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Safe Drinking Water Act Nitrite Method Feasibility Tests for the Gallery Analyzer

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Abstract

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In this thesis feasibility testing was conducted for nitrite determination by a Thermo Fisher Scientific™ Gallery™ Aqua Master discrete analyzer. These tests consisted of establishing a detection limit, reporting limit as well as initial precision and recovery for the method. In addition, a laboratory fortified matrix study was done with different matrices that are known to interfere with nitrite analysis. All the tests were conducted for the analyzer as well as a reference method, which was a manual turbidimetric photometric measurement.

The thesis was done for Thermo Fisher Scientific, as a part of a project aiming to get nitrite detection with Gallery approved as an official method for Environmental Protection Agency's Safe Drinking Water Act. The experimental studies presented in this thesis were done to ensure that the created application is suitable for drinking water samples as well as to gather data for the alternative test procedure program, which is a procedure for evaluation and approval of new methods.

Every result was within the set specifications. For instance, the limit of detection was 0.33 µg N/L for Gallery and 1.5 µg N/L for the reference method. The reporting limit was 2.5 µg N/L for Gallery and 5 µg N/L for the reference method. The recovery percentages from the laboratory fortified matrix study were accurate, between 93–104 %, which indicates the great tolerance of different nitrite interferences with Gallery. The relative percentage difference between duplicate analyses was between 0.9–2.7 % with Gallery. The results obtained from the tests verified that the application is suitable for determining nitrite from drinking water samples.

Keywords: nitrite, Griess reaction, spectrophotometry, Gallery™ Aqua Master, method detection limit, method reporting limit, laboratory fortified matrix, discrete analyzer, nitrification, denitrification, drinking water

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Tiivistelmä

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Tässä opinnäytetyössä tehtiin toimivuustestaukset nitriitin analysoimismenetelmälle Thermo Scientific™ Gallery™ Aqua Master diskreettianalysaattorille. Toimivuustestauksiin sisältyi toteamisrajan, määritysrajan ja lähtötarkkuuden sekä oikeellisuuden määrittäminen. Lisäksi tehtiin näytematriisitutkimus erilaisilla tunnetusti nitriitin analysointia häiritsevillä yhdisteillä. Kaikki testaukset tehtiin analysaattorin lisäksi referenssimenetelmällä, joka oli manuaalinen turbidimetrinen fotometrinen mittaus.

Tämä opinäytetyö tehtiin Thermo Fisher Scientificille, osana projektia, jonka tavoitteena on saada nitriitin määrittäminen Gallerylla Yhdysvaltain ympäristösuojeluviraston Safe Drinking Water Act -asetuksen viralliseksi hyväksytyksi analyysimenetelmäksi. Toimivuustestaukset tehtiin, jotta varmistutaan menetelmän soveltumisesta myös juomavesinäytteille. Lisäksi kerättiin dataa vaihtoehtoisten testimenetelmien ohjelmaan, jota käytetään uusien menetelmien arviointiin ja hyväksyntään.

Kaikki saadut tulokset olivat asetetuissa spesifikaatioissa. Esimerkiksi toteamisraja oli 0,33 µg N/l Gallerylla ja 1,5 µg N/l referenssimenetelmällä. Määritysraja oli 2,5 µg N/l Gallerylla ja 5 µg N/l referenssimenetelmällä. Nitriitin saantoprosentit näytematriisitutkimuksessa olivat oikeellisia, 93–104 %, mikä kertoo menetelmän hyvästä sietokyvystä nitriitin analysoinnin häiriötekijöille. Suhteellinen prosentuaalinen ero rinnakkaismääritysten välillä oli 0,9–2,7 %. Kokeellisesta osuudesta saadut tulokset varmistivat, että menetelmä soveltuu nitriitin analysoimiseen juomavesinäytteistä.

Avainsanat: nitriitti, Griessin reaktio, spektrofotometria, Gallery™ Aqua Master, toteamisraja, määritysraja, näytematriisitestaukset, diskreettianalysaattori, nitrifikaatio, denitrifikaatio, juomavesi

Contents

List of Abbreviations

ATP	Alternative Test Procedure
CCV	Continuous Calibration Verification
CoD	Coefficient of Determination
CWA	Clean Water Act
DA	Discrete Analyzer
ECM	Electrochemical Measurement
EPA	Environmental Protection Agency
IPR	Initial Precision and Recovery
LFB	Laboratory Fortified Blank
LFM	Laboratory Fortified Matrix
LRB	Laboratory Reagent Blank
MDL	Method Detection Limit
MRL	Method Reporting Limit
NED	N-(1-naphtyl)-Ethylenediamine Dihydrochloride
NPDWR	National Primary Drinking Water Regulations
NSDWR	National Secondary Drinking Water Regulations
QCS	Quality Control Sample
R&D	Research and Development
SDWA	Safe Drinking Water Act

1 Introduction

The subject of this thesis is Safe Drinking Water Act Nitrite Method Feasibility Tests for the Gallery Analyzer. The thesis was done in collaboration with Thermo Fisher Scientifics research and development, R&D. The experiments were conducted as a part of the R&Ds project for verification of a nitrite determination method with the Gallery analyzer focusing on drinking water sample matrices. The experiments were planned by the R&D team with Marita Jokinen as the project manager. The experiments consist of, for example, comparing methods, establishing method detection and reporting limits as well as performing a matrix interference analysis. [1, 2].

The aim of this study was to verify a suitable application for nitrite determination for the Thermo Fisher Scientific™ Gallery™ Aqua Master discrete analyzer that meets the requirements set by the Safe Drinking Water Act (SDWA). The verification of the application was achieved by performing feasibility testing for a SDWA approved reference method as well as the Gallery analyzer. In this project, a manual turbidimetric method performed by a spectrophotometer was selected as the reference method.

In addition to the experimental study, a theoretical introduction about nitrite determination with the Gallery analyzer will be created as a part of this thesis. The introduction will be published as a part of a commercial scientific document, which will be published besides the new application when the whole verification and validation process is finished.

Artificial intelligence, in the form of ChatGPT (OpenAI, GPT-5 model, 2025) and Thermo Fisher Scientific's internal application, GeneAI (OpenAI, GPT-4 model, 2023), has been used as a supportive linguistic and structural tool while creating this thesis. All factual content has been confirmed through independent sources and critical media literacy principles were utilized.

2 Theory

2.1 Nitrite

Nitrite, NO_2^- is a negatively charged ion containing one nitrogen and two oxygens. It is an inorganic molecule that appears as transparent crystals or colorless aqueous solutions. [3].

Occurrence

Nitrite occurs in nature as a part of the nitrogen cycle. Nitrogen cycle refers to a process where nitrogen is transferred between the atmosphere, biosphere, hydrosphere and geosphere. [4]. This is very important regarding life on Earth, because nitrogen is not only an important nutrient for plants and microbes, but also a crucial component of amino acids [5].

In addition to the nitrogen cycle, nitrite can also be produced by *Nitrosomonas* bacteria in a process called nitrification [6]. Nitrification is the oxidation of ammonia to nitrite and subsequently to nitrate as presented in Equation 1. Nitrification requires oxygen, making it an aerobic process. The cumulation of nitrite via nitrification requires that the reaction is incomplete. [7].

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \quad (1)$$

Besides nitrification, nitrite can also be formed in a denitrification process, which is the reverse reaction of nitrification. Denitrification is a redox reaction, where nitrate is firstly reduced to nitrite following reduction to nitrous oxide and finally nitrogen gas. The reaction is presented in Equation 2. This reaction occurs especially in environments with low oxygen levels or totally anaerobically. [7].

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2 \quad (2)$$

The amounts of nitrate and nitrite found in ground and surface water vary due to different oxygen levels of the water as well as the stability of the compounds. Groundwater has typically lower levels of dissolved oxygen, which promotes denitrification. Conversely the higher amounts of oxygen dissolved in surface water forms a more suitable environment for nitrification. Groundwater is water collected from aquifers, which are bodies of rock, sand, gravel or sediment containing water. [8]. Bodies of water that are located above the ground are considered surface water. Typical sources include, for instance, lakes, rivers, streams, reservoirs and the ocean [9].

In terms of stability, nitrate is a highly stable compound, which means that it is relatively resistant to chemical reactions. On the contrary nitrite is rather unstable, which makes it prone to reacting with other elements and compounds, especially oxidizing to nitrate. This stability can be explained by the oxidation states of nitrite (+3) and nitrate (+5). Nitrate having a greater oxidation state than nitrite makes it more stable in oxidizing conditions. On the contrary, nitrite is more stable in reducing conditions, due to its lower oxidation state. [3].

As indicated by these presented observations, nitrite is more commonly found in groundwater rather than surface water. This is due to the reducing nature of groundwater as a low oxygen environment, which favors nitrate to nitrite reduction. Nitrite can be found in both surface and ground water.

Sources

Nitrite rarely appears by itself in the environment, instead it is formed from nitrate under different conditions. Common nitrate sources are runoff from nitrate containing fertilizer, wastewater disposal, and oxidization of waste containing nitrite or ammonia. In addition to being found in drinking water, nitrate is also present in many vegetables and meat products. The nitrate is converted to nitrite, which is the main source of dietary exposure to nitrite. Additional primary sources of nitrite include preserved meat products and leafy vegetables.

Water environments that have galvanized steel pipes, low oxygen levels or even completely anoxic water, stagnant water in distribution systems as well as water treatment plants that perform disinfection by chloramination are more prone to the formation of nitrite [6]. Chloramines are disinfectants, containing ammonia and chlorine, which are used to treat drinking water with the intent to protect water quality [10]. This might promote the growth of nitrifying bacteria, as there may be ammonia released from sources such as decaying chloramine, corrosion, reactions of the pipe surface and oxidation of natural organic matter [11].

Impact on Health

The most known health risks associated with nitrite are methemoglobinemia and thyroid effects. Some studies associate reproductive and developmental toxicity, as well as carcinogenicity with nitrite exposure, but this has not been yet verified by credential sources. Young children and pregnant women are at higher risk from nitrite exposure. [12, 13].

Absorbed nitrite can form methaemoglobin by oxidizing haemoglobin. Methaemoglobin has a reduced ability for red blood cells to bind to and transport oxygen within the body. This can cause methemoglobinemia, which is particularly dangerous to infants. The condition can appear as blue discoloration of the skin due to reduced oxygen levels in the tissue [14].

In addition to methaemoglobin, nitrite can contribute to the formation of nitrosamines, some of which are known carcinogenic compounds [15]. Thyroid effects caused by nitrite are linked to the interference with iodine uptake, which can lower the thyroid hormone production [13].

Common Analysis Methods and Interferences

EPA’s approved methods for nitrite determination from drinking water are ion chromatography, automated or manual cadmium reduction, spectrophotometry and capillary ion electrophoresis [16].

Common interference sources in nitrite determination from water samples are high turbidity, high levels of dissolved organic matter, high salinity, ionic strength and metals such as copper, lead or iron. These interactions may cause false results, either lower or greater than the actual nitrite levels. For example, copper may affect the reaction by catalyzing the decomposition of the diazonium salt, which leads to lower levels of nitrite detected. [17].

The unstable nature of nitrite in aqueous solutions proposes additional complications to analysis from water samples. This is usually avoided by preparing fresh nitrite solutions daily and possibly fixing samples with an acidic preservative.

2.2 Thermo Scientific™ Gallery™ Aqua Master Discrete Analyzer

Thermo Scientific™ Gallery™ and Thermo Scientific™ Gallery™ Aqua Master are automated discrete analyzers that offer a wide range of applications for industrial and environmental analysis. The exterior of the analyzer is presented in Figure 1. The analyzers' operational principle is photometric, with a xenon lamp as the light source and a filters ranging from 340 to 880 nm. The absorbance ranges from 0 to 3.5, with a resolution of 0.001 A. There can be an additional electrochemical, ECM unit installed, which allows pH, ranging from 2 to 12 and conductivity, ranging from 20 µS/cm to 112 mS/cm to be measured. [18].

Colorimetric, turbidimetric and bichromatic reactions can be measured with Gallery analyzers. These reactions take place in disposable Thermo Scientific™ DECACELL™ cuvettes, which come in 36 rows each containing 10 cuvettes. These cuvettes can be inserted into the analyzer by opening the right-side cover and sliding the cuvette strip into its place (Figure 2, Position 5). [18].

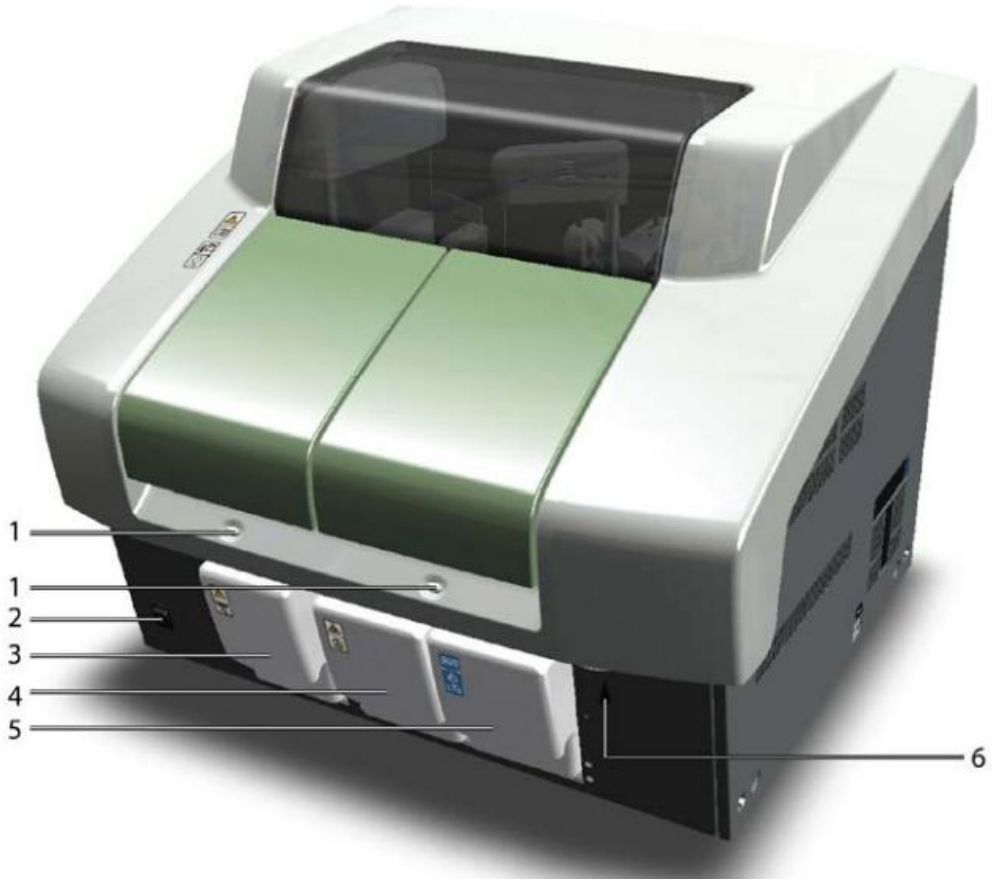

Figure 1. Thermo Scientific™ Gallery™ discrete analyzer exterior with marked positions that correspond to the following 1. operation buttons, 2. main switch, 3. cuvette waste bin, 4. waste container, 5. water container, and 6. lock handle under the main cover [19].

The analyzers use Thermo Scientific™ system reagents, which can be inserted in a reagent rack into the instrument from the left-side cover (Figure 2, Position 6). Samples can be inserted into the instrument in the same way as the reagents, in a sample rack that holds 0.5 mL, 2.0 mL or 4.0 mL sample cups. The reagent bottles have barcodes that the instrument scans internally and by which recognizes what reagent has been inserted. The interior of the instrument is presented in Figure 2.

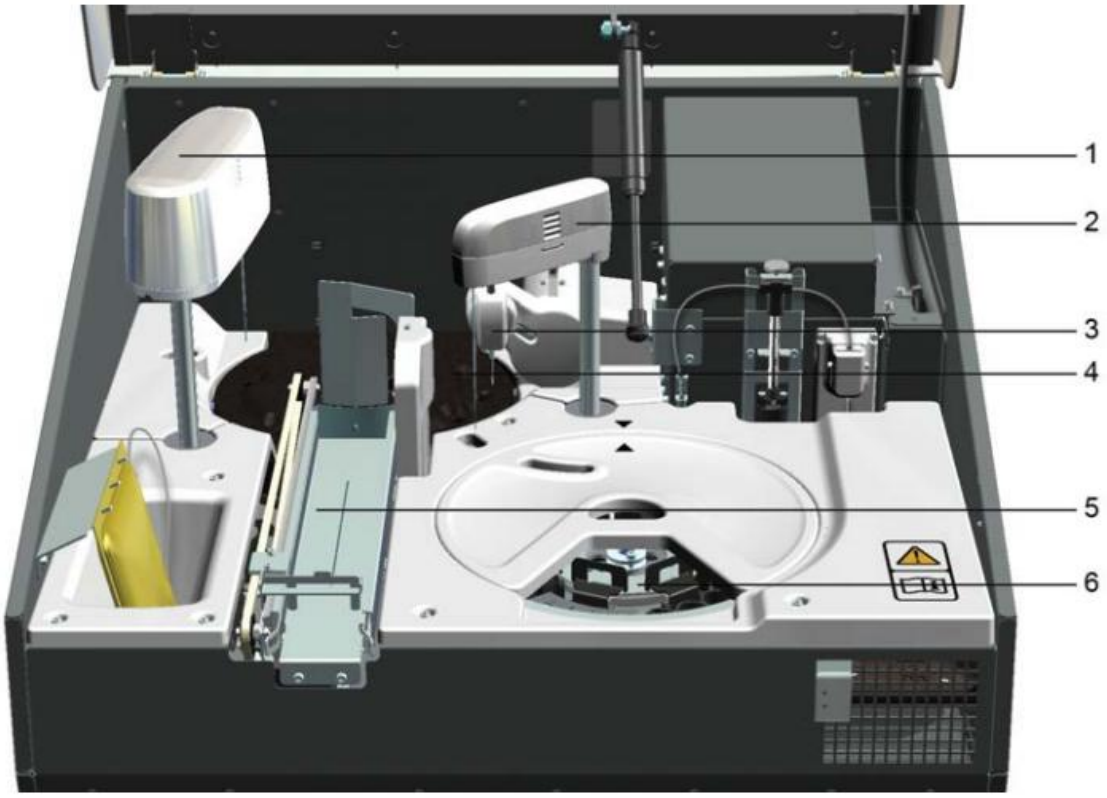

Figure 2. Thermo Scientific™ Gallery™ discrete analyzer interior, with marked positions that correspond to the following 1. ECM unit (if applicable), 2. dispenser, 3. mixer, 4. incubator, 5. cuvette loader, 6. sample and reagent racks [19].

The Thermo Scientific™ Gallery™ Aqua Master discrete analyzer can be used to determine analytes e.g. alkalinity, chloride, nitrite, sulfate and total hardness from different sample matrices e.g. drinking water, wastewater and soil samples. The advantages of conducting environmental analysis by Gallery-analyzer rather than traditional methods are rapid analysis-time, uncomplicated process, reliability of results and low waste amounts. It also allows time for other tasks, because constant supervision is not required. [20].

2.3 Griess Reaction

Both methods utilized in this study are established from the Griess reaction. The reaction was first introduced by a German chemist Johann Peter Griess in 1858. The reaction is divided into two steps, diazotization followed by a coupling reaction. In the first step, diazotization, nitrite and sulfanilamide react together in

an acidic environment. First, the nitrite-ions form nitrous acid in the acidic environment, which then reacts with the aromatic amine forming a diazonium salt. This reaction is presented in Figure 4. [21, 22]

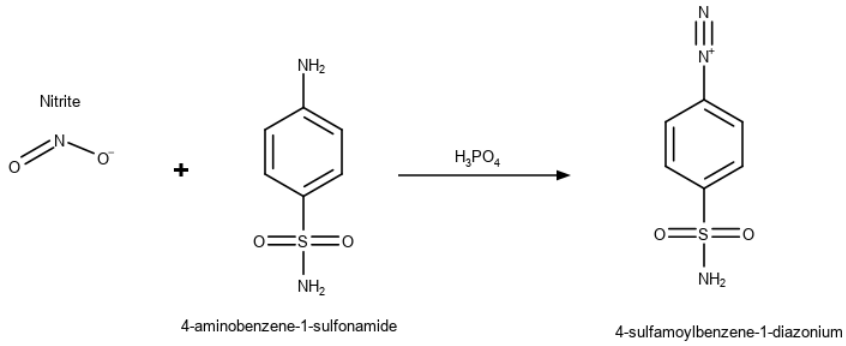

Figure 4. First step of the Griess reaction

After the diazonium salt has formed, a coupling reaction between the salt and N-(1-naphtyl)-ethylenediamine dihydrochloride, NED, occurs, which forms the azo dye. This is presented in Figure 5. The azo dye is responsible for the pink color change of the sample solution. The intensity of the color developed as the reaction proceeds is directly proportional to the amount of nitrite present in the sample.

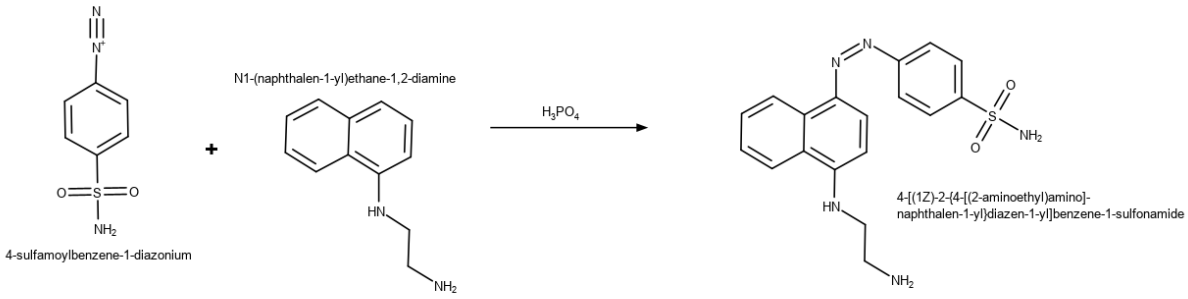

Figure 5. Second step of the Griess reaction.

The experimental study utilizes two methods, an automated and a manual turbidimetric method based on photometric measurements. The operational principle of the manual method is presented in Figure 6. The method is derived from the Standard Methods for the Examination of Water and Wastewater published by the American Public Health Association, American Water Works Association and Water Environment Federation [23].

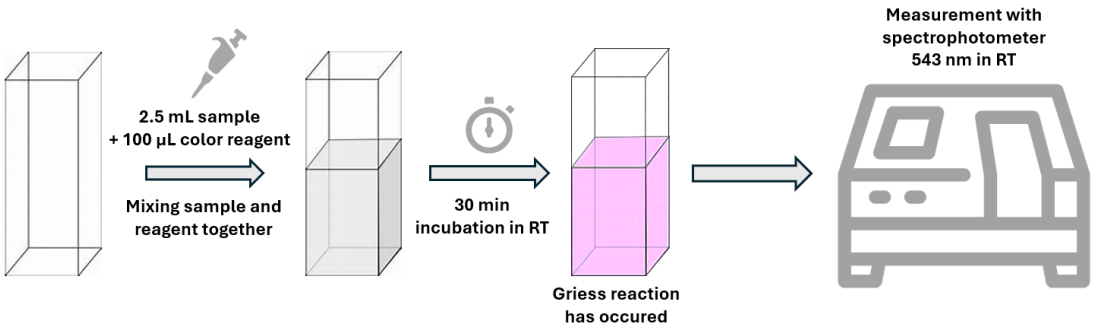

Figure 6. Principle of the manual method.

The manual method is used as an approved reference method in comparison to the fully automated analysis conducted by the Gallery Aqua Master discrete analyzer. The operational principle of the automated method is presented in Figure 7.

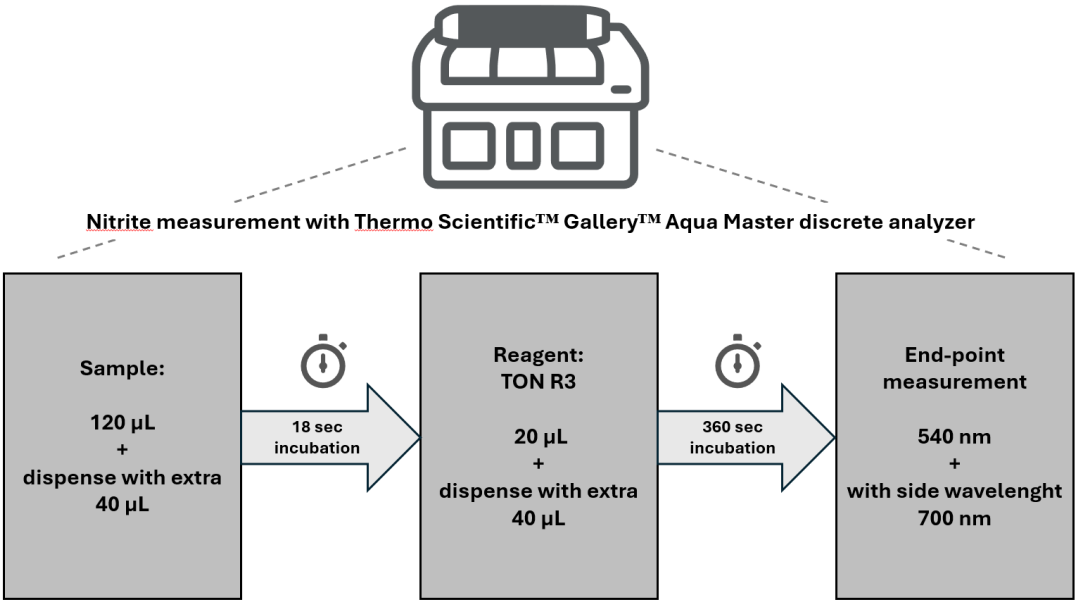

Figure 7. Principle of the automated method.

2.4 Safe Drinking Water Act

Environmental Protection Agency (EPA) is a federal agency of the United States that is in charge of enforcing and implementing federal laws that aim to protect the environment and human health, established by the Congress of the U.S.

EPA sets regulations and national standards according to these environmental laws written by the Congress. [24].

The Safe Drinking Water Act (The SDWA) is the main federal law regarding the quality of drinking water in the United States. SDWA was first approved in 1974 by the Congress and amended in 1986 and 1996 [25]. In addition to SDWA there is the Clean Water Act (CWA) regarding the quality of wastewater and ambient water. This law was first enacted in 1948 with a different title and expanded in 1972. [26].

SDWA is divided into two regulatory categories, National Primary Drinking Water Regulations (NPDWR) and National Secondary Drinking Water Regulations, (NSDWR). The NPDWR involves the controlling of contaminants that might pose a health hazard to the public, while the NSDWR addresses contaminants that are more of an aesthetic issue, e.g. odor, color or taste. [25].

The NPDWR concerns water pollutants such as fluoride, cadmium, nitrate, nitrite and arsenic. According to 40 CFR 141.62 the maximum contaminant level for nitrite is 1 mg/L as nitrogen. These regulations are enforced nationwide and do not allow for modifications. [27]. Conversely the NSDWR concerns water pollutants such as chloride, coppers, iron, manganese and sulfate (40 CFR 143.3) [28]. These regulations are merely guidelines, and each state can determine how they apply them.

2.5 Method Validation Process

With the performance of these feasibility studies, the Alternative Test Procedure (ATP) process is started. ATP refers to the process administered by the EPA of the U.S from which new methods can be approved as an official method for this area of compliance measurements. With these analyses, demonstration data is collected, an ATP application is formed and then provisioned to the EPA. After being accepted as a candidate for ATP method, method validation as well as

review and legislative approval of the method are done under the guidance of EPA. [29, 30].

3 Methods and Materials

This chapter summarizes the most important information of the materials, documents and samples utilized in the experimental study of this thesis.

Standard material and chemicals

- 85 % Phosphoric acid, Thermo Fisher Scientific (assessed purity 85.3 %), ref. 424045000
- N-(1-naphtyl)-ethylenediamine dihydrochloride, Thermo Fisher Scientific (assessed purity 99.3 %), ref. 423990250
- Potassium Phthalate Monobasic, Sigma Aldrich (assessed purity 100 %), ref. 60360
- Sulfanilamide, Sigma Aldrich (assessed purity 99.4 %), ref. S9251
- Calcium Carbonate, Sigma Aldrich (assessed purity 99.4 %), ref. 79544
- Sodium Carbonate, Sigma Aldrich (assessed purity 99.8 %) ref. S12127
- Copper(II)sulphate, Thermo Fisher Scientific, ref. 19771100
- Nitrite (as N) 1000 mg/L, Thermo Fisher Scientific, ref. 984723
- Nitrite (as NO2) 1000 mg/L / 304,5 mg/L (as N), Thermo Fisher Scientific, ref. 984722
- 37 % Hydrochloric acid, Sigma Alrich (assessed purity 37.8 %), ref. 218148

Gallery system reagents, Thermo Fisher Scientific

- TON R3, ref. 984371
- Total Hardness R1, ref. 984620
- Total Hardness R2, ref. 984621
- Total Hardness R3, ref. 984622
- Alkalinity R1, ref. 984623
- Alkalinity R2, ref. 984624

- Washing solution 4,5 %, ref. 980929 (used for daily maintenance of the analyzer).

Equipment

- Gallery™ Aqua Master discrete analyzer, Thermo Fisher Scientific software 8.3.0.0
- Multiskan GO 1510 spectrophotometer, Thermo Fisher Scientific, ref. 51119300

Application notes and methods

- CWA Nitrite Low application, D21647, version 1.1 [31]
- CWA Nitrite High application D21648, version 1.1 [32]
- Total Hardness application, D11945, version 2.0 [33]
- Alkalinity application, D11703, version 1.0 [34]
- Standard reference method (543 nm, in RT) [23].

Sample Origins

For the feasibility studies, considering the sources of drinking water for each sample was essential. This is because EPA has established certain requirements for the matrix analysis of the ATP process. The requirements are a finished drinking water sample drawn from a hard ground water source (> 250 mg/L as $CaCO_3$), a finished drinking water sample drawn from a surface water source containing elevated Total Organic Carbon (TOC) (≥2 mg/L), an artificial drinking water matrix high in ionic strength and an artificial drinking water matrix high in organic content [30].

For the samples from Finland, a website "*Vesihuoltolaitosten tunnusluvut*" created by the Finnish Environment Institute (*Fin.* SYKE) was utilized. From this website one could create a search based on the address, municipality or city. The results cover each possible drinking water supplier in the area as well as the sources for the water they supply. The foreign sample origins are estimated from

information provided by local drinking water suppliers. This information from all the samples used in this project is summarized in Table 1. [35].

Table 1. Sampling locations and information about drinking water formation.

Sample	Sampling location	Drinking water sources based on sampling location
25DW01	Rajamäki, Finland	Ground and surface water
25DW02	Lappeenranta, Finland	Ground, surface and artificial ground water
25DW03	Ruka, Finland	Ground water
25DW04	Pfaffhausen, Switzerland	Surface water [1]
25DW05	Florida, the United States	Ground water [2]
25DW06	Bryssels, Belgium	Ground and surface water [3]
25DW07	Vantaa, Finland	Ground and surface water

[1] Drinking water in the district of Pfaffhausen is supplied with approximately 95 % lake water from Lake Zurich and 3.5 % with its own spring water [36, 37].

[2] Based on Lake Worth Beach Water Quality Report of 2024, the drinking water originates from the East Coast Surficial Aquifer and Biscayne Aquifer [38, 39].

[3] VIVAQUA supplies the drinking water in Bryssels, using both groundwater and surface water. Approximately 70 % of the drinking water is formed from underground sources. [40].

4 Experimental Study

The following experimental studies were conducted to ensure that the application for nitrite determination made for CWA can be utilized for drinking water sample matrices. In addition to the results of these studies, some data from the previous studies or the CWA nitrite project are presented in this thesis. For example, the Method Detection Limit (MDL) and the Method Reporting Limit (MRL) are already previously established for Gallery.

4.1 Preparing Solutions

Here is a detailed explanation of the preparation process of the most crucial solutions used in the feasibility testing studies.

The color reagent used for the manual method was prepared according to the utilized standard method [23]. In brief, 10 mL of 85 % phosphoric acid, 1.0 g of sulfanilamide and 0.10 g of N-(1-naphtyl)-ethylenediamine dihydrochloride was added to a 100 mL volumetric flask with approximately 80 mL of deionized water. The analytes were dissolved completely, then the flask was filled to the mark and mixed thoroughly. The complete solution was transferred to a 100 mL dark Pyrex bottle and stored in +2-8°C.

For the Laboratory Fortified Matrix (LFM) study there were three solutions prepared, one to elevate the total hardness, one for elevated total organic carbon, TOC and one for elevated alkalinity. For the elevated total hardness sample an 8000 mg/L $CaCO_3$ solution was prepared by adding 0.40 g of calcium carbonate to a 50 mL volumetric flask filled with ¼ of deionized water. 10 mL of 1 M HCl was added to ensure complete dissolution and when fully dissolved the flask was filled to the mark and mixed thoroughly.

For the elevated TOC sample, a 1000 mg/L TOC-stock was prepared by adding 0.10 g of potassium phthalate monobasic to a 100 mL volumetric flask filled with ¼ of deionized water. When completely dissolved, the flask was filled to the mark and mixed thoroughly. After this, the pH was adjusted to < 2 with small increments of 85 % phosphoric acid.

For the elevated alkalinity sample, a 10 g/L (as $CaCO_3$) solution was prepared by adding 1.1 g of sodium carbonate to a 100 mL volumetric flask filled with ¼ of deionized water. When fully dissolved, the flask was filled to the mark and mixed thoroughly.

For the copper interference study a 300 mg/L copper-stock was prepared by adding 0.40 g of copper(II)sulfate to a 50 mL volumetric flask filled with ¼ of deionized water. This was dissolved completely, the flask filled to the mark and mixed thoroughly.

4.2 Calibration and Continuous Calibration Verification Solutions

All calibration solutions and continuous calibration verification (CCV) samples for both methods were made from a 5 mg N/L diluted solution, which was prepared from the 1000 mg N/L standard solution (ref. 984723).

For the calibrator of the low range, L-Cal (300 µg N/L) is analyzed and diluted automatically by the analyzer as follows: (1)+0, +2, +3, +5, +14, +29, +59 and +119. The calibrator of the high range, H-Cal (2000 µg N/L), is analyzed and diluted automatically by the analyzer as follows: (1)+3, +4, +6, +9, +19 and +39. For the CCV samples L CCV LL (2.5 µg N/L), L CCV M (50 µg N/L) and H CCV M (250 µg N/L) were prepared and analyzed according to the application (e.g. start or end of the run, between every 20 samples). The preparation of these solutions for Gallery is presented in Figure 8.

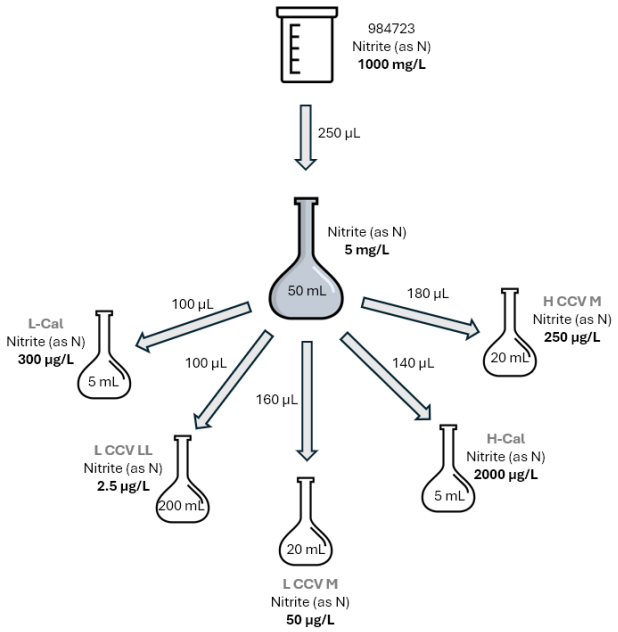

Figure 8. Gallery's Calibration and CCV solution preparation.

For the reference method performed on the manual spectrophotometer Multiskan GO, later referred to as the manual method, five calibration solutions from 5 µg N/L to 180 µg N/L were prepared. For the CCV samples, two levels, CCV LL (5 µg/L) and CCV MID (90 µg/L) were made. The detailed preparation of these solutions for the manual method is presented in Figure 9.

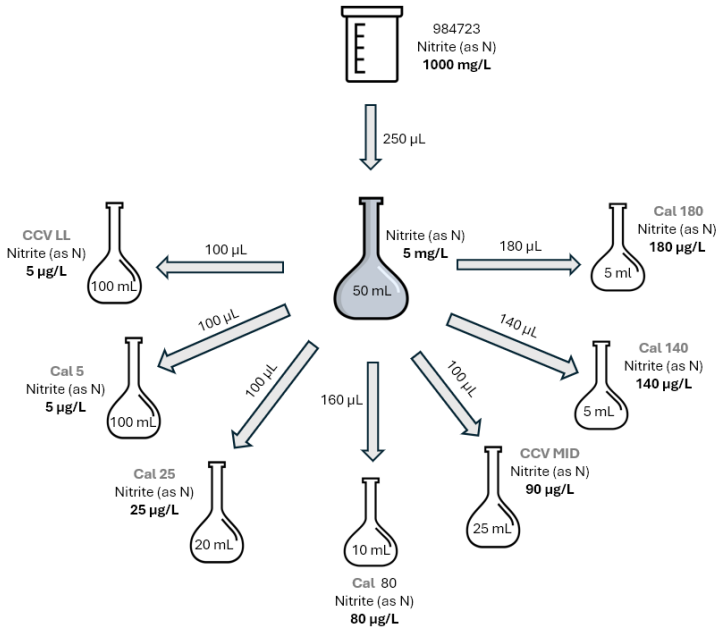

Figure 9. Manual method's calibration and CCV solution preparation.

The calibration criteria for the Coefficient of Determination (CoD), is greater than or equal to 0.995 for linear adjustment and 0.990 for second order adjustment. For the recovery results of back-calculated calibration levels and CCV samples, limits are as followed; ±10 % when the concentration is five times greater than MRL, ±20 % when the concentration is two to five times the MRL and ±50 % when concentration is less than or equal to two times the MRL. The recovery percentages are calculated according to Equation 3 [41].

$$Recovery\ \% = \frac{C_s}{C} \cdot 100, \tag{3}$$

where C_s is the measured concentration of the sample
C is the theoretical concentration of the sample

This equation is used to calculate all other recovery percentages in this feasibility study except for the samples that have been spiked with a standard material.

4.3 Quality Control and Laboratory Fortified Blank Samples

For Quality Control Samples (QCS) and Laboratory Fortified Blank (LFB) samples were prepared from a 2.5 mg N/L diluted solution, which was made from the 304.5 mg N/L nitrite standard solution. For the low range of nitrite detection, QCS 20 µg N/L, QCS 80 µg N/L, LFB 20 µg N/L, and LFB 80 µg N/L samples were made. For the high range QCS 200 µg N/L, QCS 400 µg N/L, LFB 200 µg N/L, and LFB 400 µg N/L samples were made. The detailed preparation of these solutions for the manual method is presented in Figure 10.

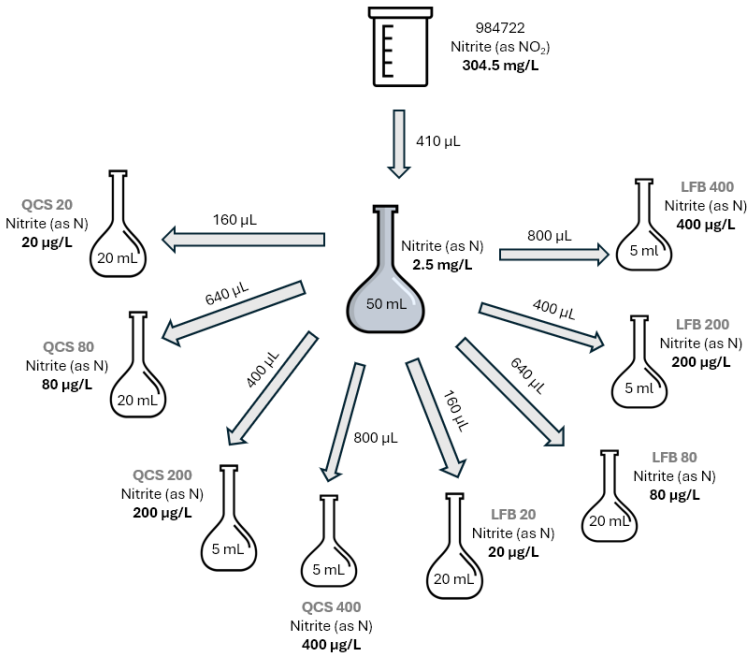

Figure 10. Gallery's QCS and LFB solution preparation.

For the manual method QCS 30 µg N/L, QCS 150 µg N/L, LFB 30 µg N/L and LFB 150 µg N/L samples were prepared. In addition, sample LFB 1200 µg N/L was prepared straight from the 304.5 mg N/L standard and diluted manually at a

ratio of 1+9 before the analysis. The detailed preparation of these solutions for the manual method is presented in Figure 11.

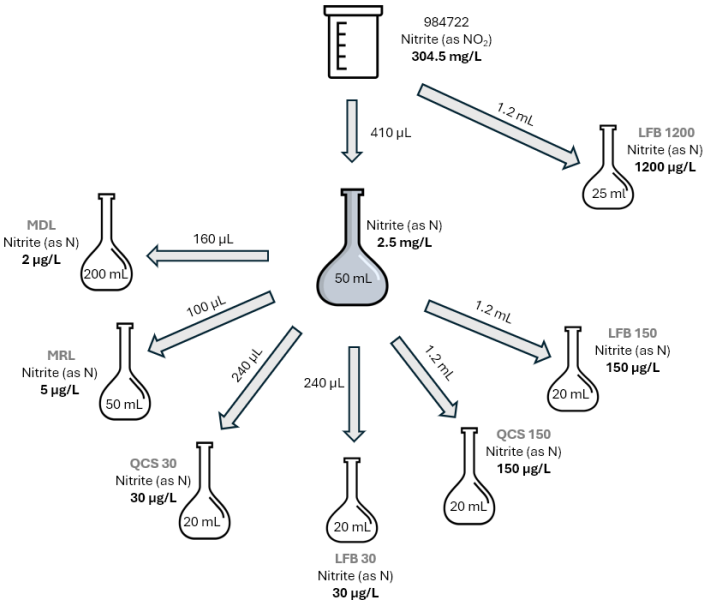

Figure 11. Manual method's QCS and LFB solution preparation.

In terms of QCS criteria, the recovery percents should be between 90–110 %. For the LFB samples, the recovery limits are ±15 % when the concentration is less than or equal to ten times the MRL and ±20 % when the concentration is greater than ten times the MRL. This assessment of the recovery percentages is for determining the accuracy of the method. For LFB samples, there is also relative standard deviation, RSD limits, which are ±10 % when the concentration is less than or equal to ten times the MRL and ±20 % when the concentration is greater than ten times the MRL. The RSD percentages are calculated according to Equation 4 [41].

$$RSD\ \% = \frac{100}{\bar{X}} \cdot \sqrt{\sum_{i=1}^{n} \frac{(X_1 - \bar{X})^2}{n-1}}, \tag{4}$$

where $\bar{X}$ is the mean of replicate measurements

X_1 is the measured value of the replicate

n is the number of replicates

This Equation is used to calculate all other RSD percentages in this feasibility study except for the samples that have been spiked with a standard material.

4.4 Initial Precision and Recovery Study

For the Initial Precision and Recovery (IPR) study 4 replicates of each LFB sample were analyzed. The samples for Gallery's low application were LFB 20 µg N/L, LFB 80 µg N/L and for the high application LFB 200 µg N/L, LFB 400 µg N/L and LFB 2000 µg N/L (automatically diluted at a ratio of 1+4). For the manual method the samples studied were LFB 30 µg N/L, 150 µg N/L and 1200 µg N/L (manually diluted at a ratio of 1+9). These samples were prepared according to the previously presented figures 10 and 11.

The IPR study was again evaluated in terms of accuracy and precision. For accuracy the limit for recovery is ±15 % when the concentration is less than or equal to ten times the and ±20 % when the concentration is greater than ten times the MRL. Precision, in contrast, is assessed through RSD, for which the limits are ±10 % when the concentration is less than or equal to ten times and ±20 % when the concentration is greater than ten times the MRL.

4.5 Method Detection Limit Study

For the Method Detection Limit (MDL) study, firstly 10 replicates of laboratory reagent blank (LRB) samples were analyzed. From those replicates an MDL estimate was calculated according to Equation 5 [41]. This estimate was then multiplied with three and rounded to an even number. The MDL sample was prepared to the concentration that was obtained from the estimate calculations.

$$MDL_{est} = 3 \times (SD_0), \quad (5)$$

where SD_0 is standard deviation of the replicate analyses (n = 10) of reagent water

Then seven LRB samples and seven samples with the calculated concentration were analyzed during a three-day period. The MDL sample value was calculated according to Equation 6 and the blank sample value according to Equation 7 [41].

$$MDL_{LFB} = t \cdot SD, \quad (6)$$

where t is 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]
SD is standard deviation of the replicate analyses

$$MDL_{LRB} = \bar{X} + t \cdot SD, \quad (7)$$

where $\bar{X}$ is the mean of replicate measurements
t is 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]
SD is standard deviation of the replicate analyses

From these two results, the MDL sample and MDL blank sample, the larger value between the options was selected for the final result. For the criteria, each replicate analyses recovery must be between 50–150 % and RSD less than or equal to 20 %.

4.6 Method Reporting Limit Study

For Method Reporting Limit (MRL) study seven replicate samples, with concentration equal to the lowest calibrator 5 µg N/L were analyzed at once. After the analysis, the half range for the prediction interval of results HR_{PIR} was calculated according to Equation 8 [41]. With the result for HR_{PIR}, the prediction interval of results, PIR limits were calculated according to equations 9 and 10 [41].

$$HR_{PIR} = 3.963 \cdot SD, \quad (8)$$

where 3.963 is a constant value for seven replicates
SD is standard deviation

$$PIR\ Upper\ Limit = \frac{\bar{X}+HR_{PIR}}{C_t} \cdot 100 \text{ and} \quad (9)$$

$$PIR\ Lower\ Limit = \frac{\bar{X}-HR_{PIR}}{C_t} \cdot 100, \quad (10)$$

where $\bar{X}$ is the mean of replicate measurements
C_s is the measured concentration of the sample
HR_{PIR} is half range for the prediction interval of results

The recovery criterion for MRL is related to the PIR limits, which should be between 50–150 %. The MRL should be less than or equal to 5 µg N/L.

4.7 Copper Interference Study

The aim of this copper interference study is to distinguish if adding copper to a sample with a known concentration of nitrite influences the recovery results of nitrite as well as the degree of the influence. For the study, two samples LFB 200 µg N/L and LFB 400 µg N/L were prepared, with the addition of 3 mg/L of Cu. For this the ~320 mg/L Cu-stock was used, detailed preparation described in subchapter 4.1. preparing required solutions. These samples were analyzed as three replicates. The criterion for the recovery is ±15 % when the concentration is over two times the MRL and ±20 % when the concentration is less than or equal to two times the MRL.

4.8 Sample Screening

As a preparative measure for the LFM study, all the drinking water samples were screened for alkalinity, total hardness and nitrite with the Gallery-analyzer. This was performed to obtain the base levels of each contaminant, from which the

samples could be selected and the amount of interference to be added could be calculated. The samples were not prepared in any way except from mixing the sample thoroughly to ensure a homogenic aliquot of the sample was obtained. This screening process does not have any criteria for the results.

On the basis of the matrix requirements, the supervisor of this thesis developed a testing plan, which includes DW1 - sample from a ground water source with elevated hardness level and ionic strength, DW2 - sample from a surface water source and containing elevated TOC-levels and organic content and DW3 – sample containing elevated alkalinity.

In addition, the plan only uses matrix samples that are prepared with real drinking water samples obtained from various places in the world. This is done to ensure that the results are as correct as possible in terms of analyzing nitrite from drinking water.

4.9 Laboratory Fortified Matrix Study

DW1 sample was prepared by pipetting 3.22 mL of the prepared 8081 mg/L $CaCO_3$-solution to a 200 mL volumetric flask and filled to the mark with sample 25DW06. The sample was analyzed for total hardness by Gallery.

DW2 sample was prepared by pipetting 490 µL of 1020 mg/L TOC-stock to a 250 mL volumetric flask and filled to the mark with sample 25DW07. 50 mL of the sample was transferred to a separate bottle, fixed and sent to an external laboratory for TOC-analysis.

DW3 was prepared by pipetting 4.79 mL of the 10.03 g/L (as CaCO3) stock to a 100 mL volumetric flask and filled to the mark with sample 25DW04. The sample was analyzed for alkalinity by Gallery. DW4 sample 25DW05 is ready to use, utilized as a water matrix sample, with high nitrite concentration.

From each of the DW1-DW4 samples two levels were prepared, low level with base nitrite spike +10 µg N/L and high level with base nitrite spike +100 µg N/L. Base spikes were done to ensure that the nitrite concentrations were above the detection limits. Except for DW2, where the nitrite level was enough for low level, so DW2 was used for DW2-L and from that was the high level prepared. The DW4 sample had so much nitrite to begin with; thus, only high level spikes were analyzed.

From the low level, two levels of spikes were prepared, +20 µg N/L and +80 µg N/L. From the high level, +300 µg N/L was prepared. Each spike level was prepared as duplicate samples. DW1 base spikes and actual spike sample preparation are displayed in Figure 12.

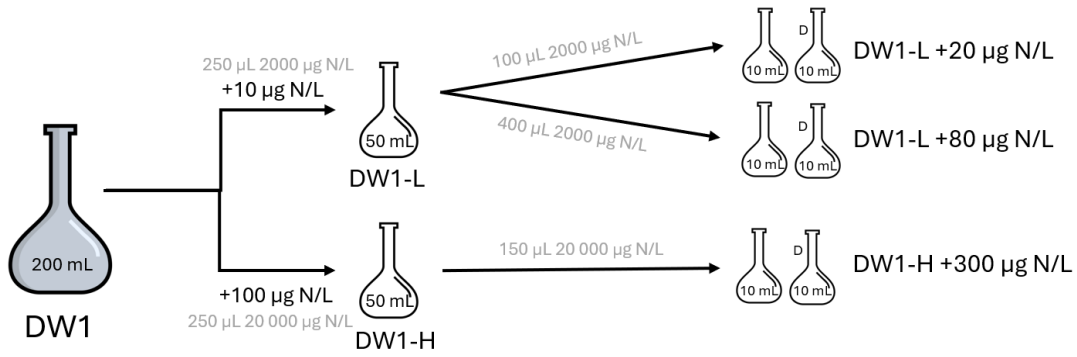

Figure 12. DW1 low ja high spike sample preparation

Each of the samples were analyzed with Gallery as well as the manual method, for acquiring reliable method comparison data. The spike samples were analyzed on the same day as they were prepared, due to the short-term stability of aqueous nitrite dilutions. From the analysis results recovery percentages and relative percentage difference, RPD are calculated according to equations 11 and 12 [41].

$$LFM\ Recovery\ \% = \frac{C_s - C \cdot f}{s} \cdot 100, \quad (11)$$

where C_s is the concentration of spiked sample
C is the concentration of unspiked sample
f is spike dilution factor*

s is the concentration of the spike, equivalent of analyte added.

*Sample volume per total volume of the spiked sample. If the added spike volume is less than 1 % of the total sample volume, the factor f can be excluded.

$$RPD\ \% = 100 \cdot \frac{|LFM - LFMD|}{\frac{1}{2}\cdot(LFM - LFM)}, \quad (12)$$

where LFM is the concentration of the LFM sample
LFMD is the concentration of the LFM sample duplicate

For the criteria of the LFM samples, accuracy and precision are both evaluated. In terms of accuracy, the recoveries should be between 80-120 %. For precision, the RPD of each set of duplicates should be ± 15 % when c > 2 x MRL and ± 20 % when c ≤ 2 x MRL.

5 Results

For the calibration results, the recoveries of the solutions were in specifications with both methods. For Gallery recoveries between 93-106 % for the low application and 93-102 % for the high application. The reference method recoveries were between 96-102 %. In terms of the coefficient of determination, Gallery's CoD ranged from 0.9994-1.000 and reference methods from 0.9997-1.000. Examples of calibration curves from the low and high applications for Gallery as well as curve for the reference method are presented in figures 13, 14 and 15.

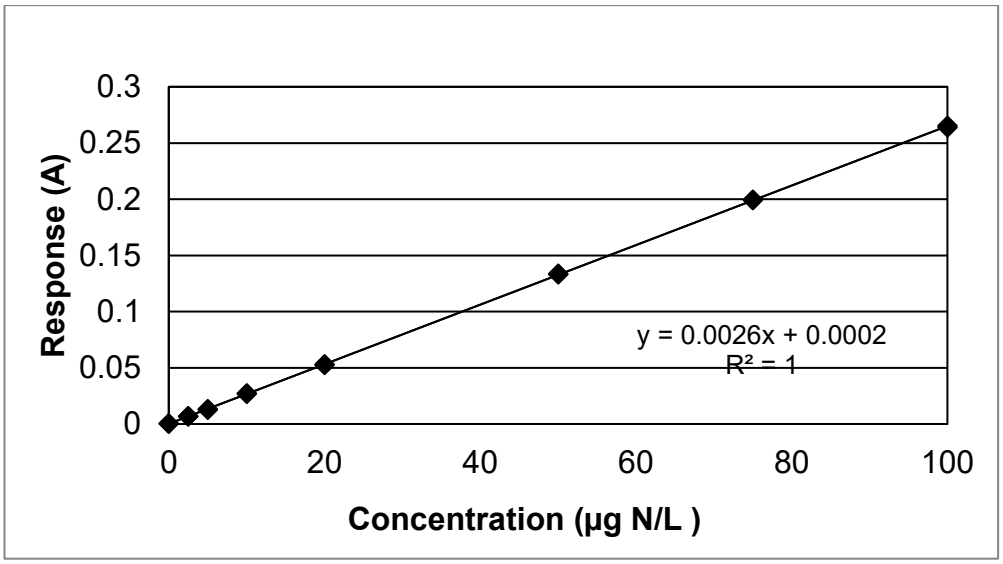

Figure 13. Calibration curve for Gallery's low nitrite application.

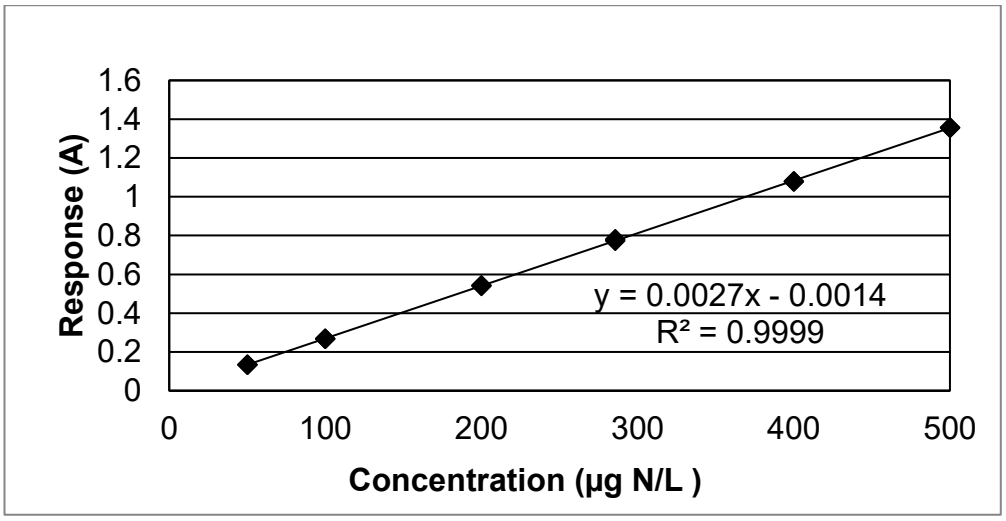

Figure 14. Calibration curve for Gallery's high nitrite application.

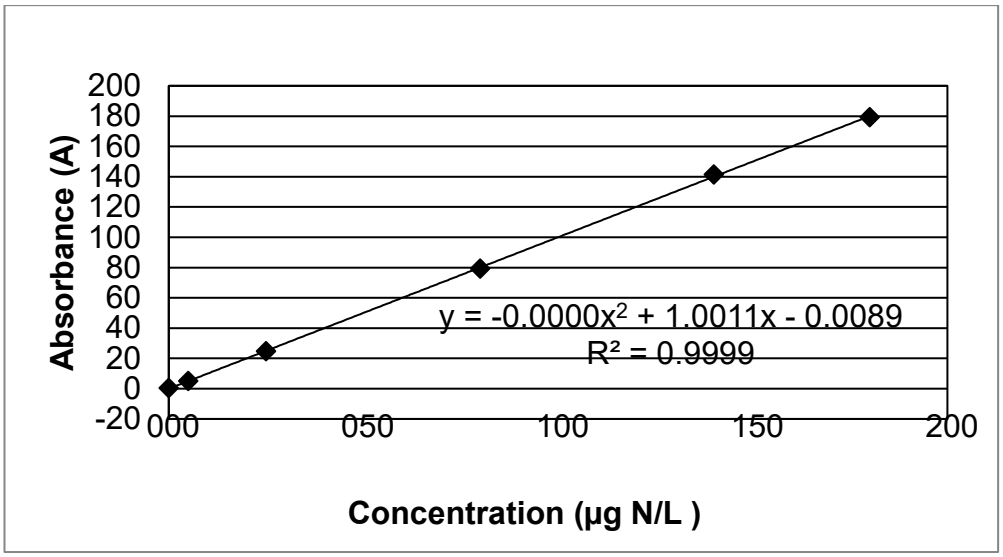

Figure 15. Calibration curve for the manual method.

An example of the reference method's calibration solutions after incubation, with the color development of the Griess reaction is presented in Figure 16.

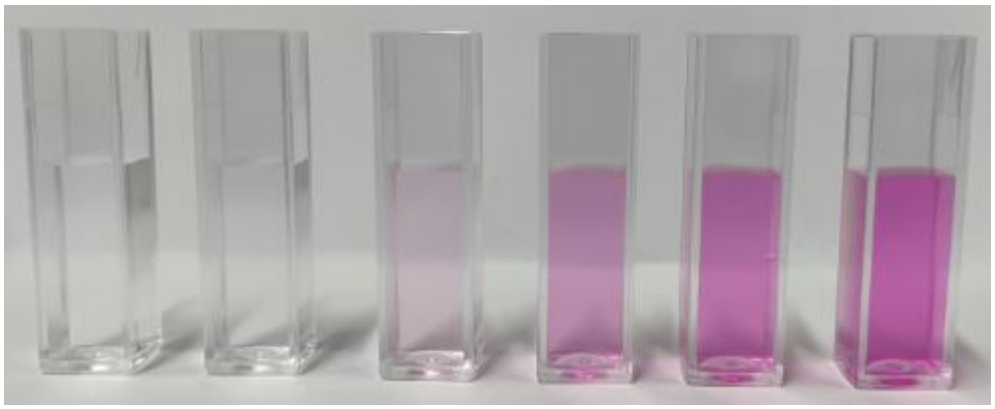

Figure 16. Calibration solutions in cuvettes, from right to left; blank, 5 µg/L, 25 µg/L, 80 µg/L, 140 µg/L and 180 µg/L.

For the QCS samples, the recoveries were 98-107 % for Gallery and 97-109 % for the reference method, whereas the recoveries of the LFB samples were 97-107 % for Gallery and 95-110 % for the reference method. More in depth results for QCS, LFB and CCV samples are presented in Table 2.

Table 2. QCS and LFB and CCV sample results for both methods.

	Sample	n	$C_{nominal}$ (µg/L)	$C_{measured}$ (µg/L)	Recovery- %	RSD- %
Gallery results	QCS 20	5	20.0	19.8-20.9	99-105	2.1
	QCS 80	5	79.9	78.9-82.6	99-103	1.5
	QCS 200	4	200	196-209	98-105	3.0
	QCS 400	4	400	394-417	98-104	2.2
	LFB 20	8	20.0	19.3-21.2	97-106	3.3
	LFB 80	8	79.9	77.4-83.4	97-104	2.2
	LFB 200	8	200	197-212	99-106	3.3
	LFB 400	8	400	387-426	97-106	3.1
	L CCV LL	8	2.5	2.42-2.62	97-105	2.4
	L CCV M	21	50.0	47.9-51.3	96-103	1.7
	H CCV M	22	250	245-263	98-105	2.5
	LRB	21	0.00	-0.02-0.18	-	-
Manual method results	QCS30	3	30.0	29.1-31.2	97-104	2.8
	QCS150	3	150	150-154	100-103	1.1
	LFB 30	12	30.0	28.4-31.4	95-105	3.7
	LFB 150	12	150	149-154	100-104	1.2
	LFB 1200	4	1220	1210-1240	99-103	1.4
	CCV LL	12	5.0	3.82-5.47	76-109	12
	CCV MID	12	90.0	89.6-95.7	100-106	2.2
	LRB	11	0.00	-0.87-0.36	-	-

The CCV and LFB samples analyzed with the reference method are presented in Figure 17.

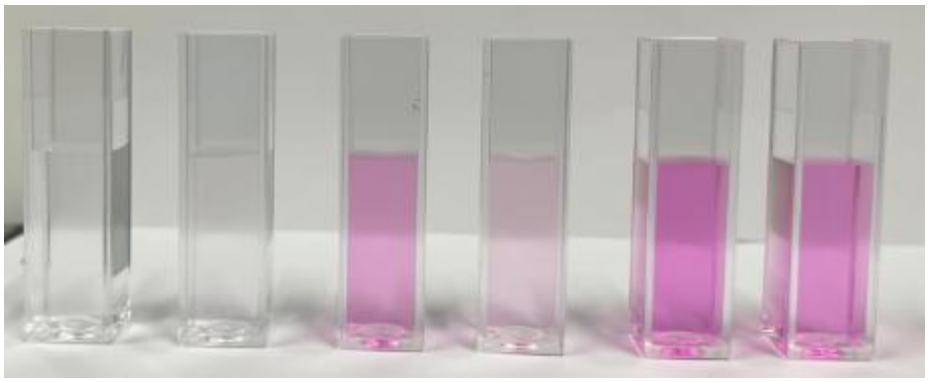

Figure 17. Continuous calibration solutions in cuvettes, from right to left; blank, CCV LL, CCV MID, LFB 30, LFB 150, LFB 1200 (1+9).

For the IPR study, Gallery's recoveries ranged from 99-107 % and RSD ranged from 0.0-0.6 %. The reference method recoveries were between 99-110 % and RSD 0.1-2.2 %. The exact results for each level of LFB sample analyzed for both methods are presented in Table 3.

Table 3. IPR results for Gallery and the manual method

	Sample	$C_{average}$ (µg/L)	SD (µg/L)	RSD- %	$C_{nominal}$ (µg/L)	Recovery- %
Gallery	LFB 20	21.0	0.1	0.5	20.0	105
	LFB 80	82.6	0.4	0.5	79.9	103
	LFB 200	212	0.3	0.1	200	106
	LFB 400	425	1.0	0.2	400	106
	LFB 2000	2040	11.6	0.6	2000	102
Manual method	LFB 30	31.5	0.25	0.008	30.0	105
	LFB 150	154	0.23	0.001	150	103
	LFB 1200	1230	0.81	0.001	1220	101

For the copper interference study recoveries for both levels of sample prepared were in specifications, with recovery percentage of 92 %. More information about the study is provided in Table 4.

Table 4. Copper interference study results for Gallery

Sample	$C_{average}$ (µg/L)	SD	RSD- %	$C_{nominal}$ (µg/L)	Recovery- %
LFB 200 + 3 mg/l Cu	183	0.616	0.3	200	92
LFB 400 + 3 mg/l Cu	369	0.701	0.2	400	92

The MRL recoveries were between 100-113 %. MRL results are presented in Table 5 and MDL results are presented in Table 6.

Table 5. MRL study results for reference method

$C_{average}$ (µg/L)	5.22
SD	0.28
RSD- %	5.3
$C_{nominal}$ (µg/L)	4.99
Recovery- %	104
HR_{PIR}	1.11
Upper PIR Limit	127 %
Lower PIR Limit	82 %

Table 6. MDL study results for reference method

	MDL Blank	MDL Sample
$C_{average}$ (µg/L)	-0.49	1.55
SD	0.52	0.47
$C_{nominal}$ (µg/L)	-	2.00
RSD- %	-	0.31
Calculated result	1.13	1.48

The results for nitrite, total hardness and alkalinity analyzed from the samples are presented in Table 7. From these samples suitable candidates for the LFM study were selected to be spiked and further analyzed.

Screening the samples, selection

Table 7. Sample screening results from Gallery

Sample	Nitrite (µg/L)	Total Hardness (µg/L)	Alkalinity (µg/L)
25DW01	0.202	28.8	28.3
25DW02	0.555	49.4	39.5
25DW03	0.593	48.4	45.2
25DW04	0.145	143	135
25DW05	131	31.5	31.3
25DW06	0.276	372	276
25DW07	11.4	59.1	37.6

The concentrations for the LFM study samples were as follows: 510 mg/L as $CaCO_3$ for the elevated hardness sample DW1, 540 mg/L as $CaCO_3$ for the TOC sample DW2 3 mg/L, for elevated alkalinity sample DW3, 130 µg/L as N for the elevated nitrite sample DW4. The LFM study results are presented in Table 8. An example of incubated spike samples for the manual method of sample DW1 is presented in Figure 18.

DW1–3 study, worst case -samples

Table 8. LFM study results for Gallery analyzer

Sample	$C_{measured}$ (µg/L)	C_{spike} (µg/L)	f	Recovery- %	RPD- %
DW1-L	10.7				
DW1-L +20 µg/L	30.3	20.1	0.990	98	0.3
DW1-L +20 µg/L D	30.4	20.1	0.990	99	

DW1-L +80 µg/L	88.5	80.4	0.960	97	0.0
DW1-L +80 µg/L D	88.5	80.4	0.960	97	
DW1-H	97.8				
DW1-H +300 µg/L	389	300	0.985	98	3.6
DW1-H +300 µg/L D	375	300	0.985	93	
DW2-L	12.3				
DW2-L +20 µg/L	32.8	20.1	0.990	103	1.5
DW2-L +20 µg/L D	32.4	20.1	0.990	100	
DW2-L +80 µg/L	92.1	80.4	0.960	100	0.6
DW2-L +80 µg/L D	92.7	80.4	0.960	101	
DW2-H	113				
DW2-H +300 µg/L	403	300	0.985	97	1.3
DW2-H +300 µg/L D	408	300	0.985	99	
DW3-L	10.7				
DW3-L +20 µg/L	30.8	20.1	0.990	101	2.2
DW3-L +20 µg/L D	31.5	20.1	0.990	104	
DW3-L +80 µg/L	91.3	80.39	0.960	101	1.4
DW3-L +80 µg/L D	92.7	80.39	0.960	103	
DW3-H	106				
DW3-H +300 µg/L	403	300	0.985	100	1.3
DW3-H +300 µg/L D	398	300	0.985	98	
DW4-H	131				
DW4-H +300 µg/L	417	300	0.985	96	0.7
DW4-H +300 µg/L D	420	300	0.985	97	

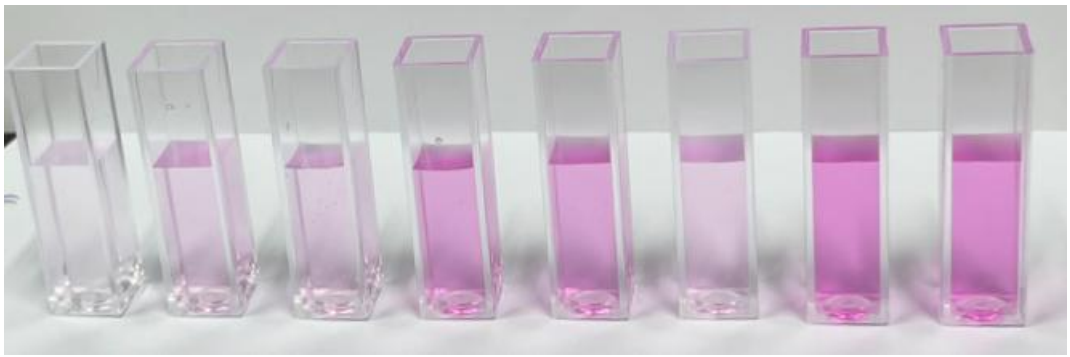

Figure 18. Example of spike samples; from right to left; DW1-L, +20, +20 D, +80, +80 D, DW1-H, +300 and +300D

In method comparison study, results obtained from both methods are compared to each other. These results are presented in Table 9. A *t*-test (paired two sample for means) was conducted on the results, and its results are presented below, in Table 9 and Figure 19.

Method Comparison Study

Table 9. Method Comparison Study results for Gallery and reference method

Sample	C(nitrite) Gallery (µg/L)	C(nitrite) Manual method (µg/L)	Bias	Recovery- %
DW1-L	10.5	11.5	-0.9	92
DW1-L +20 µg/L	29.8	33.5	-3.7	89
DW1-L +20 µg/L D	29.5	30.8	-1.3	96
DW1-L +80 µg/L	87.8	87.6	0.2	100
DW1-L +80 µg/L D	86.5	86.8	-0.3	100
DW1-H	97.1	97.7	-0.6	99
DW1-H +300 µg/L	388	391	-2.9	99
DW1-H +300 µg/L D	388	385	3.3	101
DW2-L	12.1	11.3	0.8	107
DW2-L +80 µg/L	90.5	91.3	-0.8	99
DW2-L +80 µg/L D	91.1	91.4	-0.2	100

DW2-H	205	200.3	4.9	102
DW2-H +300 µg/L	488	498	-9.9	98
DW2-H +300 µg/L D	491	499	-7.4	99
DW3-L	9.90	9.60	0.2	102
DW3-L +80 µg/L	86.4	90.7	-4.3	95
DW3-L +80 µg/L D	89.6	90.2	-0.6	99
DW3-H	101	98.0	2.7	103
DW3-H +300 µg/L	401	405	-4.2	99
DW3-H +300 µg/L D	400	396	4.4	101
DW4-H	134	130	3.3	103
DW4-H +300 µg/L	429	425	3.8	101
DW4-H +300 µg/L D	424	423	1.3	100

T-test paired two sample for means

Data from Table 8 is used for *t*-test. The hypotheses are presented in equations 13 and 14. The *t*-test is two-tailed, and the alpha is equal to 0.05. In Figure 19, a table of the *t*-test's data analysis from Excel is presented.

$$H_O\colon\ \mu_d = 0 \tag{13}$$

$$H_1\colon\ \mu_d \neq 0 \tag{14}$$

	Gallery Result	*Manual method results*
Mean	198.67	199.20
Variance	31178	31432
Observations	23	23
Pearson Correlation	1	
Hypothesized Mean Difference	0	
df	22	
t Stat	-0.696	
P(T<=t) one-tail	0.247	
t Critical one-tail	1.717	
P(T<=t) two-tail	0.494	
t Critical two-tail	2.074	

Figure 19. Screenshot from Excel's data-analysis for the *t*-test.

From the data analysis, it can be observed that the P-value 0.494 > Alpha 0.05. Thus, the P-value is greater than alpha and therefore the null hypothesis will not be rejected. From this information it can be concluded that the results derived from the two methods do not differ significantly by the 95 % confidence level.

6 Discussion

All of the studies conducted were successful, with all results falling within the specified limits. The results obtained from calibrations, i.e. from samples QCS, LFB and CCV, were rather similar between the two methods. Main observations from the results of the two methods were made regarding the MDL, MRL, IPR, OPR and LFM studies. For Gallery the limit of detection was set to 0.33 µg N/L, whereas the manual method limit was 1.5 µg N/L. The reporting limit was again lower with Gallery, 2.5 µg N/L, while the manual method was twice as much as 5 µg N/L. These results suggest that Gallery offers greater sensitivity in terms of analyzing nitrite from drinking water matrixes compared to the manual method.

The accuracy of IPR study was slightly improved with Gallery; however, the major difference can be observed in terms of the precision, where the RSD ranges from 0.0-0.6 % for Gallery and 0.1-2.2 % for the manual method. Thus, there is more variability between the measurements with the manual method compared to analysis with Gallery. Precision-wise, the IPR study displays the same pattern

that can be observed from the results, where the RSD ranges from 0.9-2.7 % for Gallery and 1.7-4.4 % for the manual method.

The results for LFM study followed the same trend, where the precision between the duplicate analyses is demonstrated through RPD. The RPD results range from 0.9-2.7 % for Gallery and 0.1-8.6 % for the manual method. Great recoveries were acquired, confirming that the method tolerates different drinking water matrixes well.

For the copper-interference analysis the recoveries of nitrate were between 92-93 %. Whereas the LFB samples with equivalent concentration had recoveries between 97-107 %. From these findings, it appears that copper interferes slightly with nitrite, though still passing the specified criteria. On the basis of the results and observations, Gallery performs as well as the manual method or even exceeding the capability of the manual method in some cases.

Besides the outcome of the studies, a considerable contrast between the methods comes from the actual execution of the tests. These findings are based on the analysis time, ease of use, environmental impact and possible error sources of the two methods.

Regarding the time required to perform the nitrite analysis, one sample takes about 10 minutes with Gallery. In addition, Gallery is capable of simultaneous analyses, which means that the 10 minutes does not multiply with each analysis and instead a few minutes will be added to the total run time. The manual method’s spectrophotometric measurement alone takes about 10 seconds, however the incubation time prior to the measurement is 30 minutes (10-120 minutes according to the standard procedure).

In terms of user-oriented approach, Gallery is more optimal with the user only needing to add the ready to use reagent and samples into the analyzer while the rest is automated. The manual method requires preparing the color reagent,

pipetting exact amounts of the sample and reagent and mixing each solution uniformly as well as incubating for approximately the same time.

These manual method experiments were done in 10-40 sample batches, with each sample mixed for approximately 30 seconds. This required thorough effort, full attention and observing a stopwatch while performing the process, whereas with Gallery constant observation of the analysis is not required, which offers the operator more time to prepare samples or conduct other tests while testing is proceeding.

The manual method leaves more room for error sources due to the high number of variables in the analysis, such as consistency with incubation time, comparable pipetting of color reagent and mixing between samples. There is also more possibility for human errors, e.g. samples being analyzed in the wrong order, improper preparation of color reagent, putting cuvette in the wrong way inside the spectrophotometer and smudges on the surface of the cuvette.

From the perspective of sustainability, analysis with Gallery is preferred due to the lower environmental impact resulting from lower amounts of harmful chemicals and reagents used in the process. In addition, a similar observation was made regarding the cuvettes used for the analyses: Gallery's DECACELL™ 240 µL and 2.5 mL macro cuvettes used for the manual method. Using smaller cuvettes not only requires smaller reagent and sample amounts but also reduces the disposal costs as well as production of waste.

Training new employees to perform these analyzes is also in favor of Gallery, due to the simplicity of the method. For example, if an employee is trained for both methods, they can perform almost every test available for Gallery, whereas the manual method as is works only for determining nitrite. This is the result of Gallery's easy and uniform operating procedures designed to ensure user-friendliness.

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Nitrite, Low Range Preliminary Application for Gallery Analyzer

The Gallery application for determination of low levels of nitrite applied in the experimental studies conducted as a part of this thesis.

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Test parameters

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DW NO2N L Version number 1 . 1

Feasibility

Date 18.9.2024

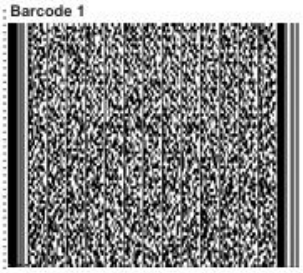
Time 9.15.50

Software version:8.3.0.0

Info

Tag	INT001
Last time changed	10.9.2024 17.08
User name	Test designer
Full name	Nitrite Low
In use	Yes
Type	Photometric
Online name	
Acceptance	Manual
Result unit	µg N/l
Number of decimals	1
Correction factor	1
Correction bias	0

Barcode 1



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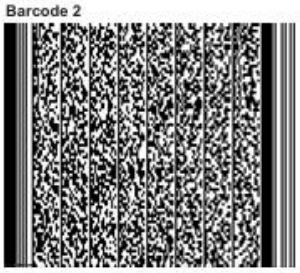

Sample type

Flow

Blank type Yes Primary dilution 1 + 0 Dispensed volume 140

Sample	Volume (µl)	Dispense with	Extra volume (µl)	Extra wash		
	120	Extra	40	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
	TON R3	20	Extra	40	Normal	None
	Barcode ID	Alarm limit (ml)	Onboard stability (days)			
	A08	2,0	1			
Incubate	Time (sec)	Actual time (sec)				
	360	360				
End-point measurement	Main wavelength (nm)	Side wavelength (nm)	Residual net abs. (A)			
	540	700	0			

*) Gallery™ includes Gallery, Gallery Aqua Master, Gallery Plus and Gallery Plus Aqua Master

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Test parameters

DW NO2N L Version number 1 . 1

Feasibility

Date 18.9.2024

Time 9.15.50

Software version:8.3.0.0

Dilution

Dilution with	Water
Primary dilution 1 +	0

Limits

	Measuring range (µg N/l) Min	Max		Next dilution ratio (1+) Low	High
Primary dilution	*	*		*	*
2nd dilution	*	*		*	*
3rd dilution	*	*		*	*
4th dilution	*	*		*	*
Test limit	*	100,00	µg N/l		
Critical limit	*	*	µg N/l		
Init. abs.	*	2,5	A		

Calibration

Calibration type	Linear
Repeat time (days)	1
Points/calibrator	Duplicate
Acceptance	Manual
Calibration order	Ascending

Nbr	Calibrator	Current lot	Concentration	Dilution 1 +
1	NO2N L-cal	Default	300	2
2	NO2N L-cal	Default	300	3
3	NO2N L-cal	Default	300	5
4	NO2N L-cal	Default	300	14
5	NO2N L-cal	Default	300	29
6	NO2N L-cal	Default	300	59
7	NO2N L-cal	Default	300	119
8	Water	Default	0	0

Abs. error (A)	*
Rel. error (%)	*
Factor limit min.	*
Factor limit max.	*
Bias limit min.	*
Bias limit max.	*
Concentration axis	Linear
Response axis	Linear
RSE max. (%)	*

QC

*) Gallery™ includes Gallery, Gallery Aqua Master, Gallery Plus and Gallery Plus Aqua Master

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Test parameters

DW NO2N L Version number 1.1
Feasibility

Date 18.9.2024
Time 9.15.50
Software version:8.3.0.0

Procedure	CalQC	QC profile	NO2N CalQC
Interval type		In use	Yes
Requests		Acceptance	Manual
Time (hh:mm)	0.00	Trigger	Manual,Calibration

Procedure	CCV LL	QC profile	NO2N CCV L
Interval type		In use	Yes
Requests		Acceptance	Manual
Time (hh:mm)	0.00	Trigger	Manual,Start of run

Procedure	Ongoing QC	QC profile	NO2N L
Interval type	Requests	In use	Yes
Requests	20	Acceptance	Manual
Time (hh:mm)	0.00	Trigger	Manual,Interval,Reagent lot change,Reagent vial change,Start of run,End of run

Procedure	Control	Current Lot	Conc.	SD	Req. count	Run group
CalQC	NO2N L CCV M	Default	50	2,5	1	All
CalQC	LRB	Default	0	0,625	1	All
CalQC	NO2N QCS 20	Default	20	1	1	All
CalQC	NO2N QCS 80	Default	80	4	1	All
CCV LL	NO2N CCV LL	Default	2,5	0,625	1	All
Ongoing QC	NO2N L CCV M	Default	50	2,5	1	All
Ongoing QC	LRB	Default	0	0,625	1	All
Ongoing QC	NO2N LFB 20	Default	20	1,5	1	1
Ongoing QC	NO2N LFB 80	Default	80	6	1	2

Procedure	Nbr of controls	SD multiplier
CalQC	1	2
CCV LL	1	2
Ongoing QC	1	2

*) Gallery™ includes Gallery, Gallery Aqua Master, Gallery Plus and Gallery Plus Aqua Master

Nitrite, High Range Preliminary Application for Gallery Analyzer

The Gallery application for determination of high levels of nitrite applied in the experimental studies conducted as a part of this thesis.

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Test parameters

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DW NO2N H Version number 1 . 1

Feasibility

Date 18.9.2024

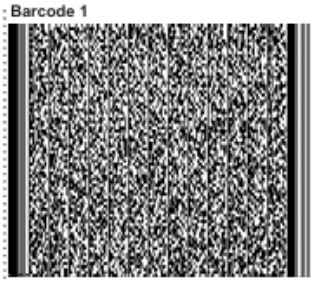
Time 9.15.24

Software version:8.3.0.0

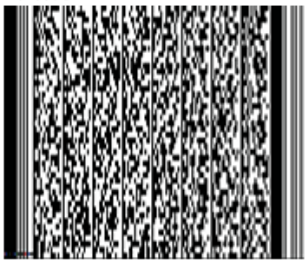
Info

Tag	INT002
Last time changed	11.9.2024 9.52
User name	Test designer
Full name	Nitrite High
In use	Yes
Type	Photometric
Online name	
Acceptance	Manual
Result unit	µg N/l
Number of decimals	0
Correction factor	1
Correction bias	0

Barcode 1



Barcode 2



Sample type

Flow

Blank type Yes Primary dilution 1 + 0 Dispensed volume 140

Sample	Volume (µl)	Dispense with	Extra volume (µl)	Extra wash		
	120	Extra	40	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
	TON R3	20	Extra	40	Normal	None
	Barcode ID	Alarm limit (ml)	Onboard stability (days)			
	A08	2,0	1			
Incubate	Time (sec)	Actual time (sec)				
	360	360				
End-point measurement	Main wavelength (nm)	Side wavelength (nm)	Residual net abs. (A)			
	540	700	0			

*) Gallery™ includes Gallery, Gallery Aqua Master, Gallery Plus and Gallery Plus Aqua Master

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Test parameters

DW NO2N H Version number 1 . 1
Feasibility

Date 18.9.2024
Time 9.15.24

Software version:8.3.0.0

Dilution

Dilution with	Water
Primary dilution 1 +	0

Limits

	Measuring range (µg N/l) Min	Max		Next dilution ratio (1+) Low	High
Primary dilution	*	500,0		*	4,0
2nd dilution	*	*		*	*
3rd dilution	*	*		*	*
4th dilution	*	*		*	*
Test limit	*	2 500,0	µg N/l		
Critical limit	*	*	µg N/l		
Init. abs.	*	2,5	A		

Calibration

Calibration type	Linear
Repeat time (days)	1
Points/calibrator	Duplicate
Acceptance	Manual
Calibration order	Ascending

Nbr	Calibrator	Current lot	Concentration	Dilution 1 +
1	NO2N H-cal	Default	2 000	3
2	NO2N H-cal	Default	2 000	4
3	NO2N H-cal	Default	2 000	6
4	NO2N H-cal	Default	2 000	9
5	NO2N H-cal	Default	2 000	19
6	NO2N H-cal	Default	2 000	39

Abs. error (A)	*
Rel. error (%)	*
Factor limit min.	*
Factor limit max.	*
Bias limit min.	*
Bias limit max.	*
Concentration axis	Linear
Response axis	Linear
RSE max. (%)	*

QC

*) Gallery™ includes Gallery, Gallery Aqua Master, Gallery Plus and Gallery Plus Aqua Master

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Test parameters

DW NO2N H Version number 1 . 1

Feasibility

Date 18.9.2024

Time 9.15.24

Software version:8.3.0.0

Procedure	CalQC	QC profile	NO2N CalQC
Interval type		In use	Yes
Requests		Acceptance	Manual
Time (hh:mm)	0.00	Trigger	Manual,Calibration

Procedure	Ongoing QC	QC profile	NO2N H
Interval type	Requests	In use	Yes
Requests	20	Acceptance	Manual
Time (hh:mm)	0.00	Trigger	Manual,Interval,Reagent lot change,Reagent vial change,Start of run,End of run

Procedure	Control	Current Lot	Conc.	SD	Req. count	Run group
CalQC	NO2N H CCV M	Default	250	12,5	1	All
CalQC	NO2N QCS 200	Default	200	10	1	All
CalQC	NO2N QCS 400	Default	400	20	1	All
Ongoing QC	NO2N H CCV M	Default	250	12,5	1	All
Ongoing QC	NO2N LFB 200	Default	200	15	1	1
Ongoing QC	NO2N LFB 400	Default	400	30	1	2

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

*) Gallery™ includes Gallery, Gallery Aqua Master, Gallery Plus and Gallery Plus Aqua Master

Parameters for the Reference Method

The parameters for determination of nitrite with the reference method are presented in Table 3.1. The protocol used in this thesis is created based on Standard Methods for the Examination of Water and Wastewater 23rd edition, part 4000 - 4500-NO2 nitrogen (nitrite) (2023, American Public Health Association, American Water Works Association, Water Environment Federation) [23].

Table 3.1. Parameters for the reference method.

Instrument	Multiskan GO 1510 spectrophotometer, Thermo Fisher Scientific, ref. 51119300
Software version	SkanIt Software RE for Microplate Readers RE, ver. 6.1.1.7
ESW version	1.00.40
Incubator temperature	25.00 °C
Wavelength	543 nm