Brown, Don

From: McCambridge, Michael
Sent: Tuesday, February 21, 2017 3:01 PM
To: Glutz, David A.
Cc: Powell, Mark; Tipsord, Marie; McGill, Richard; Brown, Don
Subject: RE: Contact info
Attachments: NECi Nitrate-Reductase Method (2-16).pdf; Thermo-Fisher Discrete Analyzer (2-18-16).pdf

Don Brown: Please enter a copy of this communication on a pending rulemaking to docket R17-12. I will replace this document with the latest version of this e-mail chain should further communication occur.

Mr. Glutz,

Attached are the requested copies of your nitrate-nitrite and orthphosphate mehtods for drinking water analysis. As mentioned, I am trying to incorporate by reference to these USEPA-approved methods in the Illinois drinking water rules for use in this State. Incorporation by reference requires that I have a copy of the document as approved by USEPA and recite where regulated entities can obtain the method. Where I can, I add an Internet address for obtaining a copy.

I could not find a copy of the orthophosphate method by an extensive search of the Thermo-Fisher corporate website. I would, hopefully, like to give a domestic address and web address for obtaining the method. For now, I will use the Finnish address.

Thank you for telling me that you are an alternative source for the NECi nitrate-reductase method. I will now see if I can find it on the Thermo-Fisher website.

Michael J. McCambridge
Attorney
Illinois Pollution Control Board
312-814-6924 (M-Th, 9:00 a.m.-7:00 p.m.)
219-614-5082 (personal cell during all other hours)

From: Glutz, David A. [mailto:david.glutz@thermofisher.com]
Sent: Tuesday, February 21, 2017 2:47 PM
To: McCambridge, Michael
Subject: [External] Contact info

Mike,

Great to talk with you just now. I'll dig into the references to find out the most efficient/direct way to find the Orthophosphate and Enzymatic Nitrate Reductase methods on GALLERY. Attached is the Nitrate Reductase method to be sure we're both looking at the correct one. I'll wait to hear from you on the phosphate method you have on file.

Kind Regards,
David Glutz
Product Specialist
Gallery Discrete Analyzers
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The world leader in serving science.

Method for Nitrate Reductase Nitrate-Nitrogen Analysis of Drinking Water Version 1.0 Revision 2.0 1February, 2016

1. Scope and Application

1.1. This test method is applicable to the determination of nitrate plus nitrite (as nitrogen) in drinking water.

1.2. The standard range of this test method is from 0.05 to 5 mg/L of nitrate-nitrogen. Samples with greater than 5 mg N/L may be diluted with deionized water (nitrate-free) to bring them into the range of the test method. In addition, for samples with nitrate-N content lower than 0.05 mg N/L, the sample volume used in the test may be increased for a low range test method from 0.01 to 1.0 mg N/L.

1.3. Quality Control and Quality Assurance (QC/QA) procedures and criteria are delineated. Precision and Bias of the method has been validated to be ±10% for all QC procedures and samples of a variety of Drinking Water matrices.

1.4. This description of the test method does not purport to address all safety concerns associated with its use. It is the responsibility of the user of this test method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2. Summary of Method

2.1. Reduction of nitrate in the sample is accomplished by using eukaryotic nitrate reductase (EC 1.1.7.1-3; NaR) to catalyze the conversion of nitrate to nitrite in the presence of NADH or NADPH as reductant, in a biochemical buffer with a pH near neutrality (Campbell et al., 2006). This version of the Method is for discrete analyzers.

2.2. The combined nitrite (both original nitrite and that generated by reduction of nitrate to nitrite) is reacted with sulfanilamide and subsequent coupling with N-(1-napthyl)ethylenediamine dihydrochloride to yield a pink color. The absorbance of the chromophore is measured at ~540 nm in a spectrophotometer.

2.3. Calibrants of certified nitrate standard are treated with the reduction step and colorizing reaction to yield a standard curve which is used to determine the nitrate-N content of samples, expressed as mg/L nitrate N. This step insures that NaR is functioning.

2.4. Nitrite content of the sample (expressed as mg/L nitrite-N) can be determined by omitting the reduction step and directly reacting the sample with the color dyes.

2.5. Finally, nitrate-N content can be calculated by subtracting the nitrite content (mg/L nitrite N) from the combined nitrate plus nitrite (mg/L nitrate+nitrite N), to yield the nitrate content (expressed as mg/L nitrate N).

3. Definitions

3.1. Nitrate Reductase (NaR): NADH:NaR (EC1.7.1.1 and CAS 9013-03-0) with 1 unit of enzyme activity defined as 1 μmol nitrite produced per minute at 30°C and pH 7 with NADH (refer to section 3.4) as electron donor. See Note following Section 7.6 for information on the type of NaR acceptable for this method.

3.2. Discrete Analyzer: a programmable, computer-controlled instrument with colorimetric detector that automates wet chemical analysis by using one or more robotic arms interfaced to high-precision volumetric dispensers to aspirate and dispense samples, standards, diluents and reagents.

3.3. Greiss Reaction: chemical formation of an azo-dye by diazotization of nitrite ion with sulfanilamide (CAS 63-74-1), at an acidic pH, and subsequent coupling with N-(1-naphthyl)ethylenediamine hydrochloride (CAS 1465-25-4). The product of this reaction is a dye that absorbs light at 540 ± 20 nm.

3.4. NADH: β-Nicotinamide adenine dinucleotide, reduced form (CAS 53-54-9), is a coenzyme found in all living cells.

3.5. EDTA: Disodium ethylenediaminetetraacetate dihydrate (CAS 60-00-4, a heavy metal chelator.

4. Interferences

4.1. Sample color that absorbs at wavelengths between 520 and 560 nm interferes with the absorbance measurements. When color is suspect, analyze a sample blank, omitting the N-(1-naphthyl)ethylenediamine dihydrochloride from the color reagent. **NOTE:** The instrumentation described in this test method may automatically correct.

4.2. Certain ions may cause interferences. However, the inclusion of EDTA in the system's phosphate buffer (section 3.6 and 7.1) overcomes most, if not all, of these heavy metal interferences.

5. Safety

5.1. General laboratory safe practices should be used in handling all samples and reagents in this test method.

5.2. When preparing the sulfanilamide color reagent in hydrochloric acid (section 7.4), the user should handle the concentrated hydrochloric acid with extra caution and wear heavy gloves and eye protection. The prepared sulfanilamide color reagent should also be handled with caution since it is ~3 N hydrochloric acid.

5.3. Potassium nitrite is toxic if swallowed, and laboratory tests suggest that it may be mutagenic or teratogenic. Gloves and safety glasses are used when handling potassium nitrite.

5.4. Potassium nitrate, unlike potassium nitrite, is not toxic to humans, unless one is chronically exposed to nitrate such as in your drinking water or food. Potassium nitrate will not explode on its own. However, it is recommended that standard laboratory safety practices such as the use of safety glasses and gloves be used when handling it.

6. Equipment

6.1. Automated Discrete Analysis System (see section 3.2).

6.1.1. Discrete Analyzer Characteristics

a. Detectors: Colorimetric detector with optical interference filters, or Spectrophotometer (fixed wavelengths, or scanning)

b. Sample delivery system: High-precision syringe or piston pump

c. Heater: Resistant or Peltier-based heater

d. Dilution device: Built-in dilution function

e. Process: Individual cuvette, sequential batch analysis or random-access single samples

6.1.2. Equivalent DA instruments include, but are not limited to:

a. Thermoscientific Instruments see:

i. Aquakem™ Photometric Analyzers: Models 200, 250, & 600

ii. Arena™ Photometric Analyzers: Models 30, 60, 20, & 20XT

iii. Gallery™ and Gallery™ Plus Automated Photometric Analyzers

b. Unity Scientific

i. SMARTCHEM® 200 and SMARTCHEM® 170 ; EASYCHEM® Plus

ii. Westco Scientific Instruments see: http://westcoscientific.com/

iii. SMARTCHEM® 200 and SMARTCHEM® 170 ; EASYCHEM® Plus

c. Astoria-Pacific

7. Reagents and Standards

7.1. Phosphate Buffer Solution: Dissolve 3.75 g of potassium dihydrogen phosphate (KH_2PO_4; CAS 7778-77-0), 0.01 g of disodium ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$), and 1.4 g potassium hydroxide (KOH; CAS 1310-58-3) in about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. Transfer this solution to a screw-cap container and store at 4°C. This solution is stable for 6 months.

7.2. β-nicotinamide adenine dinucleotide, reduced form (NADH) stock solution (2mg/mL): dissolve 0.1 g NADH in 25 mL of reagent water contained in a 50 mL volumetric flask, dilute to the mark and mix. Transfer 1-mL aliquots to 1.5 mL snap-cap colorless polypropylene vials and store at -20°C. Stable for 1 month. **NOTE:** NADH is a hygroscopic white powder that is freely soluble in water. The solids are stable if stored dry and protected from light. Neutral solutions are colorless and stable for 1 week if stored at 4°C, but decompose rapidly under basic or acidic conditions.

7.3. NADH working solution: thaw one 1-mL vial of NADH stock (refer to section 7.2) and dilute to 10 mL with phosphate buffer (refer to section 7.1). This reagent is stable for about 8 hours. Prepare sufficient NADH working solution for the number of samples and standards to be analyzed. **NOTE**: NADH inhibits color formation in the Greiss reaction (refer to section 3.3). The molar concentration of NADH in the reduction medium should be about twice that of the highest calibration standard.

7.4. Sulfanilamide (SAN) Reagent (10g/L): While stirring constantly add 300 mL of concentrated hydrochloric acid (HCl, 37% w/v)) and 10 g of sulfanilamide to about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. This solution is stable for about six months when stored in a brown bottle at 20°C.

7.5. N-(1-naphthyl)ethylenediamine dihydrochloride (NED) solution (1g/L): dissolve 1 g NED in about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. Transfer to a glass or amber screw-cap container. This solution is stable for 6 months at 20°C.

7.6. Nitrate Reductase (NaR): follow the manufacturer's instructions for preparing a solution of 1 unit NaR (refer to section 3.1) activity per mL of phosphate buffer (refer to section 10.1). Dilute 3 units NaR to 20 mL with phosphate buffer. Store the solution at 2-6°C, where it is stable for 8 hours. Prepare sufficient NaR for the total number of samples and standards to be analyzed. **NOTE:** For some NaR forms, high phenolic content humic substances (>2 mg dissolved organic carbon /L) have little effect on the NaR activity in the temperature range of 5-15°C, but become increasingly inhibitory in the temperature range of 20-40°C (Patton and Kryskalla, 2011; 2013) . Humic substances at the operation temperatures specified in this test method do not inhibit other forms of NaR, such as recombinant AtNaR2 (Campbell et al., 2006). If humic acids are expected to be present, the user must verify reduction efficiency of the NaR in use by analysis of Quality Control checks that approximate the sample matrix.

7.7. Nitrate Solution, Stock (1000 mg/L NO3-N): Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 h. Dissolve 7.218 g in about 500 mL reagent water contained in a 1000 mL volumetric flask, dilute to the mark and mix. This solution is stable for up to 2 months with refrigeration. Alternatively, certified nitrate stock solutions are commercially available through chemical supply vendors and may be used.

7.8. Nitrate Solution, Standard (10 mg/L NO3-N): Dilute 10 mL of stock nitrate solution (7.7) to 1 L with Deionized water and store in a dark bottle. Prepare fresh as needed.

7.9. Nitrite Solution, Stock (1000mg/L NO2-N): Place about 7 g of potassium nitrite (KNO_2) in a tared 125-mL beaker and dry for about 24 h to a constant weight in a desiccator containing a suitable desiccant. Adjust the weight of the dry potassium nitrite to 6.072 g. Add 50 mL of water to the beaker, stir until dissolved, and transfer quantitatively to a 1000-mL volumetric flask. Dilute to the mark with deionized water and store in a sterilized bottle under refrigeration. Prepare fresh as needed. Alternatively, certified nitrite stock solutions are commercially available through chemical supply vendors and may be used. **NOTE**: Potassium nitrite is easily oxidized; use only dry, free flowing white, or yellowish white crystalline powder of this reagent.

7.10. Nitrite Solution, Standard (10 mg/L NO2-N): Dilute 10 mL of stock nitrite solution (7.9) to 1 L with water. This solution is unstable; prepare fresh as needed.

8. Sample Collection, Preservation, and Storage

8.1. Sample preservation and holding time requirements for drinking water samples are as follows:

8.1.1. For nitrate: Chill the sample to ≤4°C and analyze within 48 hours. If the sample is chlorinated, the holding time for an unacidified sample kept at 4°C is extended to 14 days.

8.1.2. For nitrite: Do not add acid. Chill the sample to ≤4°C and analyze within 48 hours of collection.

8.1.3. For nitrate-nitrite: Acidify to pH<2 with H_2SO_4 at the time of collection, and analyze within 28 days of collection.

8.2. Sample preservation and holding time requirements for wastewater samples or other samples to be used for compliance monitoring under the Clean Water Act.

8.2.1. For nitrate: Chill the sample to ≤6 C and analyze within 48 hours of collection.

8.2.2. For nitrite: Chill the sample to ≤6 C and analyze within 48 hours of collection.

8.2.3. For nitrate-nitrite: Acidify to pH <2 with H_2SO_4 at the time of collection, and analyze within 28 days of collection.

8.3. In all cases, samples should be analyzed as soon as possible after collection.

9. Quality Control (QC)

9.1. **Requirement for QC Analysis:** To be certain that analytical values obtained using the test method are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when analyzing nitrite-nitrate in drinking water. Follow the instrument manufacturer's calibration protocols and instructions.

9.2. **Initial Demonstration of Performance (IDP):** IDP is used to characterize instrument performance and laboratory performance prior to performing analyses by this test method and periodically as specified below. The laboratory must complete the IDP tests and demonstrate compliance with the acceptance standards before beginning to analyze samples.

9.2.1. **Instrument Calibration Range (ICR):** ICR must be determined initially and verified every six months or whenever a significant change in the instrument

response is expected or observed. The initial demonstration of ICR must employ a number of standards sufficient to insure that the results are reproducible and statistically acceptable. Verification of ICR must use a minimum of a blank and three standards. If any verification data exceeds the nominal value of the standard by ±10%, ICR must be reestablished. ICR data must be fitted with a statistics program using either linear or non-linear regression analysis and an equation relating instrument response to the nominal values of the standards for the calibration obtained. The regression coefficient (R^2) must be 0.999 or greater; if it is not, determine the problem and recalibrate the instrument.

9.2.2. **Quality Control Sample (QCS):** QCS is a standard of known concentration from an external source and different from calibration standards. QCS must be analyzed initially and quarterly or as required to meet QC requirements. If the QCS analysis result is not within ±10% of the known concentration, the test has failed. The source of the problem must be identified and corrected before continuing with IDP.

9.2.3. **Reduction Efficiency (RE):** Analyze a nitrite standard (Section 7.10) for determining the Reduction Efficiency. The nitrite standard will contain 2.5 mg Nitrite-N/L (or other mid-range concentration) and the absorbance at 540 nm (or appropriate measure of the instrument response) is compared to the absorbance at 540 nm (or appropriate measure of the instrument response) of a Certified Nitrate-N Standard of the same concentration as the Nitrite-N standard:

RE = [(Nitrate-N Standard A-540)/(Nitrite-N Standard A-540) X 100].

For acceptable results, RE must be 90% or greater. If the RE is unacceptable, the source(s) of the problem must be determined and the DA re-calibrated.
NOTE: The likely source of a problem with the RE is - either the NaR solution needs to be replaced, or the NADH has expired and needs to be replaced.

9.2.4. **Method Detection Limit (MDL):** The MDL must be established for the analyte using reagent water (blank) fortified at a concentration near the detection limit of the instrument. Perform the MDL study according to "40 CFR Part 136 Appendix B". Samples should be prepared using reagent water fortified with nitrate at a concentration between 1 to 5 times the expected MDL. First, calibrate the instrument (see section 9.3). MDL is determined by analyses of seven replicates of fortified reagent water (spiked) and processed through the entire analytical method. Calculate the replicates analyte concentration in system units using the instrument calibration (see Section 10). Calculate MDL as follows:

MDL = (t) x (S)

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.143 for seven replicates) and

S = standard deviation of the seven replicate analyses.

If the concentration of the sample (spike) used to determine the MDL is greater than 5X the calculated MDL repeat the MDL determination at a lower concentration for the sample (spike) studied. MDL should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. **Calibration and Calibration Verification:** Prior to the analysis of samples, calibrate the instrument using at least four working standards containing concentrations of nitrate in reagent water that bracket the expected sample concentration. Before each analysis, verify the calibration with a mid-range second source calibrant (SSC) from an external source and different from calibration standards. The result should fall within ± 10% of the known concentration. If the SSC is not within ± 10%, determine the problem, and recalibrate the instrument.

9.4. **Initial Performance and Recovery (IPR):** If a laboratory has not performed this test method before, or if there has been a major change in the measurement system, for example, new analyst, new instrument, and so forth, a precision and bias study must be performed to demonstrate laboratory capability. Analyze four replicates of a standard solution prepared from a Certified Independent Reference Material (CRM) containing a mid-range concentration of nitrite-nitrate in water. The matrix and chemistry of the solution should be equivalent to the solution used in the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps. Calculate the mean and standard deviation of the four values and compare to the acceptable ranges provided by the manufacturer of the CRM. Do not use this test method to analyze samples unless the IPR recoveries are within the accepted limits. Acceptable limits are ±10% of the known value.

9.5. **Ongoing Performance and Recovery Sample (OPR):** To ensure that the test method is in control, analyze a OPR containing a mid-range concentration of nitrite-nitrate in water with each batch or 10 samples. If large numbers of samples are analyzed in the batch, analyze the OPR after every 10 samples. The OPR must be taken through all of the steps of the analytical method including sample preservation and pretreatment. The result obtained for the OPR shall fall within ± 10% of the known concentration. If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all the samples in the batch must be reanalyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.6. **Method Blank:** Analyze a reagent water test blank with each batch or 10 samples. The concentration of nitrite-nitrate in water found in the blank should be less than the MDL. If the concentration of nitrite-nitrate in water is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows

no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.7. **Matrix Spike (MS) and Matrix Spike Duplicate (MSD):** To check for interferences in the specific matrix being tested, perform MS and MSD on at least one sample from each batch by spiking an aliquot of the sample with a known concentration of nitrite-nitrate in water and taking it through the analytical method. The spike concentration plus the background concentration of nitrite-nitrate in water must not exceed the highest calibration standard. The spike must produce a concentration in the spiked sample that is 2 to 5 times the nitrite-nitrate in water concentration in the unspiked sample, or 10 to 50 times the detection limit of the test method, whichever is greater.

Calculate the percent recovery of the spike (P) using the following formula:

$$P = 100 \times \frac{[A \times (V_s + V) - (B \times V_s)]}{C \times V}$$

In this equation, A = nitrite+nitrate concentration (mg/L) in spiked samples, B = nitrite+nitrate concentration (mg/L) in unspiked samples, C = nitrite+nitrate concentration (mg/L) in the spiking solution, V_s = sample volume (mL) used, and V = added spiking solution volume (mL).

The percent recovery of the spike shall fall within ±10%, based on the nitrite-nitrate concentration in the spike. If the percent recovery is not within these limits, a matrix interference may be present in the sample selected for spiking. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.8. **Independent Reference Material (IRM):** In order to verify the quantitative value produced by the test method, analyze an Independent Reference Material (IRM) submitted as a regular sample (if practical) to the laboratory at least once per quarter. The concentration of the IRM should be in the concentration mid-range for the method chosen. The value obtained must fall within the control limits established by the laboratory.

10. Calibration and Standardization

10.1. Using the standard nitrate solution (section 7.8) prepare calibration standards by using the automated calibration function of the discrete analyzer (section 3.2). Table 1 specifies suggested calibrants. **NOTE**: Most discrete analyzers generate calibration standards and calibration curves automatically using Certified calibrants. Follow the

manufacturer's instructions for calibrating with individual calibration standards, if an automatic calibration function is not available.

10.2. Prepare at least one calibration standard from the standard nitrite solution (section 7.10) at the same concentration as one of the nitrate standards to verify the efficiency of the reduction. Verify that reduction efficiency is greater than 90% with each batch of enzyme.

10.3. Run the calibration analysis as described in section 11 for sample analysis.

10.4. Prepare a standard curve by plotting the absorbance of each processed calibration standard against its known concentration. **NOTE**: Most discrete analyzers generate calibration curves automatically.

11. Procedure

11.1. Removal of Color Interferences: If there is a possibility that the color of the sample might absorb in the photometric range from 540 ± 20 nm, determine the background absorbance. **NOTE**: Many discrete analyzers automatically compensate for background absorbance and turbidity on each sample. Follow the manufacturer's instructions.

11.2. Prepare a method in the discrete analyzer software following these specifications:

11.2.1. Dispense 55 µL of NaR (section 7.6) and 5 µL of sample. Mix.
NOTE: Larger volumes of samples and reagents may be used while maintaining the same ratio (See Table 2).

11.2.2. Add 12 µL of NADH (section 7.3). Mix and measure the background absorbance.

11.2.3. Incubate 600 seconds at 37°C.

11.2.4. Add 25 µL of SAN reagent (section 7.4). Mix and incubate 120 seconds at 37°C.

11.2.5. Add 25 µL of NED reagent (section 7.5). Mix and incubate 120 seconds at 37°C.

11.2.6. Measure absorbance at 540 nm, using an optional background subtraction at 700 nm. If automatic background subtraction is not available, prepare a reagent blank (with SAN only added) for background subtraction at 540 nm.

11.3. When determining nitrite alone, replace NaR reagent (section 7.6) with Phosphate Buffer (section 7.1).

12. Data Analysis and Calculations

12.1. Determine the concentration of nitrate or nitrite nitrogen in the samples in mg N/L using the computer based data handler provided with the automated discrete analyzer software. **NOTE**: The discrete analyzer will automatically calculate the net absorbance by subtracting the background absorbance from the measured absorbance of the color developed sample. Use the net absorbance to determine the concentration of nitrogen in the sample.

12.2. Where separate values are required for nitrite-nitrogen and nitrate-nitrogen, calculate the nitrate-nitrogen by subtracting the nitrite-nitrogen from the total nitrate-nitrite nitrogen content.

12.3. Report the nitrogen content in mg N/L as:
Nitrite-Nitrogen (NO_2-N), mg N/L;
Nitrate-Nitrogen (NO_3-N), mg N/L; and
Combined Nitrate-Nitrite Nitrogen (NO_3, NO_2-N), mg N/L.

13. Method Performance

13.1. The performance of this method was validated by an Inter-Laboratory Study (See Plan for Inter-Laboratory Test Trial) and results of that study are delineated here.

13.2. Method Performance Data

13.2.1. Table 3. Drinking Water Matrices for Analysis

13.2.2. Table 4. Calibration of Enzymatic Methods Summary

13.2.3. Table 5. Enzymatic Efficiency Summary

13.2.4. Table 6. Initial Performance and Recovery (IPR) Summary and Ongoing Performance and Recovery (OPR) Summary

13.2.5. Table 7. Minimum Detection Limit (MDL) Summary

13.2.6. Table 8. DW-1 and DW-1-Cl Tap Water Matrix Nitrate-N Content and Spike Analysis

13.2.7. Table 9. DW-2 High TDS Drinking Water Matrix Nitrate-N Content and Spike Analysis

13.2.8. Table 10. DW-3 High TOC Drinking Water Matrix Nitrate-N Content and Spike Analysis

13.2.9. Table 11. Reference Standard RS-1 Summary

14. Pollution Prevention

All reagents and standards should be prepared in volumes consistent with laboratory use to minimize the generation of waste.

15. Waste Management

All waste should be disposed of in a manner consistent with local regulations. **NOTE**: This method uses very small volumes of samples and reagents and, therefore, less waste is generated than with most analysis methods.

16. References

Campbell, Wilbur H., P Song, GG Barbier (2006) Nitrate Reductase for Nitrate Analysis in Water. Environmental Chemistry Letters, 4: 69-73.

Patton, C.J., and Kryskalla, J.R., 2011, Colorimetric determination of nitrate plus nitrite in water by enzymatic reduction, automated discrete analyzer methods: U.S. Geological Survey Techniques and Methods, book 5, chap. B8, 34 p. (Available on line at http://pubs.usgs.gov/tm/05b08/).

Patton, C.J., and Kryskalla, J.R., 2013, Analytical properties of some commercially available nitrate reductase enzymes evaluated as replacements for cadmium in automated, semiautomated, and manual colorimetric methods for determination of nitrate plus nitrite in water: U.S. Geological Survey Scientific Investigations Report 2013–5033, 36 p., http://pubs.usgs.gov/sir/2013/5033/.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1993. Methods for Chemical Analysis of Water and Wastes. Method 353.2. Revision 2.0. U.S. Environmental Protection Agency, Washington, DC

17. Tables, Diagrams, Forms, Flowcharts, and Validation Data

Table 1

Example Concentrations of Calibration Standards

NO_3^--N or NO_2-N, mg/L	mL of 10 mg N/L Standard per 100 mL final volume
0.01	0.1
0.05	0.5
0.1	1.0
0.5	5.0
1.0	10
2.0	20
3.0	30
5.0	50

Table 2

Scaled sample and reagent volumes for implementation at larger assay volumes.

Volume (µL)	x1	x2	x3	x4	x5
Sample	5	10	15	20	25
AtNaR2	55	110	165	220	275
NADH	12	24	36	48	60
SAN	25	50	75	100	125
NED	25	50	75	100	125
Total Assay	122	244	366	488	610

Table 3

Drinking Water Matrices for Analysis

Each Sample Matrix (except DW-1) will be provided to Participating Laboratories as "Ready to Analyze" (samples will be filtered with 0.45 µm filter and preserved by acidifying) and in sufficient volume to permit multiple analysis. We will supply each lab with Chlorine Standard Solution, 25-30 mg/L as Cl_2, 2 mL PourRite Ampules (NIST)

Sample Matrix	Identifier	Number Replicates	Spikes*	Laboratories
Finished drinking water: from cold water tap of participating laboratories on day of analysis	DW-1	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	All
Local Tap Water with 2-4 mg/L free chlorine added	DW-1 + Cl_2	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	All
Drinking water with high TDS (~ 500 ppm)	DW-2	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	Lab 3
Drinking water with high TOC (~2 ppm)	DW-3	7	3 MS & MSD At 0.5 mg N/L At 1.0 mg N/L At 2.5 mg N/L	Lab 2
ERA #698 Standard Reference	SR-1**	7	NONE	All

*Each MS and MSD sample will be analyzed once. See Appendix D for method of preparing spikes and the layout of a set of analysis of typical DW-1 and its spikes.

SR-1 is Reference Standard of known Nitrate-N mg/L concentration (not acidified) and **must be analyzed by all Participating Laboratories. **SR-1 will require dilution with reagent water.**

Table 4

Calibrants and Standard Calibration Curve Equation for Participating Labs

	Lab 1	Lab 2	Lab 3
Calibration	**NaR-R**	**NaR-R**	**NaR-R**
Reagent Blank	0.00	0.00	0.00
Calibrant 1	0.05	0.05	0.05
Calibrant 2	0.25	0.10	0.10
Calibrant 3	0.50	0.25	0.50
Calibrant 4	0.75	0.50	1.00
Calibrant 5	1.25	1.25	2.50
Calibrant 6	2.50	2.50	5.00
Calibrant 7	3.75	5.00	
Calibrant 8	5.00		
Linear Regression	**Nitrate (mg N/L) = (Absorbance Sample - Intercept) / Slope**		
Slope	0.118	**Not Used in These Analyses**	
Intercept	0.022		
R^2	0.99989		
Polynomial Regression	**Nitrate (mg N/L) = (A x Conc x Conc) + (B x Conc) + C *for Lab 1* or = (A x A-550 x A-550) + (B x A-550) + C *for Lab 2* Or = (A x Area x Area) + (B x Area) + C *for Lab 3***		
Constant A	---	-0.1988	0.0018733
Constant B	---	15.0219	0.5099
Constant C	---	-0.5308	0.1713
R^2	---	1.00000	0.99993

Table 5

All Data from All Labs for

Enzymatic and Cadmium Reduction Efficiency

Evaluated using Certified Standards for 2.50 Nitrate-N mg/L and 2.50 Nitrite-N mg/L

Reduction Efficiency of greater than 90% is acceptance criterion.

	Lab 1	Lab 2	Lab 3*
	NaR-R	NaR-R	NaR-R
Nitrate Absorbance 540 nm, 550 nm, Or Area/sec	0.318	0.2080 0.1982 0.1909 0.1929 0.1792	5.4019 9.1622
Nitrite Absorbance 540 nm, 550 nm, Or Area/sec	0.341	0.1963 0.1892 0.1853 0.1881 0.1787	5.7256 8.9972
Catalytic Reduction Efficiency (NO_3/NO_2) Percent	93.2551	105.9603 104.7569 103.0221 102.5518 100.2798	94.3464 101.8339

*Lab 3 ran a series of 10-12 of these tests, which are not shown here.

Table 6

All Data Reported by Each Lab for

Initial Performance and Recovery (IPR)

and

Ongoing Performance and Recovery (OPR)

	Lab 1	Lab 2*	Lab 3
	NaR-R	NaR-R	NaR-R
IPR Nitrate-N mg/L	2.01	2.01	2.01
Number IPR	4	16	8
Mean IPR	2.0705	1.9682	1.9827
Mean IPR Recovery (%)	103.0075	97.9201	98.6412
OPR Nitrate-N mg/L	2.01	2.50	2.01
Number OPR	7	15	14
Mean OPR	**2.1388**	**2.4769**	**2.4877**
Standard Deviation of Mean	0.03685	0.07204	0.11536
Relative Standard Deviation (%)	1.7233	2.9085	4.6372
Mean Recovery (%)	106.4065	99.0740	99.5083

Table 7

Minimum Detection Limit

7 Replicates

MDL = Standard Deviation x 3.143

Abbreviations: DA, Discrete Analyzer; FIA, Flow Injection Analyzer

	Lab 1	Lab 2	Lab 3
	NaR-R	NaR-R	NaR-R
Analytical Equipment	DA	DA	FIA
Spike mg Nitrate-N/L	0.050	0.025	0.010
MDL mg Nitrate-N/L	0.0097	0.006507	0.009301
Ratio Spike/MDL	5.1453	3.8422	1.0751

Table 8. A. DW-1 and DW-1-Cl - Lab 1 Tap Water Matrix Nitrate-N Content and Spike Analysis

Lab 1 NaR Reduction Method DW1 (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

A-540	Nitrate-N mg/L	Mean ± Standard Deviation Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.059	0.3207		A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.059	0.3215	0.3237 ± 0.003698 RSD 1.1424%	0.179	1.3392	0.4859	97.1829	0.9830	0.179	1.3392	1.0155	101.5514	0.3947	0.366	2.9467	2.6230	104.9206	0.4451
0.06	0.3282																
0.06	0.3279		0.178	1.3352	0.4907	98.1429		0.178	1.3352	1.0115	101.1514		0.367	2.9584	2.6347	105.3886	
0.06	0.3235																
0.06	0.3255																
0.059	0.3185																

Lab 1 NaR Reduction Method DW1-Cl

A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
0.058	0.3117		A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.058	0.3122	0.3185 ± 0.01625 RSD 5.1020%	0.118	0.8191	0.5006	100.1200	1.4082	0.175	1.3087	0.9902	99.0200	1.6923	0.367	2.9515	2.6330	105.3200	0.3564
0.063	0.3547																
0.059	0.3175		0.117	0.8121	0.4936	98.7200		0.177	1.3256	1.0071	100.7100		0.368	2.9609	2.6424	105.6960	
0.059	0.3136																
0.058	0.3126																
0.058	0.3072																

Table 8. B. DW-1 and DW-1-Cl - Lab 2 Data Set 2 Tap Water Matrix Nitrate-N Content and Spike Analysis

Lab 2 NaR Reduction Method DW1 (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

A-550 nm	Nitrate- N mg/L	Mean ± Standard Deviation Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0825	0.9304	0.94569 ± 0.016307 RSD 1.7243%															
0.0835	0.9464		0.1151	1.4501	0.5044	100.8837	3.5318	0.1437	1.9045	0.9588	95.8841	4.3690	0.2315	3.2907	2.3451	93.8021	2.4482
0.0828	0.9352																
0.0852	0.9735		0.1140	1.4326	0.4869	97.3825		0.1464	1.9474	1.0017	100.1668		0.2352	3.3489	2.4032	96.1270	
0.0838	0.9512																
0.0823	0.9272																
0.0841	0.9560																

Lab 2 NaR Reduction Method DW1-Cl

A-550 nm	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %	A-550	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0820	0.9224	0.8546 ± 0.03249 RSD 3.8017%															
0.0775	0.8505		0.1091	1.3546	0.5000	99.9911	3.8968	0.1361	1.7839	0.9293	92.9273	0.8506	0.2241	3.1744	2.3198	92.7913	2.0549
0.0770	0.8425																
0.0773	0.8473		0.0562	0.4791	0.4808	96.1691		0.1366	1.7919	0.9372	93.7211		0.1880	2.3527	2.2726	90.9040	
0.0764	0.8330																
0.0783	0.8633																
0.0758	0.8234																

Table 8. C. DW-1 and DW-1-Cl - Lab 3 Tap Water Matrix Nitrate-N Content and Spike Analysis (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

Lab 3 NaR Reduction Method DW1 (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

Area V/s 540 nm	Nitrate- N mg/L	Mean ± Standard Deviation Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.53691	0.03439	0.03438 ± 0.0006398 RSD 1.8608%	1.4411	0.5674	0.5674	106.6035	2.5541	2.5271	1.1292	1.094818	109.4818	0.3283	5.2217	2.5423	2.507918	100.3167	1.9232
0.52026	0.03541																
0.53262	0.03376																
0.53577	0.03385		1.4678	0.58112	0.5468	109.3615		2.5341	1.1328	1.098418	109.8418		0.367	2.9584	2.6347	105.3886	
0.52710	0.03393																
0.52747	0.03426																
0.53226	0.03509																

Lab 3 NaR Reduction Method DW1-Cl (Abbreviation: RSD = Relative Standard Deviation; RPD = Relative Percent Difference)

Area V/s A-540	Nitrate-N mg/L	Mean ± Std. Dev. Nitrate-N mg/L	0.5 mg N/L Spike					1.0 mg N/L Spike					2.5 mg N/L Spike				
			A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %	A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.5681	0.1190	0.1166 ± 0.004657 RSD 3.9943%	1.4923	0.5938	0.4772	95.44171	0.05210	2.4589	1.0938	0.9772	97.7208	0.05118	5.2544	2.5597	2.4431	97.7243	0.2213
0.5703	0.1201																
0.5688	0.1194																
0.5627	0.1185		1.4875	0.5913	0.4747	94.94571		2.4578	1.0933	0.9767	97.6709		5.2558	2.5543	2.4377	97.5083	
0.5471	0.1082																
0.5539	0.1117																
0.5685	0.1192																

Table 9

DW-2 High TDS Drinking Water Matrix Nitrate-N Content and Spike Analysis

Abbreviation: RPD = Relative Percent Difference Lab 3 NaR Reduction Method DW-2

Nitrate-N mg/L	Mean Nitrate-N mg/L	Standard Deviation mg N/L	Relative Standard Deviation
0.2907	0.2959	0.004749	1.6048%
0.2973			
0.3023			
0.2985			
0.2939			
0.2992			
0.2893			

0.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
1.944	0.7900	0.4941	98.8226	0.0486
1.9437	0.7898	0.4939	98.7746	

1.0 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
2.8302	1.2489	0.9530	95.3003	0.3875
2.8375	1.2526	0.9567	95.6703	

2.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
5.8573	2.8417	2.5458	101.8321	0.4251
5.8371	2.8309	2.5350	101.4001	

Table 10

DW-3 High TOC Drinking Water Matrix Nitrate-N Content and Spike Analysis

Abbreviation: RPD = Relative Percent Difference Lab 2 NaR Reduction Method DW-3

Nitrate-N mg/L	Mean Nitrate-N mg/L	Standard Deviation mg N/L	Relative Standard Deviation
0.0052	0.003814	0.007267	190.5184%
0.0068			
-0.0110			
0.0084			
0.0036			
0.0020			
0.0117			

0.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0621	0.4981	0.4943	98.8571	3.8104
0.0633	0.5173	0.5135	102.6971	

1.0 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.0934	0.9980	0.9942	99.4186	2.4332
0.0919	0.9741	0.9703	97.0286	

2.5 mg N/L Spike				
A-540	mg N/L	Spike mg N/L	Recovery %	RPD %
0.1836	2.4145	2.4107	96.4274	1.5558
0.1861	2.4523	2.4485	97.9394	

Table 11

Standard Reference - SR-1 (Seven Replicates Analyzed)

ERA 698 Certified Value **SR-1 = 5.14 ± 1.83% mg Nitrate-N/L**

	Dilution	Diluted Mean mg Nitrate-N/L	Standard Deviation mg Nitrate-N/L	Relative Standard Deviation %	Final mg Nitrate-N/L	Compared to ERA
Lab 1 - NaRR	Auto-diluted 1:10	5.3989	0.03375	0.6251	5.3989	105.0195%
Lab 1 - CdR	NONE	5.0937	0.07998	1.5701	5.0937	99.0992%
Lab 2 - NaRR	Auto-diluted 1:4	4.9795	0.07552	1.5166	4.9795	96.8783%
Lab 2 - CdR	1:4	1.0128	0.02132	2.1052	5.0640	98.5214%
Lab 3 - NaRR	1:2	2.5629	0.005100	0.1990	5.1258	99.7218%
Lab 3 - CdR	1:2	2.5902	0.01816	0.7011	5.1800	100.7782%

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APPLICATION NOTE:

Drinking Water Orthophosphate for Thermo Scientific Gallery discrete analyzer

Name of the method:	Drinking Water Orthophosphate
Reference:	Standard Methods (SM) 4500-P E. Phosphorous /Ascorbic Acid Method. EPA approved method under 40 C.F.R. § 141.23 (National Primary Drinking Water Regulations, NPDWR, based on SDWA, Safe Drinking Water Act).[1]
Intended use:	This paper presents Drinking Water Orthophosphate method for Thermo Scientific Gallery™ discrete analyzer. The method was designed to follow the standard method SM 4500-P E. as closely as possible.
Revision number:	5
Revision date (mm/dd/yyyy):	02/18/2016

1. Scope and Application

1.1 This automated method covers the determination of orthophosphate (CAS: 14265-44-2) in drinking water with the Thermo Scientific™ Gallery™ discrete analyzer.

1.2 The method is based on reaction that is specific for the orthophosphate ion.

1.3 The applicable range for this method is from 0.0125 – 0.5 mg PO4-P/L. An extension of the range to 5 mg PO4-P/L is achieved when 1:10 automated dilution is configured. The automated dilution feature must be confirmed with acceptable analysis of quality control samples by the user.

2. Summary of Method

2.1 Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphorous to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

2.2 Only orthophosphate forms a blue color in the test. Polyphosphates (and some organic phosphate compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphate compounds may be converted to the orthophosphate form by persulfate digestion. These conversion methods are not covered in this Application Note.

Thermo Fisher Scientific, Analyzers & Automation, R&D Industrial diagnostics
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3. Definitions

3.1 Units and symbols from the international metric system (SI) are used. Definitions, acronyms, and abbreviations are explained as they occur for the first time.

4. Interferences

4.1 No interference is caused by copper, iron or silicate at concentrations many times greater than their reported concentration in seawater. However, high iron concentrations can cause precipitation of, and subsequent loss, of phosphorous.

4.2 Arsenates react with the molybdate reagent to produce a blue color similar to that formed with orthophosphate. Thus, it should be considered when present in concentrations higher than orthophosphate. Concentrations as low as 0.1 mg as/L can interfere with the orthophosphate determination.

4.3 Hexavalent chromium and nitrite-ion can interfere with the orthophosphate determination yielding in approximately 3 % lower results at concentrations of 1 mg/L and 10 % to 15 % lower results at 10 mg/L concentrations.

4.4 Sample turbidity is not to be removed by filtration prior to analysis for orthophosphate, unless dissolved reactive phosphorous is studied. Samples for total phosphorous may be filtered only after digestion. Sample color that absorbs in the photometric range used for analysis will also interfere. The sample blank is measured automatically in the application for sample color and turbidity correction.

5. Safety

5.1 The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

5.2 The following chemicals have the potential to be toxic or hazardous. Consult MSDS (Material Safety Data Sheet) for details.

5.2.1 Sulfuric acid (contained in Phosphate R1) (CAS# 7664-93-9)

5.2.2 Ammonium molybdate tetrahydrate (contained in Phosphate R1) (CAS# 12027-67-7)

5.2.3 Antimony potassium tartrate (contained in Phosphate R1) (CAS# 28300-74-5)

5.2.4 Ascorbic acid (contained in Phosphate R2) (CAS# 50-81-7)

5.2.5 Potassium phosphate monobasic (CAS# 7778-77-0)

6. Equipment and Supplies

6.1 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2 Water purification system for producing suitable water for autoanalyzers. Refer to instrument user manual.

6.3 Thermo Scientific Gallery, automated discrete photometric system.

6.4 Filter, wavelength 880 nm.

6.5 DECACELL™ Cuvettes for Thermo Scientific Gallery. DECACELL cuvettes must always be used with Thermo Scientific Gallery. Cuvettes are for single use only.

6.6 Washing solution 4.5 % hypochlorite solution, 4 × 20 mL, is used for daily instrument cleansing.

7. Reagents and Standards

Reagents for Gallery:	THERMO SCIENTIFIC ORDERING NUMBERS
	984366 Phosphate R1, 4 × 20 mL
	984367 Phosphate R1, 20 × 20 mL
	984368 Phosphate R2, 4 × 20 mL
Standards for Gallery:	THERMO SCIENTIFIC ORDERING NUMBER
	984729 Phosphate (as P) Std, 1000 ppm, 500 mL (calibration)
	984726 Phosphate (as PO4) Std, 1000 ppm, 500 mL (QCS)

7.1 Preparation of reagents needed in this method is described under. Also ready to use reagents are available for this method.

7.2 Reagent water — Distilled or deionized water, free of the analyte of interest. Water stored in bottles should be substituted by fresh water after one week.

7.3 Phosphate R1 — Prepared by adding 15 mL of ammonium molybdate solution (20 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ /500 mL water) to 50 mL of sulfuric acid solution (70 mL conc. H_2SO_4 /500 mL water) followed by 5 mL addition of antimony potassium tartrate solution (1.5 g $K(SbO)C_4H_4O_6\cdot \frac{1}{2}H_2O$ /500 mL water) to this mixture.

7.4 Phosphate R2 — Ascorbic acid solution containing 1.76 g ascorbic acid in 100 mL water. This solution is stable for 5 days when refrigerated between 2 -8 °C.

7.5 Phosphate (as P) Std, 1000 ppm — Phosphate standard 1000 mg/L as P is for calibrating the Gallery instrument.

7.6 Phosphate calibration solutions — Prepare an appropriate series of standards by diluting suitable volumes of Phosphate standard (stock) with distilled water, or use automated Gallery calibration dilution feature.

7.7 Quality Control Solution (QCS) — A second source standard from an external source, e.g. Phosphate (as PO4) Std, 1000 ppm (326.2 ppm as P). Dilute to appropriate concentration with distilled water. Do not use Phosphate calibration solutions as QCS-samples.

7.8 Laboratory Fortified Blank (LFB) — Phosphate standard (stock) 1000 mg/L or self made standard solution. Dissolve 0.4394 g of predried (105 °C for one hour) potassium phosphate monobasic (KH_2PO_4) in distilled water and dilute to 1000 mL. 1.0 mL of this solution contains 0.1 mg P. Dilute to appropriate concentration with distilled water. Do not use Phosphate calibration solutions as LFB-samples.

8. Sample Collection, Preservation, and Storage

8.1 Sample containers may be of plastic material or of Pyrex glass. All bottles must be thoroughly cleaned and rinsed with distilled water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal. Do not store samples containing low concentrations of phosphorous in plastic bottles because orthophosphates may be adsorbed to the walls of plastic bottles.

8.2 Samples should be analyzed as soon as possible, after collection, within 48 hours at maximum. Samples must be cooled and maintained at 4 °C.

9. Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of laboratory reagent blanks, continuing calibration check standards, fortified blanks and fortified samples as duplicates as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 The initial demonstration of capability (IDC) is used to characterize instrument performance in an individual laboratory by determination of calibration curve and analysis of quality control samples (QCS) and laboratory performance by determination of method detection limit (MDL), minimum reporting level (MRL) and the initial precision and recovery (IPR) test.

9.2.1 Calibration Curve — Calibration curve must be determined initially and reanalyzed at least every six months or whenever a significant change in instrument response is observed or expected. The calibration curve must use a minimum of five standards and a blank to ensure that the resulting curve is fitted correctly. The Gallery analyzer fits the calibration first as a linear curve. The user can then fit the calibration to 2nd degree curve, if the fitting is better thereby. The correlation coefficient of the calibration curve should be equal to or greater than 0.995.

9.2.2 Quality Control Sample (QCS) — When beginning the use of this method, whenever new standard materials are used, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the analyses of a secondary standard solution (QCS) from an external source. If the determined concentrations are not within ± 15 % or ± 20 % when the concentration is ≤ 2x MRL of the stated values, performance of the determinative step of the method is

unacceptable. The source of the problem must be identified and corrected before proceeding with the initial determination of MDL or continuing with on-going analyses.

9.2.3 Method Detection Limit (MDL) — An MDL should be established using reagent water (blank) fortified at a concentration of one to five times the estimated instrument detection limit (MDLest). The estimate is calculated as three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

$$MDLest = 3 \times (S_0)$$

where S_0 = standard deviation of the replicate analyses (n=10) of reagent water.

Prepare a laboratory fortified blank sample (LFB) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit (recommended 1 – 5 x MDLest). Perform all calculations defined in the method and report the concentration values in the appropriate units. Each individual result must be within 70 – 130 % of the theoretical value. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where t = Student's t-value for a 99 % confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for 7 replicates]
S = standard deviation of the replicate analyses

MDL should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.2.4 Minimum Reporting Level (MRL) Confirmation — The minimum concentration that can be reported by a laboratory as a quantified value for the method analyte in a sample following analysis. The MRL must be at or above the level of the lowest fortified calibrator, where it must meet the criteria set for MRL confirmation. It would also have to be considered that criteria for Laboratory Reagent Blank (LRB) must be met (LRB ≤ 1/3 x MRL).

Fortify and analyze seven replicate LFBs at the proposed MRL concentration.
Calculate the mean (*Mean*) and standard deviation (s) for these replicates. Determine the Half Range for the Prediction Interval of Results (HR_{PIR}) using the equation:

$$HR_{PIR} = 3.963s$$

Where s = standard deviation
3.963 = constant value for seven replicates

Calculate the upper and lower limits for the Prediction Interval of Results (PIR = *Mean* +HR_{PIR}) from the results and confirm that the results meet the criteria. Accepted results confirm the MRL to be validated.

$$PIR\ Upper\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ \ Concentration} \times 100$$

$$PIR\ Lower\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ \ Concentration} \times 100$$

Criteria: The Upper PIR Limit must be ≤ 150 % recovery.
The Lower PIR Limit must be ≥ 50 % recovery

9.2.5 Initial Precision and Recovery (IPR) — For initial precision and recovery test the laboratory should analyze four replicate volumes of reagent water spiked with the analyte of interest (LFB). Calculate accuracy as percent recovery and precision as relative standard deviation (% RSD) as shown under.

$$\%RSD = \frac{100}{\bar{X}} \times \sqrt{\sum_{i=1}^{n} \frac{(X_1 - \bar{X})^2}{n-1}}$$

where $\bar{X}$ = mean of replicate measurements
X_1 = measured value of the replicate
n = number of replicates

$$\text{Recovery}\% = \frac{C_s - C}{s} \times 100$$

where Cs = spiked sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample

Criteria for % Recovery: ±15% (or 85 - 115 %), when c is > 2 x MRL and
±20% (or 80 - 120 %), when c is ≤ 2 x MRL
Criteria for % RSD: ±15%

9.3 Assessing laboratory performance with ongoing QC includes the use of Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB), Continuing Calibration Check (CCC)

9.3.1 Laboratory Reagent Blank (LRB) — The laboratory should analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment.

Criteria for LFB: Must be below 1/3 of MRL

9.3.2 Laboratory Fortified Blank (LFB) — The laboratory should analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery as shown in 9.2.5. If

the recovery of any analyte falls outside the required control limits, the source of the problem should be identified and resolved before continuing analyses. LFB analyses data is used to assess laboratory performance against the required control limits.

Criteria for % recovery: ±15% (or 85 - 115 %), when c is > 2 x MRL and
±20% (or 80 - 120 %), when c is ≤ 2 x MRL

When enough data from LFB samples analyzed as part of method ongoing QC is gathered the laboratory can assign its own control limits from the standard deviation of the results.

9.3.3 Continuing Calibration Check (CCC) — For all determinations the laboratory should analyze the CCC (a mid-range check standard) and a calibration blank immediately following calibration, after every tenth sample and at the beginning and end of the sample run. Analysis of the CCC solution and calibration blank immediately following calibration must verify that the instrument is within ± 10 % of calibration. Subsequent analyses of the CCC solution must verify the calibration is still within ± 10 %. This procedure is done automatically by the analyzer. **Note!** Application has manual result acceptance instead of automatic acceptance to ensure CCC protocol is followed.

If the calibration cannot be verified within the limits specified in the application, the analyzer gives a message of outlier result. In such case reanalyze the CCC solution by triggering the Ongoing QC procedure. If the second analysis of the CCC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable CCC solution must be reanalyzed, even if the resumed CCC is acceptable.
The analysis data of the calibration blank and CCC solution must be kept on file with the sample analyses data.

Criteria: CCC % recovery ±10% (or 90 -110 %)

9.3.4 Quality Control Samples (QCS) — Standard solution samples (9.2.2), that must reach laboratory acceptance criteria are analyzed with each calibration, whenever new standard materials are used, on a quarterly basis or as required to meet data-quality needs in addition to the IDC measurements.

Criteria: ±15% (or 85 - 115 %), when c is > 2 x MRL and
±20% (or 80 - 120 %), when c is ≤ 2 x MRL

9.4 Laboratory Fortified Matrix Sample

9.4.1 Laboratory Fortified Matrix Sample (LFM) (also termed Matrix Spike, MS and Matrix Spike Duplicate MSD) — The laboratory must add a known amount of analyte to a minimum of 10 % of the routine samples. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times

the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank in section 9.3.2.

Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 85-115 %. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control, the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related. The Gallery analyzer has a standard addition feature for difficult sample matrixes.

$$\text{LFM Recovery \%}, R = \frac{(C_s \cdot f) - C}{s} \times 100$$

where C_s = spiked sample concentration
f = spike dilution correction
C = sample background concentration
s = concentration equivalent of analyte added to sample

Note! If the added spike volume is less than 1% of the total LFM sample volume, the factor f can be excluded.

Criteria: LFM % Recovery: ±15%

9.4.2 Laboratory Fortified Matrix Sample (LFM) Precision — The precision of the LFM determinations is also assessed by measuring LFM samples in three replicates followed by calculation of relative standard deviation (% RSD) of the results as shown in 9.2.5.

In routine laboratory performance evaluation LFM duplicate samples (LFMD, also termed Matrix Spike Duplicate or MSD) are used. Precision is then calculated as follows:

$$\%RSD = 100 \times \frac{LFM - LFMD}{½ \times (LFM + LFMD)}$$

where LFM = analyte concentration measured in LFM sample
LFMD = analyte concentration measured in LFM duplicate

Criteria: % RSD ±20%

10. Calibration and Standardization

10.1 Dilute Phosphate standard (stock) (Thermo Scientific) with distilled water to get a suitable phosphate standard for calibration. Use the automated Gallery calibration dilution feature for a calibration curve. Alternatively, prepare a series of at least five standards, covering the desired range, and a blank, for a calibration curve.

10.2 Process standards and blanks as described in Section 11.0 Procedure.

10.3 The Gallery analyzer plots automatically instrument response against standard concentration. The user must accept this calibration curve before the analyzer starts to measure blanks and samples. The calibration correlation coefficient shall be equal to or greater than 0.995.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable control sample (QCS). If measurements exceed ± 15 % of the established LFB value, the analysis should be terminated and the instrument recalibrated. Periodic reanalysis of the QCS is recommended quarterly at minimum. Ongoing QC is done automatically by the analyzer and it includes analyzing LRB, CCC and LFB samples with each batch of ten samples. In addition LFM samples are to be done in duplicates with every batch of ten samples.

11. Procedure

11.1 Preparation before analysis — Add all required reagents, samples, other consumables and requests for tests following the instrument instructions according to the manufacturer.

11.2 Gallery Drinking Water Orthophosphate test flow — To 120 µL of sample or standard or blank 14 µL of Phosphate R1(Thermo Scientific) is added and solution is mixed. After blank measurement, 6 µL of Phosphate R2 (Thermo Scientific) is added and solution is mixed. After 9 minutes incubation, the absorbance is measured at 880 nm. The application is shown in Appendix A.

12. Data Analysis and Calculation

12.1 The Gallery analyzer plots automatically instrument response against standard concentration making a calibration curve. The instrument computes sample concentration by comparing sample response with the standard curve.

12.2 Results are reported in mg P/L.

13. Method Performance

13.1 According to a validation study of the Gallery Drinking Water Orthophosphate method, single laboratory result for MDL was 0.00036 mg/L as P and MRL was confirmed to be 0.0125 mg/L as P. Initial precision was analyzed to be 0.1-1.0 % RSD and accuracy 102-103 % recovery at concentrations 0.1 - 0.4 mg/L as P.

13.2 Precision for LFB samples was 0.2 – 1.1 % RSD and for LFM samples 0.1 – 0.7 % RSD corresponding to sample concentrations 0.1 – 0.4 mg/L as P.

13.3 Accuracy for LFB samples was 102 – 103 % recovery and for LFM samples 92 – 98 % recovery corresponding to sample concentrations 0.1 – 0.4 mg/L as P.

13.4 CCC standard recovery was 100 – 105 % and LRB was always below MDL.

13.5 Method limits for LFB and LFM samples are 15 % RSD and 85 - 115 % Recovery and for CCC 90 - 110 % Recovery. LRB must be below MDL.

14. Pollution Prevention

14.1 The instrument uses small amounts of reagents, which reduces the quantity of wastes significantly compared to manual methods or flow analyzers. The small packing size facilitates the use of reagents during their shelf lives and thus reduces disposal cost of unused materials.

15. Waste Management

15.1 Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner.

15.2 The containers for cuvette and liquid waste must be emptied and rinsed with water at the end of the day.

16. References

16.1 4500-P E. PHOSPHOROUS/Ascorbic acid method. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association (APHA), 2012.

16.2 Code of Federal Regulations 40 § 141.23. Inorganic chemical sampling and analytical requirements.

16.3 Code of Federal Regulations 40 § 136, Appendix B — Definition and Procedure for the Determination of the Method Detection Limit – Revision 1.11.

16.4 4020 Quality Assurance/Quality Control. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association (APHA), 2012.

16.5 1020-B.4 Method Detection Level Determination and Application. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association (APHA), 2012.

17. Tables, diagrams, flowcharts and validation data

17.1 Test parameters and test flow for Gallery Drinking Water Orthophosphate method are presented in Appendix A.

17.2 Method performance data

17.2.1 Calibration and QCS-samples

Calibration curve was prepared between 0 - 0.5 mg P/L for DW o-PO4P application. Calibration was done with automatic dilution from working standard solution of 1.5 mg P/l and laboratory reagent water. Calibration was verified with QCS-samples.

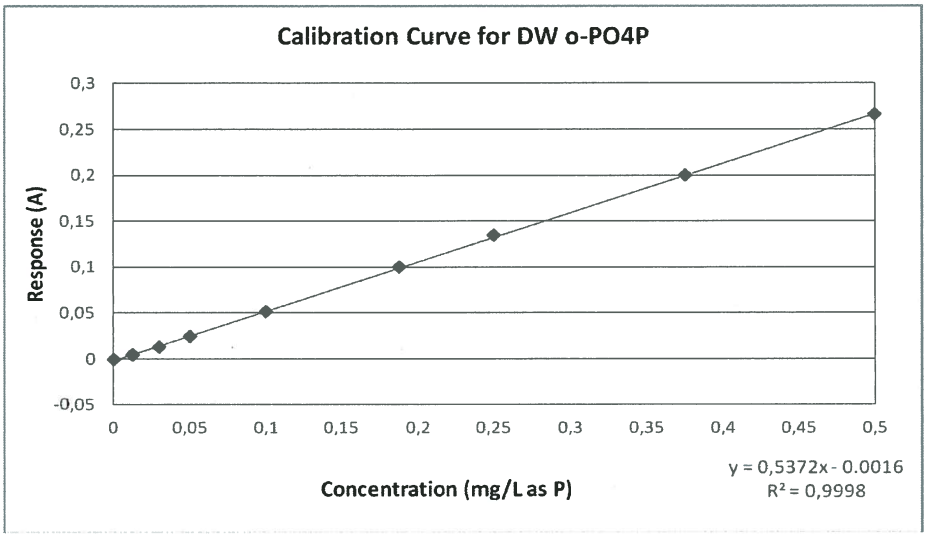

Figure 1. Calibration curve for DW o-PO4P application.

Table 1. Results for Phosphate QCS-samples after calibration.

Orthophosphate as phosphorous		
QCS-sample	**Duplicate % RSD**	**% Recovery**
PO4P 0.1mg/l	0.13 %	96 %
PO4P 0.4mg/l	0.19 %	103 %

17.2.2 Method Detection Limit (MDL)

MDL was established using reagent water (blank) fortified at a concentration approximately five times the estimated instrument detection limit (MDLest). The estimate was calculated as three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

$$MDLest = 3 \times (S_0)$$

where S_0 = standard deviation of the replicate analyses (n=10) of reagent water.

Table 2. Values for determining MDLest for DW o-PO4P application.

Sample	Results (mg/L)
LRB m in.	0.001800
LRB m ax.	0.002122
average =	0.00188
n =	10
std. deviation (SD) =	0.00013
MDLest calculated =	**0.000402**
MDLest assigned=	**0.0022**

NOTE! The MDLest had to be assigned higher than the calculated MDLest, because the highest individual LRB result of this test was as high as 0.002122 mg/l as P, and criteria for LRB sample in this test is, that it should never be above the MDL est.

A laboratory fortified blank sample (LFB) at a concentration which was approximately five times the MDLest, was analyzed in seven replicates. Each individual result had to be within 70 – 130 % of the theoretical value. MDL was calculated as follows:

$$MDL = (t) \times (S)$$

where t = Student's t-value for a 99 % confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for 7 replicates]
S = standard deviation of the replicate analyses

Table 3. Values for determining MDL for DW o-PO4P application.

Sample	Result (mg/L)	% Recovery
LFB 0.01 mg/l PO4P	0.00793	79 %
LFB 0.01 mg/l PO4P	0.00791	79 %
LFB 0.01 mg/l PO4P	0.00795	80 %
LFB 0.01 mg/l PO4P	0.00768	77 %
LFB 0.01 mg/l PO4P	0.00796	80 %
LFB 0.01 mg/l PO4P	0.00803	80 %
LFB 0.01 mg/l PO4P	0.00797	80 %
average =	**0.007919**	**79 %**
n =	**7**	
std. deviation (SD) =	**0.000114**	
% RSD =	**4.5 %**	
MDL =	**0.00036**	

17.2.3 Minimum Reporting Level (MRL)

Seven replicate LFBs were analyzed at the proposed MRL concentration.
The mean (*Mean*) and standard deviation (s) for these replicates were calculated. The Half Range for the Prediction Interval of Results (HR_{PIR}) was determined using the equation:

$$HR_{PIR} = 3.963s$$

Where s = standard deviation
3.963 = constant value for seven replicates

The upper and lower limits for the Prediction Interval of Results (PIR = *Mean* +HR_{PIR}) were calculated from the results.

$$PIR\ Upper\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ \ Concentration} \times 100$$

$$PIR\ Lower\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ \ Concentration} \times 100$$

Criteria: The Upper PIR Limit must be ≤ 150 % recovery.
The Lower PIR Limit must be ≥ 50 % recovery

Table 4. Results for MRL confirmation for DW o-PO4P application.

Sample	Result (mg/L)	% Recovery
LFB 0.01 mg/l PO4P	0.010088	101 %
LFB 0.01 mg/l PO4P	0.009663	97 %
LFB 0.01 mg/l PO4P	0.009568	96 %
LFB 0.01 mg/l PO4P	0.009378	94 %
LFB 0.01 mg/l PO4P	0.009891	99 %
LFB 0.01 mg/l PO4P	0.009616	96 %
LFB 0.01 mg/l PO4P	0.009605	96 %
average =	**0.00969**	97 %
n =	**7**	
std. deviation (s) =	**0.000232**	
HR_{PIR} =	**0.000921**	
Upper PIR Limit =	**106.1 %**	
Lower PIR Limit =	**87.7 %**	
% RSD=	**2.4 %**	

Calculated limits met the criteria and confirmed the MRL to be validated.

17.2.4 Initial Precision and Recovery (IPR)

Four replicate volumes of reagent water spiked with the analyte of interest (LFB) were analyzed at three different concentrations. % recovery and % RSD were calculated. Results are shown in table 7.

Criteria for % recovery: 85 % - 115 %, when c is > 2 x MRL and
80 % - 120 %, when c is ≤ 2 x MRL
Criteria for % RSD: ±15%

Table 5. Results for initial precision and recovery (IPR) for DW o-PO4P application.

Sample	Result (mg/L)	% Recovery
LFB 0.1 mg/l	0.1027	103 %
LFB 0.1 mg/l	0.1028	103 %
LFB 0.1 mg/l	0.1027	103 %
LFB 0.1 mg/l	0.1028	103 %
average	**0.1027**	**103 %**
std.deviation	**0.000074**	
% RSD	**0.1 %**	
Acceptable	**YES**	**YES**
LFB 0.3 mg/l PO4P	0.3056	102 %
LFB 0.3 mg/l PO4P	0.3020	101 %
LFB 0.3 mg/l PO4P	0.3096	103 %
LFB 0.3 mg/l PO4P	0.3051	102 %
average	**0.3056**	**102 %**
std.deviation	**0.0031**	
% RSD	**1.0 %**	
Acceptable	**YES**	**YES**
LFB 0.4 mg/l	0.4074	102 %
LFB 0.4 mg/l	0.4083	102 %
LFB 0.4 mg/l	0.4088	102 %
LFB 0.4 mg/l	0.4083	102 %
average	**0.4082**	**102 %**
std.deviation	**0.00059**	
% RSD	**0.1 %**	
Acceptable	**YES**	**YES**

17.2.5 Precision and accuracy from LFB

Precision (%RSD) and accuracy (% Recovery) were determined using LFB samples at 0.1, 0.3 and 0.4 mg/L concentrations. Results are shown in table 8.

Criteria for % recovery: 85 - 115 %, when c is > 2 x MRL and
80 - 120 %, when c is ≤ 2 x MRL
Criteria for % RSD: ±15%

Table 6. Precision and accuracy data for DW o-PO4P application.

Sample: **Concentration:**	LFB 0.1 mg/l PO4P 0.1 mg P/L	LFB 0.3 mg/l PO4P 0.3 mg P/L	LFB 0.4 mg/l PO4P 0.4 mg P/L
average =	0.10257	0.30541	0.40891
n =	10	10	10
std. dev. (SD) =	0.000209	0.003242	0.000767
% RSD =	0.2 %	1.1 %	0.2 %
Avg. % Recovery =	103 %	102 %	102 %
Acceptable	**YES**	**YES**	**YES**

17.2.6 Precision and accuracy from LFM samples

Laboratory Fortified Matrix (LFM) samples were prepared by spiking three different tap water samples with Phosphate standard solution to gain spike concentrations of 0.1 and 0.4 mg/L as P for each sample. The analyte concentrations were high enough to be detected above the original sample and were not less than four times the MDL. The added analyte concentrations were chosen to be the same as those used for LFB. Precision was estimated by analyzing four replicates and calculating the %RSD. Accuracy was estimated from LFM and corresponding unspiked samples by analyzing them and calculating the % Recovery using the next equation. Results for LFM precision and accuracy are shown in table 9.

$$\text{LFM Recovery\%}, R = \frac{(C_s \cdot f) - C}{s} \times 100$$

where C_s = spiked sample concentration
f = spike dilution correction
C = sample background concentration
s = concentration equivalent of analyte added to sample

Note! Because the added spike volume was less than 1% of the total LFM sample volume (0.4 % and 0.12 % of total LFM sample volume), the factor f could be excluded.

Criteria for LFM % recovery: 85 - 115 %, when c is > 2 x MRL and
80 - 120 %, when c is ≤ 2 x MRL

Criteria for LFM % RSD: ±15%

Table 7. Laboratory Fortified Matrix (LFM) sample results for DW o-PO4 application.

Sample	Result avg. n = 3 (mg/L)	Spike. mg/L	% Recovery	% RSD. n =3	Acceptable
TW-02	0.0021				
TW-02 +0.1 mg/l PO4P	0.0946	0.10	92 %	0.7 %	**YES**
TW-02 +0.4 mg/l PO4P	0.3906	0.40	97 %	0.1 %	**YES**
TW-03	0.0026				
TW-03 +0.1 mg/l PO4P	0.0944	0.10	92 %	0.3 %	**YES**
TW-03 +0.4 mg/l PO4P	0.3943	0.40	98 %	0.2 %	**YES**
TW-04	0.0028				
TW-04 +0.1mg/l PO4P	0.0989	0.10	96 %	0.7 %	**YES**
TW-04 +0.4mg/l PO4P	0.3754	0.40	93 %	0.3 %	**YES**
Average =			**95 %**	**0.4 %**	**YES**

17.2.7 Continues Calibration Check (CCC)

Continues Calibration Check (CCC) solution was analyzed in intervals of every 10th sample, at the beginning and end of run. CCC sample concentration was from the mid calibration area – 0.25 mg/L. CCC sample result summary is in table 10.

Criteria for CCC % recovery: 90 – 110 %

Table 8. PO4P CCC result summary. Sample: PO4P CCC, concentration 0.25 mg/L.

	Analyzer 1	Analyzer 2
Average:	0.254	0.256
% Recovery	102 %	102 %
n =	20	6
std. deviation (SD) =	0.0033	0.0048
% RSD=	1.29 %	1.87 %
Minimum result:	0.249	0.252
Maximum result:	0.261	0.261
Acceptable	**YES**	**YES**

17.3 The validation study results showed the Drinking Water Orthophosphate method for Thermo Scientific Gallery discrete analyzer is equally effective in meeting the QC acceptance criteria given in the reference method.

Table 9. Performance of the Drinking Water Orthophosphate method for Thermo Scientific Gallery discrete analyzer compared to reference method acceptance criteria.

QC acceptance criteria for SM 4500-P E	**Performance of DW o-PO4P for Gallery analyzer**
Minimum detectable concentration: 10 µg P/l	MDL calculated: 0.00036 mg P/L MRL: 0.0125 mg P/L
Recovery IPR: NA (laboratory specific) % Recovery CCC: 90 – 110 % Recovery LFB: Mean ± 3 x SD (n ≥ 20) Recovery LFM: NA (laboratory specific)	% Recovery IPR: 102 – 103% % Recovery CCC: 100 – 105% % Recovery LFB: 102 – 103% % Recovery LFM: 92 – 98 %
% RSD IPR: NA (laboratory specific) % RSD LFB: NA (laboratory specific) % RSD LFM: NA (laboratory specific)	% RSD IPR: 0.1 – 1.0 % % RSD LFB: 0.2 – 1.1 % % RSD LFM: 0.1 – 0.7 %
Method Blank: ≤ ½ x MRL	Method Blank (LRB): ≤ 1/3 x MRL

APPENDIX A.

Test flow for DW o-PO4P application in the Gallery analyzer.

Thermo SCIENTIFIC

Test parameters

DW o-PO4P Version number 1.1

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Gallery™ application

Thermo Fisher Scientific Oy

Prior to change

Date 15.2.2016 **User** Test designer

Time 14:02:15 **Software version: 5.3**

Info

Tag	INT001
Last time changed	15.2.2016 14:01
User name	Test designer
Full name	DW o-Phosphate < 0.5 mg/l
In use	No
Type	Photometric
Online name	
Acceptance	Manual
Result unit	mg/L as P
Number of decimals	3
Correction factor	1
Correction bias	0
Sample type	

Barcode 1 **Barcode 2**

Flow

Blank type Yes Primary dilution 1 + 0 Dispensed volume 140

ThermoFisher SCIENTIFIC

Thermo SCIENTIFIC

Test parameters

DW o-PO4P Version number 1.1

Gallery™ application
Thermo Fisher Scientific Oy
Prior to change

Date 15.2.2016 **User** Test designer
Time 14:02:15 **Software version: 5.3**

Sample	Volume (µl)	Dispense with	Extra volume (µl)	Extra wash
	120	Extra	60	No

Incubate	Time (sec)	Actual time (sec)
	18	18

End-point blank	Blank resp. min.(A)	Blank resp. max.(A)
	*	*

Reagent	Reagent	Volume (µl)	Dispense with	Extra volume (µl)	Syringe speed	Replacing reagent
	Phosphate R1	14	Extra	20	Normal	None
	Barcode ID	Alarm limit (ml)	Onboard stability (days)			
	A04	2,0	5			

Incubate	Time (sec)	Actual time (sec)
	120	117

Reagent	Reagent	Volume (µl)	Dispense with	Extra volume (µl)	Syringe speed	Replacing reagent
	Phosphate R2	6	Extra	20	Normal	None
	Barcode ID	Alarm limit (ml)	Onboard stability (days)			
	A05	2,0	5			

Incubate	Time (sec)	Actual time (sec)
	540	540

End-point measurement	Main wavelength (nm)	Side wavelength (nm)	Residual net abs. (A)
	880	None	0

Dilution

Dilution with	Water
Primary dilution 1 +	0

Limits

	Measuring range (mg/L a		Next dilution ratio (1+)	
	Min	Max	Low	High
Primary dilution	*	0,5000	*	9,0
2nd dilution	*	*	*	*
3rd dilution	*	*	*	*
4th dilution	*	*	*	*

Test limit	*	5,0000	mg/L as
Critical limit	*	*	mg/L as
Init. abs.	*	2,5	A

Thermo SCIENTIFIC

Test parameters

DW o-PO4P Version number 1.1

Gallery™ application
Thermo Fisher Scientific Oy
Prior to change

Date 15.2.2016 **User** Test designer
Time 14:02:15 **Software version: 5.3**

Calibration

Calibration type	2nd order
Repeat time (days)	1
Points/calibrator	Duplicate
Acceptance	Manual

Abs. error (A)	*
Rel. error (%)	*
Factor limit min.	*
Factor limit max.	*

Nbr	Calibrator	Current lot	Concentration	Dilution 1 +
1	PO4P-0	Default	0,0000	0
2	PO4P-cal	Default	1,5000	119
3	PO4P-cal	Default	1,5000	49
4	PO4P-cal	Default	1,5000	29
5	PO4P-cal	Default	1,5000	14
6	PO4P-cal	Default	1,5000	7
7	PO4P-cal	Default	1,5000	5
8	PO4P-cal	Default	1,5000	3
9	PO4P-cal	Default	1,5000	2

Coeff. of det. min. 0,995

QC

Procedure	Ongoing QC	QC profile	PO4P
Interval type	Requests	In use	Yes
Requests	10	Acceptance	Manual
Time (hh:mm)	0:00	Trigger	Manual,Interval,Reagent lot change,Reagent vial change

Procedure	QCS	QC profile	PO4P QCS
Interval type		In use	Yes
Requests		Acceptance	Manual
Time (hh:mm)	0:00	Trigger	Manual,Calibration

Procedure	Control	Current Lot	Conc.	SD	Req. count
Ongoing QC	LRB	Default	0,000	0,0021	1
Ongoing QC	PO4P-CCC	Default	0,250	0,0125	1
Ongoing QC	PO4P-LFB 0.1	Default	0,100	0,0075	1
Ongoing QC	PO4P-LFB 0.4	Default	0,400	0,0300	1
QCS	PO4P-QCS 0.1	Default	0,100	0,0075	1
QCS	PO4P-QCS 0.4	Default	0,400	0,0300	1

Procedure	Nbr of controls	SD multiplier
Ongoing QC	1	2
QCS	1	2