nitrate and nitrite, calculated as N or NO_3-N+NO_2-N. All results presented in this study are reported as TON.

US/EPA: United States Environmental Protection Agency

TFS: Thermo Fisher Scientific

R&D: Research and development

FINAS: The national accreditation body in Finland

Reduction efficiency: Efficiency percentage (%) of reduction of sample nitrate to nitrite.

Materials And Methods

Instruments

Thermo Scientific Aquakem 250, a discrete photometric autoanalyser (Thermo Fisher Scientific Oy, Vantaa, Finland).

FOSS Tecator FiaStar 5000 analyser (FOSS, Hillerod, Denmark) with Prepacked FOSS Cd-column (n:o 5000 3139).

Thermo Scientific Gallery, a discrete photometric autoanalyser (Thermo Fisher Scientific Oy, Vantaa, Finland).

Applications

Application for TON Cd reduction (FOSS FiaStar) is a FINAS accredited method for nitrate nitrogen, sum of nitrite nitrogen and nitrate nitrogen analysis. Method is based on SFS-EN ISO 13395:1997.

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

See Table 1 for the sample types analysed with different methods and Table 2 for the parameters, like reagent and sample volumes, used in the different TON Vanadium application test flows. Applications and method details for TON Vanadium reduction (Aquakem and Gallery) can be requested from the address info.cdx.fi@thermofisher.com

Several applications were used depending on sample matrix and concentration range (Table1).

Reagent- and sample ratios in TON Vanadium applications are listed in Table 2.

Reagents and calibrator

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

Standard solution stock used was NO_3-N 200 mg N/l prepared by dissolving 1.444 g KNO_3 to a litre of distilled water.

Also a solution 200 mg/l N, prepared by dissolving 0.6068 g $NaNO_3$ to a litre of distilled water, was used.

For the determination of reduction efficiency, TFS used a commercial standard stock solution, Nitrite as N 100 mg/l.

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 low salinity, and therefore differs from ocean water.

Four high saline ocean waters were additionally tested by Thermo Fisher Scientific Vantaa R&D laboratory, with TON Vanadium seawater application. Samples (Table 3) were additionally spiked to show the test linearity and recovery.

Number of samples and sample TON concentrations

93 brackish water samples were analysed, the concentration range being 2.2 - 157 µg/l. Two replicates were analysed with TON Vanadium method and single samples with TON Cd method.

The number of natural water samples was 128 of which 24, from concentration range 110 - 4100 µg/l, were analysed with TON-V 5mg application. These 24 samples were analysed in replicates with both TON Vanadium (TON-V 5mg) and TON Hydrazine methods. 104 of the natural water samples, from concentration range 0.5 µg/l - 320 µg/l, were tested in replicates with TON Vanadium method (NO32-VV200). 99 of these samples were analysed as single samples with TON Cd and 37 as replicates with TON Hydrazine method.

Table 1: Methods and sample types for correlation study

Sample type	Hydrazine application (Aquakem 250)	Cadmium application (FIAStar)	Vanadium application (Aquakem 250)
Waste water	x	-	TON-V 2mg TON-V 5mg
Natural water	x	x	TON-V 2mg TON-V 5mg
Low concentration natural water	x	x	NO32-VV200
Finnish brackish water	-	x	NO32-VV200
Household water	x	-	TON-V 2mg TON-V 5mg
Chlorinated swimming pool water	x	-	TON-V 5mg
Ocean Water	-	-	TON-V SW *

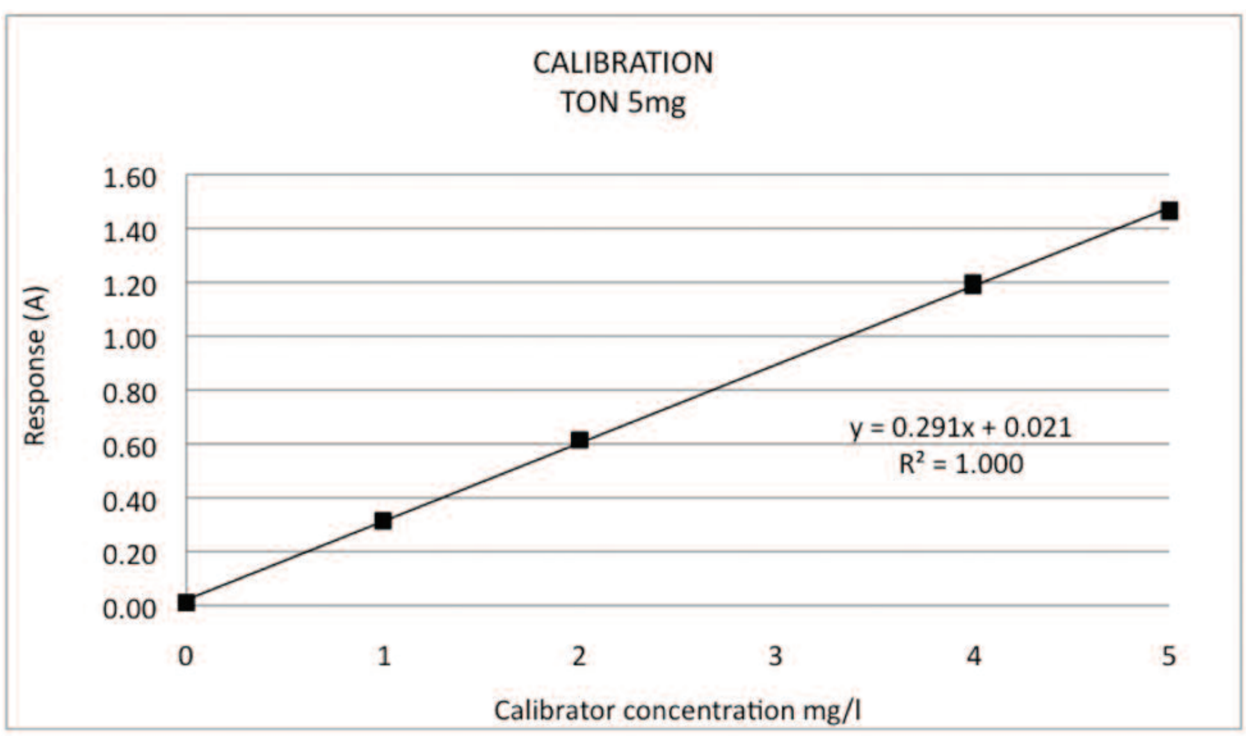
**The ocean water samples were studied with Gallery by TFS –Vantaa R&D laboratory*

Table 2: TON Vanadium applications

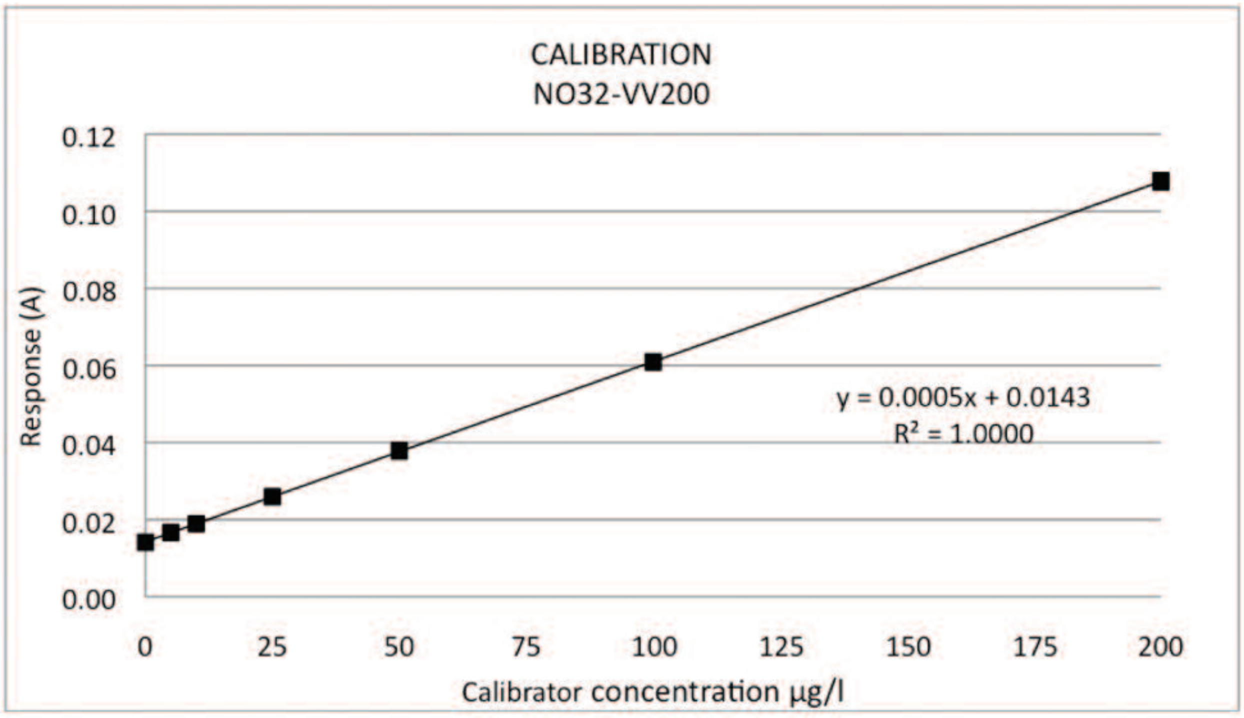
Application	Test flow							
	V (R1), µl	V (R2), µl	Inc. 1, s	Blank	V (Sample), µl	Inc. 2, s	Mix	End Point
NO32-VV200	80	40	300	x	40 + 80 water	1200	x	540 nm
TON-V 2mg	84	42	300	x	10 + 64 water	1200	x	540 nm
TON-V 5mg	84	42	300	x	10 + 64 water	1200	x	540 nm
TON-V SW	120, Combination reagent R1+R2 (1:1)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

Table 3: Seawater samples.

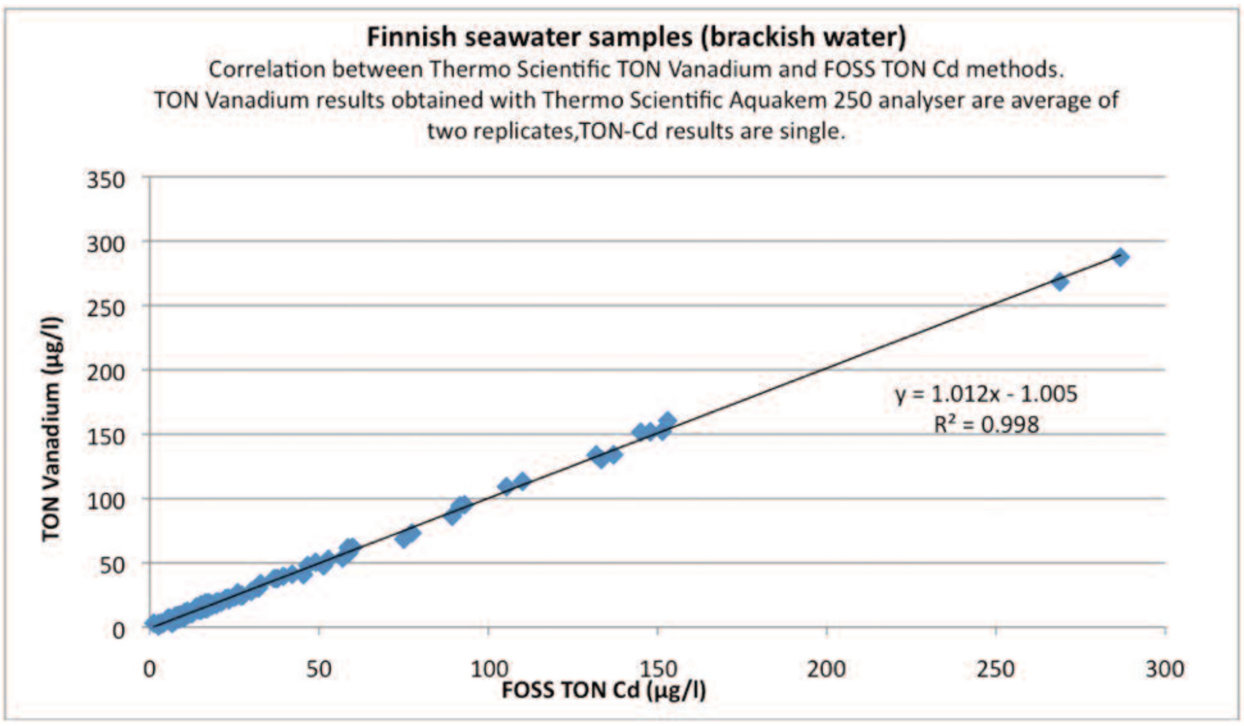
Sample	Details
Similan 1	Andaman Ocean near Similan, Koh Lak, Thailand (from sea/reef 26 m deep)
Similan 2	Andaman Ocean near Similan, Koh Lak, Thailand (from sea/reef 26 m deep)
Similan 3	Andaman Ocean near Similan, Koh Lak, Thailand (from sea/reef surface)
GOA	Indian Ocean, Goa (from beach)



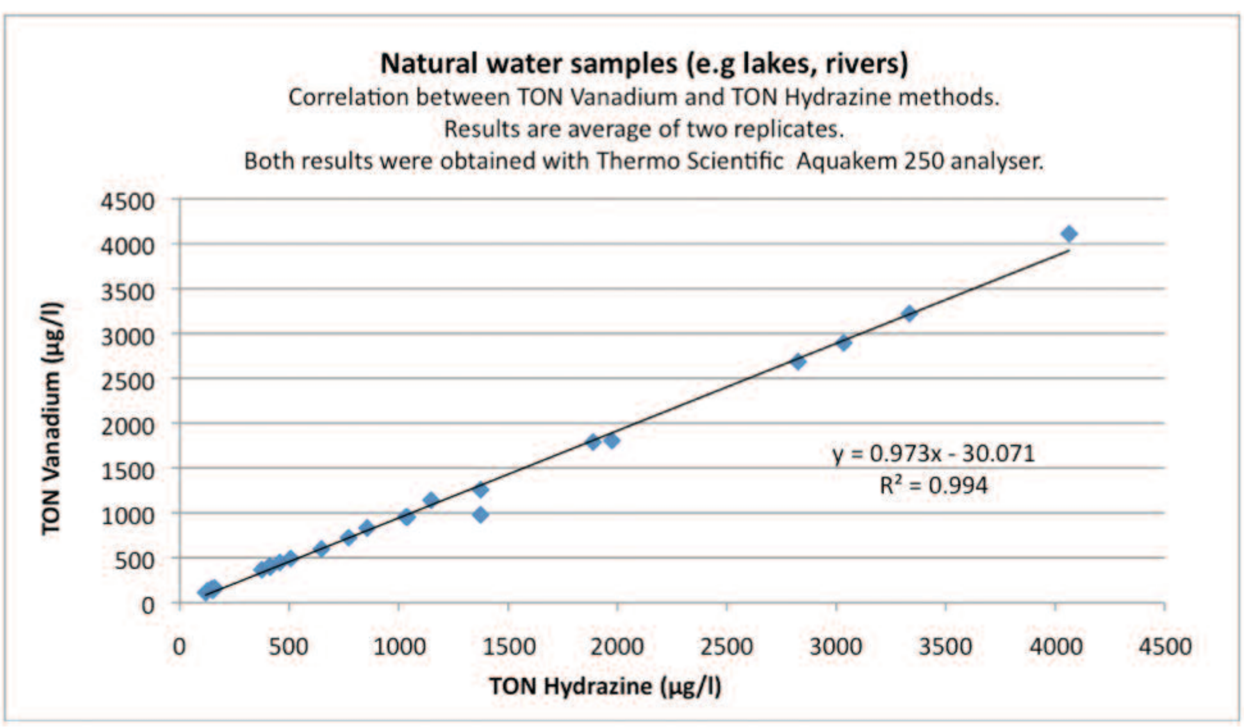
Picture 1: Calibration curve for TON-V 5mg application.



Picture 2: Calibration curve for NO32-VV200 application.



Picture 3: Brackish water sample correlation between TON Vanadium and TON Cd (93 samples, concentration range 2.2-157 mg/l).



Picture 4: Natural water sample correlation between TON Vanadium and TON Hydrazine (24 samples, concentration range 119-4064 µg/l).

Household waters tested, 11 pcs, were from 0.022 - 4.11 mg/l and waste water samples, 20 pcs, from 1.2 – 13.7 mg/l. Two swimming pool waters were tested and they were from concentration range 1.5 mg/l. All were done in two replicates to both methods, TON Vanadium and TON Hydrazine.

Controls

Routinely, water based control samples were also run (0.01, 0.1, 0.4, 1 and 4 mg/l) with appropriate application. In addition a certified reference standard VKI RW1, concentration 100 µg/l, was analysed.

Results and Discussion

Linear Calibration

Picture 1 shows a typical calibration picture performed with the TON Vanadium method, application TON-V 5 mg. In Picture 2, a calibration with application NO32-VV200 is shown. Both calibrations are performed with Aquakem 250 analyser with automated calibrator dilution options.

Comparison study results and discussion

Samples were analysed side by side with Thermo Scientific Aquakem discrete autoanalyser by vanadium chloride reduction or hydrazine reduction or with FOSS Tecator FiaStar 5000 analyser by Cd-reduction.

Note that depending on the sample type, some of the correlation graphs (Pictures 3-8) show TON Vanadium method correlation to TON Hydrazine, some to TON Cd. Unit in the pictures can be µg/l or mg/l, depending on the sample concentrations.

Brackish water samples

Brackish water samples were analysed with TON Vanadium and TON Cd methods. Based on the Picture 3, we can conclude that these two methods show a very good correlation. Recoveries (%) of results are between 62 % and 122 %.

Natural water samples

Natural water samples were analysed with TON Vanadium, TON Hydrazine and TON Cd methods. Based on the Picture 4 and 5, we can conclude that the natural water samples in wide concentration range show a very good correlation between TON Vanadium and TON Hydrazine methods. Also the correlation between TON Vanadium and TON Cd from low concentration natural water is very good, see Picture 6 for details. Recoveries (%) vary from 71 % to 115 % when comparing TON Vanadium method to TON Hydrazine method, and from 73 % to 121 % when compared to TON Cd method.

Household water samples

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

Waste water samples

Waste water samples were analysed with TON Vanadium and TON Hydrazine methods. As seen in Picture 8, waste water sample correlation between TON Vanadium and TON Hydrazine shows a very good correlation in the concentration range where the samples were analysed (1-2 mg/l and 11-15 mg/l). Recovery (%) of samples varies from 94 % to 118 %. It is very likely that also the concentration between 2-11 mg/l shows similar correlation. In this correlation study both methods were analysed with the same discrete Aquakem analyser and the results show the true correlation of the chemistries rather than two instrument technologies.

Swimming pool water samples

Additionally two swimming pool waters were analysed with TON Vanadium and TON Hydrazine methods. As seen in Table 4, a fairly good correlation was obtained also from this pair of samples. Recovery % was 91 % with TON Vanadium compared to TON Hydrazine.

Table 4: Swimming pool water 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 %**

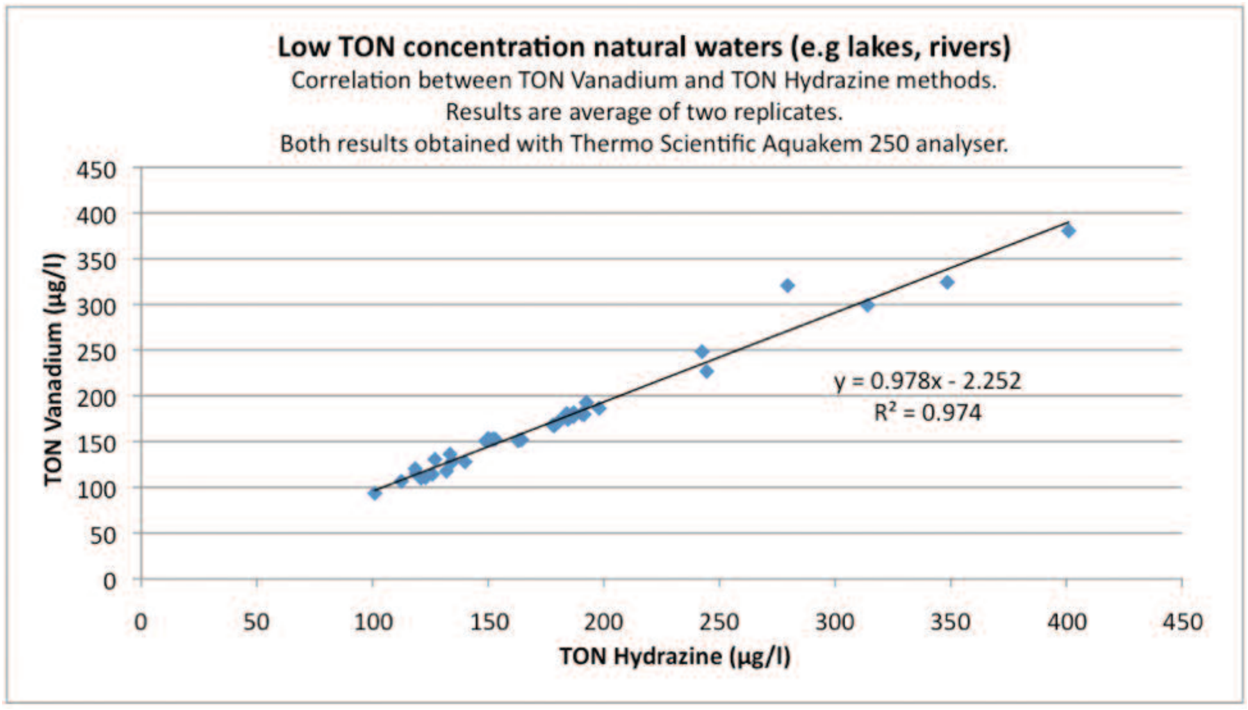
Seawater sample spikes

We also wanted to study the recovery of TON in high saline sea water samples. This study was performed by using nitrate spikes to the sea water samples. These seawater results were performed at TFS-Vantaa R&D laboratory.

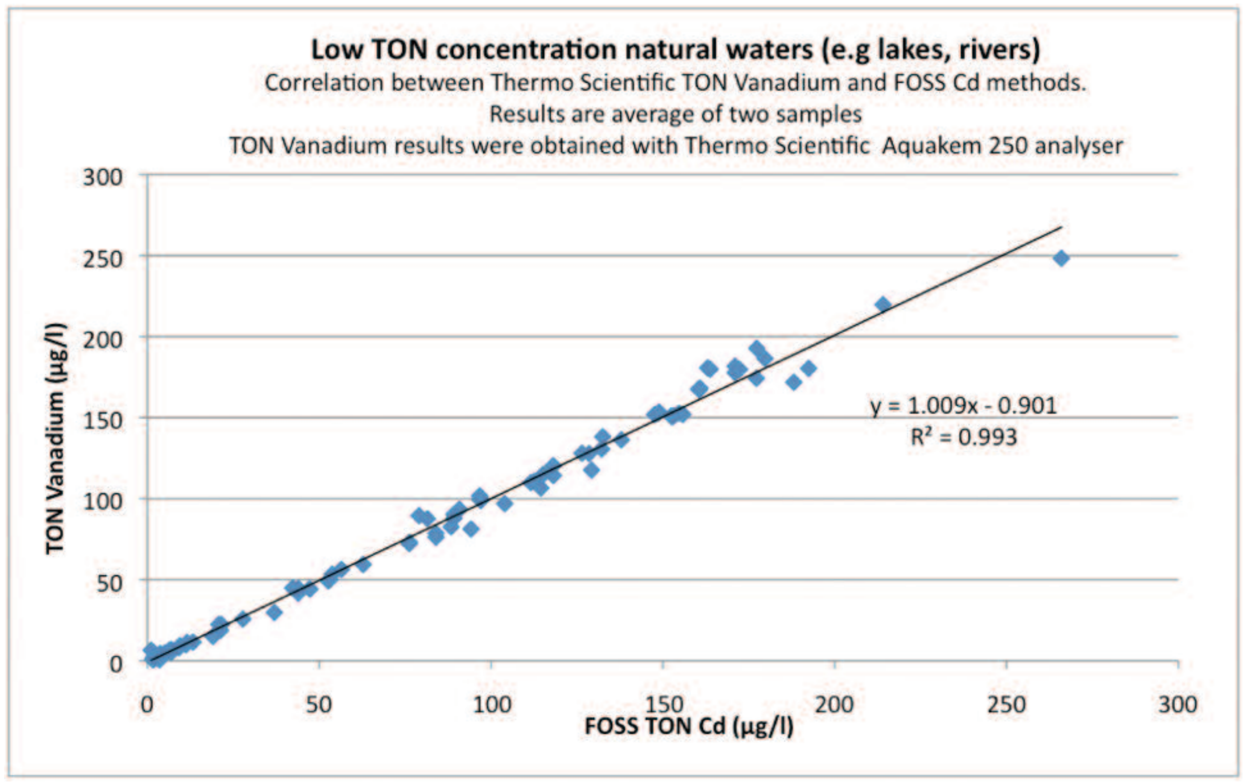
As seen in Table 5, TON Vanadium seawater application gave good spike recovery (%) to all analysed seawater samples (92 %-103 %). Also the reduction efficiency (100 µg/l NO_3-N vs. 100 µg/l NO_2-N) was good (105 %).

Table 5: Seawater spike samples with TON Vanadium (4 samples, spike 0, 40 or 80 µg/l NO_3-N or NO_2-N).

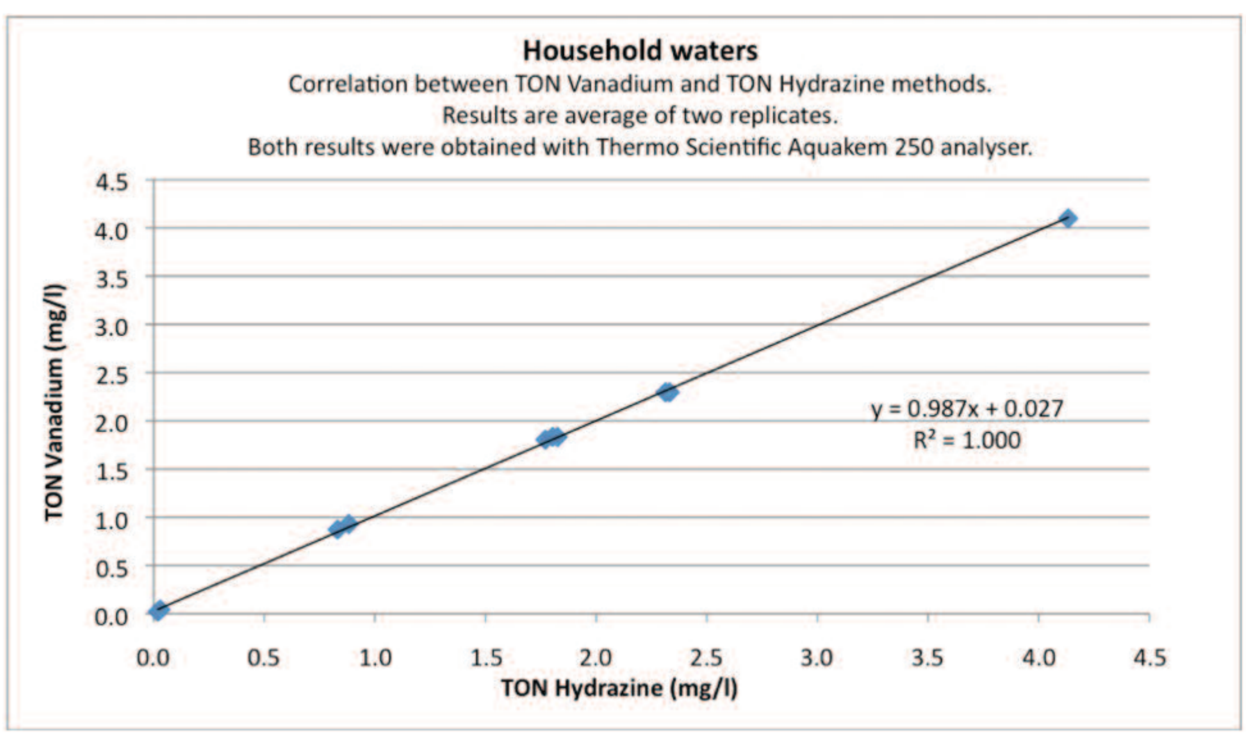
Sample or sample + spike	Result, µg/l	Recovery-%
Similan 1	0	
Similan 1 +40 µg NO_3-N /l	41	**103 %**
Similan 1 +80 µg NO_3-N /l	81	**102 %**
Similan 1 +40 µg NO_2-N /l	37	**93 %**
Similan 1 +80 µg NO_2-N /l	80	**99 %**
Similan 2	0	
Similan 2 +40 µg NO_3-N /l	39	**98 %**
Similan 2 +80 µg NO_3-N /l	82	**102 %**
Similan 2 +40 µg NO_2-N /l	38	**95 %**
Similan 2 +80 µg NO_2-N /l	80	**99 %**
Similan 3	-2	
Similan 3 +40 µg NO_3-N /l	37	**98 %**
Similan 3 +80 µg NO_3-N /l	79	**102 %**
Similan 3 +40 µg NO_2-N /l	37	**99 %**
Similan 3 +80 µg NO_2-N /l	78	**101 %**
GOA	-3	
GOA +40 µg NO_3-N /l	34	**92 %**
GOA +80 µg NO_3-N /l	78	**101 %**
GOA +40 µg NO_2-N /l	34	**92 %**
GOA +80 µg NO_2-N /l	72	**94 %**
st NO_3-N 100 µg/l	101	
st NO_2-N 100 µg/l	96	
reduction efficiency (100 µg/l)	**105 %**	
Dist.water	-2	



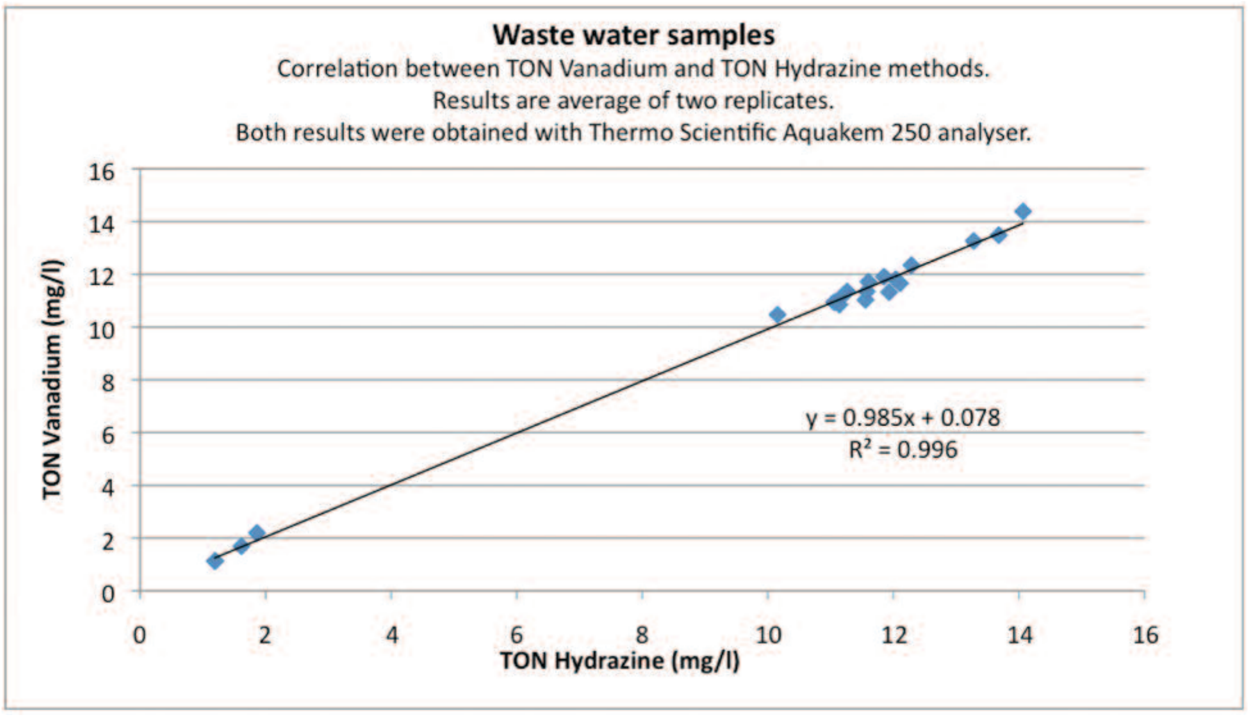
Picture 5: Low concentration natural water sample correlation between TON Vanadium and TON Hydrazine (37 samples, concentration range 101 – 401 µg/l).



Picture 6: Low concentration natural water sample correlation between TON Vanadium and TON Cd (99 samples, concentration range 0.5-320 µg/l).



Picture 7: Household water sample correlation between TON Vanadium and TON Hydrazine (12 samples, concentration range 0.022 - 4.11 mg/l).



Picture 8: Waste water sample correlation between TON Vanadium and TON Hydrazine (20 samples, concentration range 1.2-13.7 mg/l).

Table 6: Summary of the results.

	TON Vanadium compared to TON Hydrazine method			
	Natural water samples	Low natural water samples	Household water samples	Waste water samples
N	24	37	11	20
Concentration range	110-4100 µg/l	101-401 µg/l	0.022-4.11 mg/l	1.2-13.7 mg/l
Slope	0.973	0.978	1.009	0.985
R2	0.994	0.974	0.993	0.996
Recovery %	71 %-106 %	89 %-115 %	98 %-141 %	94 %-118 %
Average recovery %	**95 %**	**96 %**	**109 %**	**100 %**

	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
R2	0.998	0.993
Recovery %	62 %-122 %	73 %-121 %
Average recovery %	95 %	99 %

Conclusion

This evaluation study shows that the new TON Vanadium method for discrete analyser is a suitable method for several types of water samples. Good correlation was obtained when compared to laboratory accredited TON Hydrazine method and to the laboratory accredited FIA technique, which uses the Cadmium column for the reduction step (Table 6.).

The amount of different sample types used in this study and the actual results from the comparison of methods clearly demonstrate that the new TON Vanadium method is a viable alternative for analysis of the most common Finnish water laboratory matrixes and additionally high saline sea water samples from Indian and Andaman Oceans. This is a significant advantage as the current TON method based on hydrazine reduction is not capable of analysing samples from seawater or other high salinity matrixes.

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

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

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