Application note | 001746

Automated wet chemical analysis

Automated method to detect the sum of nitrate + nitrite (TON) in drinking water using the Thermo Scientific Gallery discrete analyzer

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Keywords

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Application benefits

- Meets requirements of regulated nitrate + nitrite total oxidized nitrogen methods
- Minimizes use of hazardous reagents and their associated costs and safety risks when carrying out nitrate + nitrite methods
- Automation saves time and reduces errors compared to manual approaches

Goals

- Analyze nitrate + nitrite in drinking water samples using the Thermo Scientific™ Gallery™ and Gallery™ Plus discrete analyzers in compliance with the United States Environmental Protection Agency (U.S. EPA) Safe Drinking Water Act (SDWA).
- Demonstrate that the results obtained using the Gallery discrete analyzer-based automated method correlate well with and meet the QC acceptance criteria in the EPA-approved reference method.
- Analyze spiked surface and tap waters to establish that the Gallery discrete analyzer-based automated method is applicable to different types of water samples.

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Introduction

Compliance with standards and regulations is essential in a world continuously faced with environmental challenges. Analysis of drinking water for contaminants such as inorganic anions, cations, heavy metals, organic pollutants, and nutrients is required to protect public health. In the United States, the quality of drinking water is regulated and supervised by the U.S. EPA. The water-quality parameters of health concerns and their analysis are regulated under the 40 CFR Part 141 National Primary Drinking Water Regulations.[1]

Measuring nitrate levels in drinking water is important because, after ingestion, nitrate is reduced to nitrite, which disrupts the blood's ability to carry oxygen, resulting in methemoglobinemia. Methemoglobinemia is especially serious for infants under the age of six months.[2] The U.S. EPA sets the Maximum Contaminant Level (MCL) for nitrate at 10 mg/L.[1] Accurate determination of nitrate concentrations in drinking water requires measurement of the sum of nitrate + nitrite, referred to as total oxidized nitrogen (TON). Traditional methods based on cadmium reduction columns reduce nitrate to nitrite, followed by quantitation of nitrite via the Griess reaction. However, these methods have several limitations. Cadmium is a carcinogenic heavy metal, and the column used in these methods must be replaced every few measurements to reduce the risk of harmful exposure. Column regeneration requires handling additional hazardous chemicals and produces waste that must be carefully disposed of at a significant cost. Also, these processes, as well as downstream analyses using flow injection or colorimetric analyzers, involve time-consuming manual steps that are potential sources of measurement inaccuracies. These challenges have triggered the need to develop a different way of doing the analysis.

In 2016, the U.S. EPA approved the alternative non-toxic enzymatic nitrate + nitrite in drinking water method, developed by NECi and published in the U.S. Code of Federal Regulation.[4] Not only is the method less hazardous in comparison to many other nitrate methods, but it can also be performed using Thermo Scientific discrete analyzer technology. The enzymatic method uses nitrate reductase to catalyze the reduction of nitrate to nitrite via NADH (reduced nicotinamide dinucleotide) to drive conversion. The resulting nitrite then reacts with the Griess reagents, producing an intensely colored compound that can be quantified using a photometer at wavelength 540 nm ±20 nm.

The Thermo Scientific™ Gallery™ and Gallery™ Plus Aqua Master discrete analyzers support the National Environmental Laboratories Accreditation Conference (NELAC) and U.S. EPA-approved enzymatic reduction methods for safer TON measurements. The Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for Thermo Scientific Gallery Discrete Analyzer[5] details an equivalent method for testing drinking water for nitrate + nitrite in a non-hazardous and environmentally friendly way. The method uses the Gallery or Gallery Plus Aqua Master discrete analyzer in combination with ready-to-go test procedures and the required enzyme kit (Part# 984187 TON (enz)). The 40 CFR Part 141 compliant method makes it possible to monitor hazardous nitrate levels in compliance with the SDWA regulations while accessing the benefits of discrete analyzer technology. In addition to providing a greener and safer approach to TON measurement, the method is easily automated and allows simultaneous analysis of multiple parameters from the same sample aliquot, automatic spiking procedures, flexible test and QC parameter configuration, and flexible result reporting with versatile features for configuring report templates.[6]

Experimental

Materials and methods

Additional details about the necessary equipment and supplies; reagents, calibrators, and control preparation; and test parameters are provided in the Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer.[5]

Equipment

- Gallery or Gallery Plus automated photometric discrete analyzer. Note: The Gallery and Gallery Plus Aqua Master discrete analyzers provide equivalent performance and can also be used to carry out the Thermo Scientific method.[5]
- Other necessary laboratory equipments and supplies are listed in the Thermo Scientific method.[5]

Reagents

Thermo Scientific™ Gallery™ system reagents for environmental and industrial analysis, catalog number 984187 TON (enz) by enzymatic reduction system reagents were used. The kit contains the AtNaR enzyme and NADH needed for the method.

In addition, deionized water, phosphate buffer, sulfanilamide reagent (SAN) and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) reagents were used.

Reagents were prepared according to the Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer.[5]

Calibrator and controls

- 984725 Nitrate (as N) Std, 1000 ppm, 500 mL standard solution was used to prepare the calibrators.
- 984724 Nitrate (as NO_3) Std, 1000 ppm, 500 mL (225.9 mg/L as N) was used to prepare the quality control (QC) samples and ongoing QC samples.
- 984723 Nitrite (as N) Std, 1000 ppm, 500 mL was used to prepare reduction efficiency samples.

Test parameters

Table 1 provides the automated test flow using the 984187 TON (nitrate + nitrite) by enzymatic reduction system reagents. Table 2 provides the calibration parameters. As an integrated and automated platform, the Gallery discrete analyzer workflow eliminates the need to handle hazardous reagents and increases laboratory productivity by freeing staff to work on other tasks.

Table 1. Automated test workflow

Automated test flow: DW TON enz	
AtNaR enzyme	68 µL
Sample	6 µL
NADH	15 µL
Incubate	600 s
SAN	31 µL
Incubate	18 s
Blank	540 nm (700 nm)
Incubate	120 s
NED	31 µL
Incubate	120 s
End-point measurement	540 nm (700 nm)

Table 2. Calibration parameters

Test range	Up to 5 mg/L as N
Primary range	Up to 25 mg/L as N
Calibration	2nd order
QC	Automated calibration QC, ongoing QC, and spike samples

Samples

In addition to analyzing several different standard samples, the study included analysis of tap water, surface water, and groundwater spiked with known amounts of analyte. Certified reference material ERA #698 was also tested.

Results and discussion

Calibration and calibration verification

Calibrators were prepared from a standard nitrate solution and analyzed using the Gallery discrete analyzer. The analyzer automatically plots responses against standard concentrations to create the calibration curve. Figure 1 presents the calibration curve obtained.

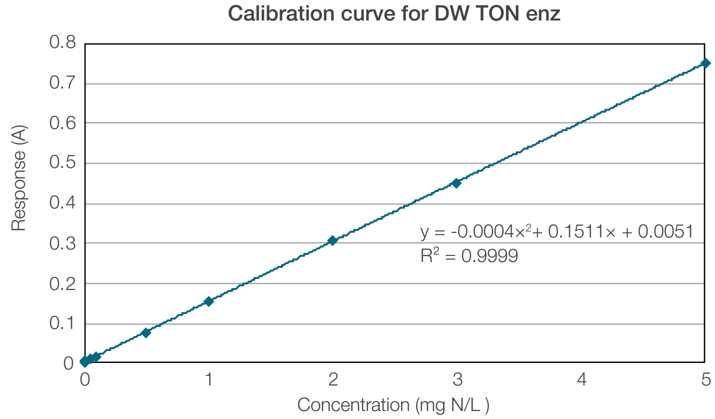


Figure 1. Calibration curve obtained per the Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer.

Each calibration was verified by the analysis of second-source standard samples against the preset limits of the method. The method's efficiency in reducing nitrate to nitrite was evaluated by analysis of nitrite standard samples of the same nitrogen concentration as the nitrate standard samples. The reduction efficiency (RE) percent was calculated as:

$$RE = \frac{C_{NO3\text{-}N}}{C_{NO2\text{-}N}} \times 100\%$$

where $C_{NO3\text{-}N}$ = QCS sample result concentration, mg N/L.
$C_{NO2\text{-}N}$ = Nitrite standard sample result concentration, mg N/L.

Table 3 lists the Quality Control Sample results for calibration verification and the calculated RE%.

Table 3. Quality control sample (QCS) and reduction efficiency results

QCS + reduction efficiency			
QCS-sample	**Result (mg N/L)**	**% Recovery**	**RE %**
NO3N 1 mg/L	0.97	97%	—
NO3N 4 mg/L	3.75	94%	—
NO2N 1 mg/L	0.97	—	101%
NO2N 4 mg/L	3.63	—	103%

Method detection limit (MDL)

The MDL was defined using low-concentration standard samples per the EPA approach described in 40 CFR Part 136 Appendix B.[7] The MDL results are listed in Table 4.

Table 4. Method MDL

MDL sample: 0.020 mg/l NO3-N Std, 7 replicates	
Average, mg/L	0.0154 (0.0141–0.0168)
% Recovery	77% (70–85%)
Std. deviation (SD), mg/L	0.0012
MDL calculated, mg/L	**0.0038**
MDL applied, mg/L	**0.0090***

* Result for MDL was 0.0038 mg/L when performed according to 40 CFR Part 136 Appendix B.[8] However, the MDL of 0.009 mg N/L was applied because the method criteria specific to that laboratory reagent blank (LBR) must be below the MDL.

Minimum reporting level (MRL)

The MRL was estimated to be three times the MDL. Seven replicate laboratory-fortified blanks (LFBs) were analyzed at this concentration to confirm MRL per the EPA method (Table 5). The MRL confirmation procedure was done per Winslow, S. D., *et al.*[8]

Table 5. MRL confirmation results

Sample: 0.027 mg/L NO3-N Std, 7 replicates	
% Recovery	98% (94–101%)
% RSD (n=7)	3.0%
The MRL was confirmed to be 0.027 mg/L	

Initial calibration range (ICR)

The initial calibration range and extended range were tested using the standard samples. Shown in Figure 2, the results verified that across the measured range the analyzer response per standard concentration met the method criteria (coefficient of determination (R^2) of 0.999 or greater) and that the difference between the results and nominal values do not differ more +/-10%.

Certified reference material (CRM)

To verify the quantitative performance of the method, CRM was analyzed. The verification was performed using the initial precision and recovery (IPR) test for the method, a common procedure when onboarding a method. The CRM results are presented in Table 6.

Table 6. CRM results

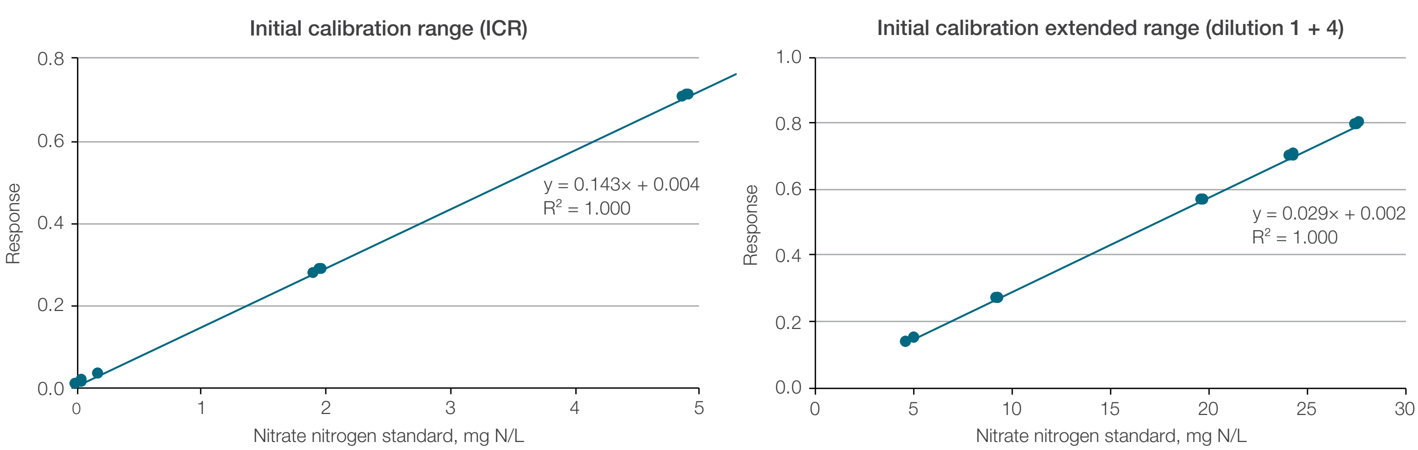
Sample: ERA #698, target: 9.61 mg/L *Analyzed in four replicates diluted to the primary range*	
% Recovery	92%
% RSD (n=4)	1.2%

Ongoing QC

To control method performance between calibrations, nitrate standard samples were automatically analyzed by the Gallery discrete analyzer in intervals of ten. Results from this procedure, also known as ongoing precision and recovery (OPR), are provided in Table 7.

Table 7. Precision and accuracy data for automated ongoing QC analyses

	NO3-N Std 1 mg/L	NO3-N Std 4 mg/L
% Recovery	97% (94–102%)	97% (94–102%)
n	24	24
% RSD	1.9%	1.4%



Recovery of samples within initial and extended calibration ranges: 92–110%

Figure 2. Correlation of result responses and the nominal values for ICR samples.

Spike samples

The accuracy of the method in the sample matrix was tested by analyzing spike samples. Tap water (LFM 1), surface water (LFM 2), and groundwater were spiked with known concentrations of nitrate standard. The results of the spike sample analyses are provided in Table 8.

Table 8. Laboratory fortified matrix (LFM) sample results

Sample	Results mg/L	% RPD	Spike % Recovery
Tap water	0.276	–	–
Tap water +0.7 mg/L	0.912	–	91%
Tap water +0.7 mg/L D	0.913	0.1%	91%
Surface water	0.900	–	–
Surface water +2 mg/L	2.893	–	100%
Surface water +2 mg/L D	2.881	0.4%	99%
Groundwater	0.320	–	–
Groundwater +1 mg/L	1.244	–	92%
Groundwater +1 mg/L D	1.243	0.1%	92%

Conclusions

Table 9 summarizes the results from the performance study of the Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer. The results demonstrated that the Gallery discrete-analyzer-based automated method meets the QC acceptance criteria in the reference method. A good correlation with the EPA-approved reference method was obtained. The results also showed that the approach is a suitable method for nitrate + nitrite testing in different types of waters such as surface and tap waters.

The Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer offers laboratories an approach to compliant nitrate + nitrite testing that minimizes the challenges associated with traditional cadmium reduction methods. The discrete analyzers automate the entire analysis, delivering high-throughput results without time-consuming cadmium packing or regeneration steps. Conveniently, Gallery system reagents reduce the need to handle hazardous chemicals, improving safety, and removing a source of experimental error. The greener enzymatic method eliminates hazardous waste disposal, further reducing costs per analysis. The Gallery and Gallery Plus Aqua Master discrete analyzers provide equivalent performance and can also be applied to the Thermo Scientific method.

Table 9. Performance of the Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer compared to reference method acceptance criteria

	QC acceptance criteria for reference method *Method for Nitrate Reductase Nitrate-Nitrogen Analysis of Drinking Water*[3]	Performance of the Thermo Scientific Drinking Water Method: Drinking Water Total Oxidized Nitrogen for the Thermo Scientific Gallery Discrete Analyzer[5]
MDL	To be determined by each laboratory according to 40 CFR Part 136 Appendix B. *(MDL results in reference method validation were between 0.0066 and 0.0097 mg N/L)*	MDL: 0.0038 mg N/L when done according to 40 CFR Part 136. Appendix B. MDL 0.009 mg N/L was applied*
MRL	NA	0.027 mg N/L
Reduction efficiency	90–110%	101–103%
Method blank	<MDL	*max. 0.0088 mg/L < MDL (0.009 mg N/L)
% Recovery initial calibration range (ICR)	±10% of true value, the equation between instrument responses and the nominal values of the standards must have regression coefficient (R^2) ≥ 0.999	92–110% for both primary and extended ranges (0.027–25 mg N/L). The R^2 was 1.000 for both ranges.
% Recovery QCS	±10%	94–97%
Recovery initial precision and recovery (IPR)	From CRM certificate *(ERA #698, lot: S234-698 acceptance limits 8.49–10.5 mg N/L)* or ±10% of known value, whichever is more restrictive.	Recovery IPR: 8.70–8.95 mg N/L (91–93% recovery)
% Recovery ongoing precision and recovery (OPR)	±10%	91–102%
% Recovery spike sample	±10%	91–100%
% RSD IPR	NA	1.2%
% RSD OPR	≤10%	1.4–1.9%
Relative percent difference (% RPD) spike duplicates	≤10%	0.1–0.4%

*The method criteria state that the LRB sample should never be higher than the MDL. The highest individual LRB result of the test was 0.0088 mg N/L.

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