

**Standard Operating Procedure for
Determination of Dissolved Inorganic Nitrate plus Nitrite (NO₃+NO₂) in
Fresh/Estuarine/Coastal Waters Using Cadmium Reduction
(References EPA 353.2)**

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**Revision 2018-1
Replaces Revision 2017-4
Effective May 1, 2018**

**I attest that I have reviewed this standard operating procedure and agree to comply
with all procedures outlined within this document.**

Employee (Print) Employee (Signature) Date

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Revised by: _____ Date: _____

Reviewed by: _____ Date: _____

Laboratory Supervisor: _____ Date: _____

Changes affecting Revision 2018

Section 1.2: Changed MDL definition to reflect new EPA Federal Register changes

Section 9.2.4: Changed MDL procedures to match EPA changes. Added sub sections 9.2.4.1 through 9.2.4.6.

Determination of Dissolved Inorganic Nitrate plus Nitrite (NO₃+NO₂) in Fresh/Estuarine/Coastal Waters Using Cadmium Reduction

1. SCOPE and APPLICATION

- 1.1 Cadmium reduction is used to quantitatively reduce dissolved nitrate to nitrite which is then measured by colorimetric quantitative analysis of a highly colored azo dye. The method is used to analyze all ranges of salinity.
- 1.2 A Method Detection Limit (MDL) of 0.0007 mg NO₃+NO₂-N/L was determined using the MDL method as specified in the EPA Federal Register 821-R-16-006, titled Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. The Quantitation Limit/Reporting Limit for NO₃+NO₂ was set at 0.0056 mg NO₃+NO₂-N/L.
- 1.3 The method is suitable for NO₃+NO₂ concentrations 0.0007 to 0.056 mg NO₃+NO₂-N/L. See Appendix 1 for addition ranges and configurations for this method.
- 1.4 This procedure should be used by analysts experienced in the theory and application of aqueous inorganic analysis. A three month training period with an analyst experienced in the analysis of nitrate plus nitrite in aqueous samples by cadmium reduction is required.
- 1.5 This method can be used for all programs that require analysis of dissolved inorganic nitrate plus nitrite.
- 1.6 This procedure references EPA Method 353.2 (1979).

2. SUMMARY

2.1 Filtered samples are passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite, both that which was reduced from nitrate and nitrite that was originally present, is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye.

3. DEFINITIONS

- 3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)
- 3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)
- 3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

- 3.4 Analytical Range – 0.0056 to 0.056 mg NO₃+NO₂-N/L, using black/black sample pump tube and yellow/yellow ammonium chloride diluent pump tube at a Standard Calibration setting of 9.00. See Appendix 1 for additional concentration ranges and configurations.
- 3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)
- 3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)
- 3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)
- 3.8 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)
- 3.9 Calibration Blank – A volume of reagent water fortified with the same matrix as the calibration standards, without analyte added.
- 3.10 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)
- 3.11 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)
- 3.12 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)
- 3.12.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.
- 3.12.2 Initial Calibration Verification (ICV) – An individual standard, which may be the same compound used as the calibrating standard, but not from the same vendor unless confirmed as different lots, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration

curve. ICV shall be analyzed in the middle of the calibration curve.

- 3.12.3 Continuing Calibration Verification (CCV) – An individual standard which which may be the same as the calibrating standard and is analyzed after every 10 field sample analysis.
- 3.13 Certified Reference Material – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025) CRM shall be analyzed in the middle of the calibration curve.
- 3.14 Colorimeter – Detector found in Bran & Luebbe Single-Channel Industrial Colorimeter. Color is quantitatively detected with 199-B021-01 phototubes using 550 nm monochromatic filters and 50 mm long flow cell with 1.5 mm internal diameter. Comparisons are made between signals from the colored solution in the flow cell to the signal of air in the reference cell. Signals from the Colorimeter are transmitted to a Recorder.
- 3.15 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)
- 3.16 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)
- 3.17 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)
- 3.18 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.
- 3.19 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)
- 3.20 External Standard (ES) – A pure analyte (potassium nitrate (KN O₃)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.
- 3.21 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

- 3.22 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.
- 3.23 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.
- 3.24 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.25 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., reagent water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.
- 3.26 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)
- 3.27 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. This is also referred to as MDL. (ACS)
- 3.28 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD such that it is \geq the lower standard This is also referred to as the Quantitation Limit.
- 3.29 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).
- 3.30 Manifold – The module whose configuration of glass connectors, fittings, mixing coils, tubing and Cadmium-Copper reduction column precisely reduces the nitrate in the sample to nitrite, followed by color production.

- 3.31 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical’s toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.32 May – Denotes permitted action, but not required action. (NELAC)
- 3.33 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. (Standard Methods)
- 3.34 Must – Denotes a requirement that must be met. (Random House College Dictionary)
- 3.35 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)
- 3.36 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.
- 3.37 Proportioning Pump – A peristaltic pump that mixes and advances samples and reagents through prescribed precision pump tubes proportionately for the reactions to take place and for the concentration to be measured.
- 3.38 Quality Control Sample (QCS) – A sample of analyte of known and certified concentration. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also referred to as CRM.
- 3.39 Recorder – A graphic recorder used to record electronic output from the colorimeter.
- 3.40 Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.
- 3.41 Sampler – An automated rotational device that moves sample cups sequentially to aspirate an aliquot into the proscribed analytical stream. As the loaded sample tray rotates, a metal probe dips into the sample cup and aspirates sample for a preset time, rises from the sample cup and aspirates air for approximately one second and goes into a reagent water-filled wash receptacle, where reagent water is aspirated. After another preset interval, the probe rises from the wash receptacle, aspirates air and moves into the next sample cup. The sampler moves at a rate of 40 samples per hour with a sample to wash solution ratio of 9:1.
- 3.42 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

- 3.43 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)
- 3.44 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)
- 3.45 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also referred to as CRM.










4 INTERFERENCES

- 4.1 Suspended matter in the sample will restrict flow through the apparatus. All samples must be filtered See Section 8.
- 4.2 Concentrations of sulfide, iron, copper or other metals above several milligrams per liter lower reduction efficiency, yielding inaccurate concentrations for those samples and, also, subsequent analyses. Frequent checks of column efficiency and re-analyses of affected samples are necessary.

5 SAFETY

- 5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes must be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Associate Director of Administration and Facilities Maintenance of the incident. Contact the CBL Associate Director of Administration and Facilities Maintenance if additional treatment is required.
- 5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.
- 5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.
- 5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Table 1

| Chemical | Health Hazard | Fire Hazard | Instability Hazard | Specific Hazard | |
|---|---------------|-------------|--------------------|-----------------|---|
| Sodium Hydroxide | 3 | 0 | 1 | ALK, COR |  |
| Copper Sulfate | 2 | 0 | 0 | |  |
| Ammonium Chloride | 2 | 0 | 2 | |  |
| Sulfanilamide | 1 | 1 | 0 | | |
| N-1-naphthylethylenediamine dihydrochloride | 1 | 0 | 0 | | |
| Brij-35 | 0 | 0 | 0 | | |
| Phosphoric Acid | 3 | 0 | 1 | ACID |  |
| Hydrochloric Acid | 3 | 0 | 2 | ACID, COR |  |
| Cadmium | 3 | 0 | 0 | |  |
| Potassium nitrate | 1 | 0 | 0 | OXY |  |
| Sodium nitrite | 2 | 0 | 1 | OXY |  |
| Chloroform | 3 | 0 | 0 | |  |

On a scale of 0 to 4, the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)
HAZARD RATING

Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable is heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer = OXY

6 EQUIPMENT AND SUPPLIES

6.1 Technicon Bran & Luebbe AutoAnalyzer II sampler (now owned by Seal Analytical), proportioning pump, manifold and colorimeter capable of analyzing for nitrate plus nitrite are used in this laboratory. A PMC Industries Flat Bed Linear recorder is used to record electronic output from the colorimeter.

6.2 Freezer, capable of maintaining $-20 \pm 5^{\circ}$ C.

6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives. This laboratory cleans all lab ware related to this method with a 10% HCl (v/v) acid rinse.

7 REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.

7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.

7.3 Alkaline Water –

| | |
|----------------------------------|---------------|
| Sodium hydroxide (NaOH, pellets) | 0.20±0.02 g |
| Reagent water | up to 1000 mL |

Add 0.20 g of sodium hydroxide pellets to 1000 mL of reagent water.

Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. The reagent is stable for six months.

7.4 Copper Sulfate Reagent, 2% –

| | |
|--|-----|
| Copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) | 2 g |
|--|-----|

Reagent water up to 100 ml
 In a 100 mL volumetric flask, dissolve 2 g of copper sulfate in ~80 mL of reagent water. Dilute to 100 mL with reagent water. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. The reagent is stable for six months.

7.5 Ammonium Chloride Reagent –

Ammonium Chloride (NH₄Cl) 10 g
 Reagent water up to 1000 mL
 Copper Sulfate Reagent, 2% 6 drops
 Sodium Hydroxide 2 pellets

In a 1000 ml volumetric flask, dissolve 10 g of concentrated ammonium chloride to ~800 ml of Reagent Water. Dilute to 1000 mL with Reagent Water. Attain a pH balance of 8.5. Add 6 drops of Copper Sulfate Reagent, 2% and 2 pellets NaOH. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. The reagent is stable for six months.

7.6 Color Reagent –

Sulfanilamide (C₆H₈N₂ O₂S) 10 g
 Phosphoric Acid (H₃PO₄), concentrated (80%) 10000 mL
 N-1-naphthylethylenediamine dihydrochloride (C₁₂H₁₄N₂·2HCl) 0.5 g
 Reagent water up to 1000 mL
 Brij-35, 30% 1 mL

In a 1000 mL volumetric flask, add 100 mL concentrated phosphoric acid and 10 g of sulfanilamide to ~500 mL reagent water. . Add 0.5 g of N-1-naphthylethylenediamine dihydrochloride and dissolve. Dilute to 1000 ml with reagent water and add 5 mL of 30% Brij-35. Write name of preparer, preparation date, reagent manufacturers, manufacturers' lot numbers in the Analytical Reagent log book. Make fresh every 3 months. Store at 4°C.

7.7 Nitrate Stock Standard, 5000 μM –

Potassium nitrate (KNO₃), primary standard grade, dried at 45°C 0.253 g
 Reagent water up to 500 mL

In a 500 mL volumetric flask, dissolve 0.253 g of potassium nitrate in ~400 mL of reagent water. Dilute to 500 mL with reagent water (1 mL contains 5 μmoles N). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 6 months or when < 20% remains in bottle.

7.8 Secondary Nitrate Standard –

Stock Nitrate Standard 0.80 mL
 Reagent water up to 100 mL

In a volumetric flask, dilute 0.80 mL of Stock Nitrate Standard to 100 mL with reagent water to yield a concentration of 40 μM NO₃ –N/L (0.56 mg N/L). Write name of preparer, preparation date, standard manufacturer,

manufacturer lot number in the Analytical Standard log book. Make fresh every 4 weeks.

7.9 Working Nitrate Standard – Dilute 1, 2.5, 5, 7.5 and 10 mL of Secondary Standard to 100 mL with reagent water to yield concentrations of 0.4 $\mu\text{M N}$ (0.0056 mg N/L), 1.0 $\mu\text{M N}$ (0.014 mg N/L), 2.0 $\mu\text{M N}$ (0.028 mg N/L), 3.0 $\mu\text{M N}$ (0.042 mg N/L) and 4.0 $\mu\text{M N}$ (.056 mg N/L). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 4 weeks.

7.10 Stock Nitrite Standard –

Sodium nitrite (NaNO_2), primary standard grade, dried at 45°C

0.1725 g

Reagent water

up to 500 mL

In a 500 mL volumetric flask, dissolve 0.1725 g of sodium nitrite in ~400 mL of reagent water. Dilute to 500 mL with reagent water (1 mL contains 5 $\mu\text{moles N}$). Add 1 mL of chloroform as a preservative. Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 6 months or when < 20% remains in bottle.

7.11 Secondary Nitrite Standard –

Stock Nitrite Standard

0.70 mL

Reagent water

up to 100 mL

In a volumetric flask, dilute 0.70 mL of Stock Nitrite Standard to 100 mL with reagent water to yield a concentration of 35 $\mu\text{M NO}_2\text{-N/L}$ (0.49 mg N/L). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 4 weeks.

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for NO_3+NO_2 should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.

8.2 Water collected for NO_3+NO_2 should be acidified to a pH of <2 and cooled to 4°C. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed.

8.3 Acidified NO_3+NO_2 samples may be stored up to 28 days at 4°C.

8.4 Non acidified NO_3+NO_2 samples may be refrigerated at 4°C for no longer than one day.

8.5 Prior to analysis, check samples and adjust pH accordingly. Samples shall be between 5 and 9.

9 QUALITY CONTROL

9.1 The laboratory 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 continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

- 9.2.1 The initial demonstration of capability (iDOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.
- 9.2.2 Linear Dynamic Range – LDR (Linear Calibration Range) should be established for NO_3+NO_2 using appropriate calibration curve of a blank and five standards.
- 9.2.3 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and every batch, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3s$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.
- 9.2.4 Method Detection Limits (MDLs) – Initial MDLs should be established for NO_3+NO_2 using a spiked water sample, typically two to 10 times the estimated MDL and no more than ten times higher than the estimated MDL. Process a minimum of seven spiked samples and seven blank samples through all steps of the method. The samples used for the MDL must be prepared and analyzed in at least three batches on three separate calendar dates. If multiple instruments are used for this analysis, MDLs must include data/calculations from all instruments.
- 9.2.4.1 Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.
- 9.2.4.2 Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.
- 9.2.4.3 Compute the MDLs (the MDL based on spiked samples) as follows:

$$\text{MDL}_S = t_{(n-1, 1-\alpha=0.99)} S_s$$

where:

MDL_s = the method detection limit based on spiked samples

$t_{(n-1, 1-\alpha=0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile

t statistic and a standard deviation estimate with n-1 degrees of freedom.

S_S = sample standard deviation of the replicate spiked sample analyses.

9.2.4.4 Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where $n \geq 100$, sort the method blanks in rank order. The $(n * 0.99)$ ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are ... 1.5, 1.7, 1.9, 5.0, and 10, then $164 \times 0.99 = 162.36$ which rounds to the 162nd method blank result.

Therefore, MDL_b is 1.9 for n =164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result).

Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = X^- + t_{(n-1, 1-\alpha=0.99)}S_b$$

where:

MDL_b = the MDL based on method blanks

\bar{X} = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1, 1-\alpha=0.99)}$ = the Student's t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses.

9.2.4.5 The verified MDL is the greater of the MDLs or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the Initial MDL determination with six degrees of freedom.)

9.2.4.6 MDLs should be determined annually, whenever there is a significant change in instrumental response or a significant change in instrument configuration. Data for annual MDL calculation and verification is analyzed at least quarterly, throughout the year.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of reagent water treated the same as the samples. An amount of analyte above the MDL found in LRB indicates possible reagent or laboratory environment contamination. LRB data are used to assess and correct contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and every batch, to verify data quality and acceptable instrument performance. If the determined concentrations are not within $\pm 3s$ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these QCS/SRM samples shall be used to determine batch acceptance.

9.3.3 The QCS are obtained from a source external to the laboratory and different from the source of calibration standards.

- 9.3.4 Control Charts – The Accuracy Control Chart for QCS/SRM samples and reagent blanks is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. (Reagent blanks in cadmium segmented flow method are baseline blanks. Since the baseline is subtracted from each peak, it is not appropriate to chart these reagent blanks.) The accuracy chart includes upper and lower warning levels ($WL=\pm 2s$) and upper and lower control levels ($CL=\pm 3s$). These values are derived from stated values of the QCS/SRM. The standard deviation (s) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter QCS/SRM results on the chart each time the sample is analyzed.
- 9.3.5 Calibration Verification Initial and Continuing (ICV/CCV)– Immediately following calibration (ICV) and following every 10 samples (CCV), two calibration verifications of $4.0 \mu\text{M NO}_3$ (.056 mg N/L) are analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards (KNO_3), and are to be within the expected value $\pm 3s$. Failure to meet the criteria requires correcting the problem, including reanalysis of any affected samples. If not enough sample exists, the data must be qualified if reported. See Appendix 1 for additional CCVs.
- 9.3.6 Reduction Efficiency Verification (REV) – The REVs are made from NaNO_2 , $35 \mu\text{M NO}_2$ (0.49 mg N/L) and are to be within the expected value $\pm 3s$ of the equivalent CCV, $35 \mu\text{M NO}_3$ (0.49 mg N/L). Failure to meet the criteria requires correcting the problem.

9.4 Assessing Analyte Recovery - Percent Recovery

- 9.4.1 Analyte recovery is assessed through percent recoveries of laboratory spikes of samples.
- 9.4.2 Percent Recovery = (Actual/Expected) x 100

9.5 Assessing Analyte Precision – Relative Percent Difference (RPD)

- 9.5.1 Analyte replication is assessed through duplicate analyses of samples – Relative Percent Difference.
- 9.5.2 $RPD = (| \text{Laboratory Duplicate Result 1} - \text{Laboratory Duplicate Result 2} |) / [(\text{Laboratory Duplicate Result 1} + \text{Laboratory Duplicate Result 2}) / 2] \times 100$

9.6 Corrective Actions for Out of Control Data

- 9.6.1 Control limit – If one measurement exceeds Accuracy Control Chart CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.
- 9.6.2 Warning limit – If two out of three successive points exceed Accuracy Control Chart WL, analyze another sample. If the next

point is within WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

- 9.6.3 Trending – If seven successive Accuracy Control Chart measurements are on the same side of the central line, discontinue analyses and correct the problem.
- 9.6.4 When external QCS samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.
- 9.6.5 When external CCV samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.7 General Operation - To assure optimal operation and analytical results, the Reagent Blank and CCV are tracked daily in the raw data file, copied to Reagent Blank and CCV Control Charts.

Table 2

| QC Indicator | Acceptance/ Action Limits | Action | Frequency (Batch) |
|--|----------------------------------|--|--|
| Correlation Coefficient | ≥ 0.995 | If <0.995 , evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier. | 1 per batch if acceptable. |
| Quality Control Sample (QCS)/ Certified Reference Material (CRM) | $\pm 10\%$ | If QCS value is outside $\pm 10\%$ of the target value reject the run, correct the problem and rerun samples. | Beginning of run and every 20 samples. |
| Initial Calibration Verification (ICV) | $\pm 10\%$ | Recalibrate if outside acceptance limits. | Beginning of run following standard curve. |
| Continuing Calibration Verification (CCV) | $\pm 10\%$ | If outside 10%, correct the problem. Rerun all samples following the last in-control CCV. | After every 10 samples. |
| Method Blank/Laboratory Reagent Blank (LRB) | \leq Method Quantitation Limit | If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit. | Following the ICV and after every 10 samples prior to the CCV. |

| | | | |
|--|-------|---|-------------------------|
| | | | |
| Laboratory Fortified Sample Matrix Spike | ± 10% | If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a “matrix induced bias” qualifier. | After every 10 samples. |
| Laboratory Duplicate | 10% | If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis. | After every 20 samples. |

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Five point calibrations are used with the Technicon Bran & Luebbe AutoAnalyzer II.

10.2 Working Nitrate Standards – Dilute 1, 2.5, 5, 7.5 and 10 mL of Secondary Standard to 100 mL with reagent water to yield concentrations of 0.4 µM N (0.0056 mg N/L), 1.0 µM N (0.014 mg N/L), 2.0 µM N (0.028 mg N/L), 3.0 µM N (0.042 mg N/L) and 4.0 µM N (0.056 mg N/L).

10.3 Prepare standard curve by plotting response on recorder of each and every standard processed through the manifold against NO₃ –N/L concentration in standards.

Compute sample NO₃ +NO₂ –N/L concentration by comparing sample response on recorder with standard curve. If NO₃ –N/L concentration is required, subtract NO₂ –N/L concentration from NO₃ +NO₂ –N/L concentration. The coefficient of determination (Pearson’s r value) for the calibration curve as well as the calculated concentration of each calibrator is reviewed. The calculated value of each calibrator must be within ten percent of the expected value. The coefficient of determination (Pearson’s r value) for the calibration curve must be greater than 0.995.

11 PROCEDURE – NEW REDUCTION COLUMN PREPARATION

- 11.1 Prepare Copper-Cadmium Column – Use good quality cadmium filings of 25-60 mesh size.
- 11.2 Clean 10 g of cadmium with 20 mL of acetone. Rinse twice with 20 mL of reagent water. Next, clean cadmium with 50 mL of 1 N Hydrochloric Acid for 1 minute. Cadmium turns silver in color. Decant Hydrochloric Acid and wash the cadmium with another 50 mL of 1 N Hydrochloric Acid for 1 minute.
- 11.3 Decant 1 N Hydrochloric Acid and wash the cadmium several times with reagent water.
- 11.4 Decant reagent water and add 20 mL of 2% (w/v) Copper Sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). Wash the cadmium until no blue color remains in the solution.
- 11.5 Decant Copper Sulfate solution and add another 20 mL of 2% (w/v) Copper Sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). Wash the cadmium until no blue color remains in the solution. The cadmium will be dark brown in color.
- 11.6 Decant Copper Sulfate solution and wash thoroughly (~10 times) with reagent water.
- 11.7 Set up Manifold, following general procedure of manufacturer in the following prescribed order.
- 11.8 Insert a glass wool plug at the outlet end of the column. Fill the reductor column tubing (22 cm length of 0.110-inch ID Tygon tubing) with reagent water and transfer the prepared cadmium granules to the column using a Pasteur pipette or some other method that prevents contact of cadmium granules with air. Do not allow any air bubbles to be trapped in column. Pack entire column uniformly with filings such that, visually, the packed filings have separation gaps $\leq \sim 1\text{mm}$.
- 11.9 Ammonium Chloride Reagent initiates analytical sample stream from 1.20 mL/min Yellow/Yellow pump tube.
- 11.10 Air is injected from 0.32 mL/min Black/Black pump tube.
- 11.11 Sample is added from 0.32 mL/min Black/Black pump tube.
- 11.12 Mixing occurs in five turn coil.
- 11.13 Air bubbles are de-bubbled from analytical sample stream using 0.60 mL/min Red/Red pump tube.
- 11.14 De-bubbled analytical sample stream passes through 22 cm reductor column.
- 11.15 Air is injected from 0.32 mL/min Black/Black pump tube.
- 11.16 Color Reagent is added from 0.32 mL/min Black/Black pump tube.
- 11.17 Mixing occurs in twenty-two turn coil.
- 11.18 Analytical sample stream enters 1.5 mm ID, 50 mm long Flow Cell pulled by 0.80 mL/min waste line. Bubbles and remainder of sample stream exit by gravity.
- 11.19 Color of analytical sample stream is quantitatively read at 550 nm by Colorimeter with 199-B021-01 Phototube, electronic output recorded on strip chart of Recorder.

- 11.20 Attach pump tubes to end rails of Proportioning Pump. Put platen on Proportioning Pump. With reagent water running through the sample line and Ammonium Chloride Reagent running through its designated line, attach the column. Make sure there are no air bubbles in the valve and attach the column to the intake side of the valve first. Open the valve to allow Ammonium Chloride Reagent stream to flow through the column. Allow reagent water to run through the Color Reagent line.
- 11.21 Turn on Colorimeter and Recorder.
- 11.22 Check for good flow characteristics (good bubble pattern) after insertion of air bubbles beyond the column. If the column is packed too tightly, an inconsistent flow pattern will result. Allow Ammonium Chloride Reagent to flow through Column, manifold and Colorimeter for one hour.
- 11.23 At conclusion of that hour, condition the column with approximately 100 mg N/L (KNO_3) for 5 minutes, followed by approximately 100 mg N/L (NaNO_2) for 5 minutes. Turn Baseline Knob on Colorimeter to obtain 0 deflection on Recorder.
- 11.24 Attach Color Reagent line to Color Reagent. At Colorimeter Standard Calibration setting of 1.00, note deflection on Recorder. Reject Color Reagent if deflection is more than 8 out of total 100 chart units. Turn Baseline Knob on Colorimeter to obtain 0 deflection on Recorder.
- 11.25 At Colorimeter Standard Calibration setting of 1.00, analyze Secondary Nitrate Standard ($35 \mu\text{M NO}_3\text{-N/L}$ (0.49 mg N/L)) and Secondary Nitrite Standard ($35 \mu\text{M NO}_2\text{-N/L}$ (0.49 mg N/L)). If peak height of Secondary Nitrate Standard is <90% of peak height of Secondary Nitrite Standard, prepare new cadmium reduction column.
- 11.26 Set Colorimeter Standard Calibration setting at 9.00. Analyze Working Nitrate Standards. Prepare standard curve by plotting response on recorder of standards processed through the manifold against $\text{NO}_3\text{-N/L}$ concentration in standards.
- 11.27 Analyze samples. Compute sample $\text{NO}_3\text{-N/L}$ concentration by comparing sample response on Recorder with standard curve.
- 11.28 At the end of the run, at Colorimeter Standard Calibration setting 1.00, analyze Secondary Nitrate Standard ($35 \mu\text{M NO}_3\text{-N/L}$ (0.49 mg N/L)) and Secondary Nitrite Standard ($35 \mu\text{M NO}_2\text{-N/L}$ (0.49 mg N/L)). If peak height of Secondary Nitrate Standard is <90% of peak height of Secondary Nitrite Standard, reject all sample concentrations and prepare a new cadmium reduction column.
- 11.29 Allow reagent water to flow through the sample line for 10 minutes. Close the valve to the column, diverting flow. Allow reagent water to flow through sample, Ammonium Chloride and Color Reagent lines for one minute. Turn Proportioning Pump switch to fast pump for its allotted time.
- 11.30 Turn off Sampler, Colorimeter and Recorder. Release and remove Proportioning Pump platen. Release pump tube holders from end rails.

12 PROCEDURE – DAILY OPERATION

- 12.1 Attach pump tubes to end rails of Proportioning Pump. Put platen on Proportioning Pump. Allow reagent water to run through the sample line, Ammonium Chloride Reagent to run through its line and reagent water to run through the Color Reagent line. Check for good flow characteristics (good bubble pattern). Open the valve to allow Ammonium Chloride Reagent stream to flow through the column.
- 12.2 Turn on Colorimeter and Recorder. Set Colorimeter Standard Calibration setting to 1.00. Let liquid pump through the column, Manifold and Colorimeter for 45 minutes.
- 12.3 At the conclusion of the 45 minutes, turn Baseline Knob on Colorimeter to obtain 0 deflection on Recorder.
- 12.4 Attach Color Reagent line to the Color Reagent. At a Colorimeter Standard Calibration setting of 1.00, note deflection on the Recorder. Reject Color Reagent if deflection is more than 8 out of total 100 chart units. Turn Baseline Knob on the Colorimeter to obtain 0 deflection on Recorder.
- 12.5 At Colorimeter Standard Calibration setting 1.00, analyze Secondary Nitrate Standard (35 μM NO_3^- /L (0.49 mg N/L)) and Secondary Nitrite Standard (35 μM NO_2^- /L (0.49 mg N/L)). If the peak height of Secondary Nitrate Standard is <90% of the peak height of Secondary Nitrite Standard, prepare a new cadmium reduction column.
- 12.6 Analyze ICV/CRM sample by dilution in order for the concentration to be in the middle of the calibration curve.
- 12.7 Set Colorimeter Standard Calibration setting at 9.00. Analyze Working Nitrate Standards. Prepare standard curve by plotting response on recorder of standards processed through the manifold against NO_3^- /L concentration in standards in Excel.
- 12.8 Analyze samples. Compute sample NO_3^- /L concentration by comparing sample response on Recorder with standard curve in Excel.
- 12.9 At the end of the run, at a Colorimeter Standard Calibration setting of 1.00, analyze Secondary Nitrate Standard (35 μM NO_3^- /L (0.49 mg N/L)) and Secondary Nitrite Standard (35 μM NO_2^- /L (0.49 mg N/L)). If the peak height of Secondary Nitrate Standard is <90% of the peak height of Secondary Nitrite Standard, reject all sample concentrations and prepare a new cadmium reduction column.
- 12.10 Analyze CRM sample every 10 samples by dilution in order for the concentration to be in the middle of the calibration curve.
- 12.11 Allow reagent water to flow through the sample line for 10 minutes. Close the valve to the column, diverting flow. Allow reagent water to flow through the sample, Ammonium Chloride and Color Reagent lines for one minute. Turn Proportioning Pump switch to fast pump for its allotted time.
- 12.12 Turn off Sampler, Colorimeter and Recorder. Release and remove Proportioning Pump platen. Release pump tube holders from end rails.

13 DATA ANALYSIS AND CALCULATIONS

13.1 Upon completion of all analysis, results are saved to a Microsoft Excel daily report file. The file is named by the run date. The daily report file for analytical batch of January 1, 2015 would be named 010115AAIINO23. Peak heights for each sample on chart recorder paper are noted and entered into the report file. Compute sample NO_3 -N/L concentration by comparing sample response on chart recorder paper with standard curve in Excel. The analyst examines each row of data. Results are eliminated that are outside the limits of the calibration range.

14 REFERENCES

- 14.1 Technicon Industrial Method No. 158-71 W/A Tentative. 1977. Technicon Industrial Systems. Tarrytown, New York, 10591.
- 14.2 USEPA. 1979. Method No. 353.2 *in* Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

| Range | Pump Tubes | umoles NO3/L | mg N/L | ml 40uM KNO3 std/100ml | CCV |
|---------------------------|------------------|--------------|--------|--------------------------------------|-------------------|
| Low | | 0 | 0 | Reagent Water | 0.056 mg NO23-N/L |
| | Blk/Blk sample | 0.4 | 0.0056 | 1.0 | |
| | Yel/Yel NH4Cl | 1 | 0.014 | 2.5 | |
| | Std Cal. 9.0 | 2 | 0.028 | 5.0 | |
| | | 3 | 0.042 | 7.5 | |
| High | | 4 | 0.056 | 10.0 | 0.21 mg NO23-N/L |
| | Orn/Grn sample | 0 | 0 | Reagent Water | |
| | Yel/Blu NH4Cl | 2 | 0.028 | 5.0 | |
| | Std Cal. 9.0 | 3 | 0.042 | 7.5 | |
| | | 4 | 0.056 | 10.0 | |
| | | 7 | 0.098 | 0.14 Primary KNO3 | |
| | | 10 | 0.14 | 0.2 Primary KNO3 | |
| | | 15 | 0.21 | 0.3 Primary KNO3 | |
| XHigh Dilution Loop | | | | ml Primary KNO3 Std/100ml | 2.8 mg NO23-N/L |
| | Orn/Wht sample | 0 | 0 | Reagent Water | |
| | Yel/Yel NH4Cl | 35 | 0.49 | 0.7 | |
| | Yel/Yel DI | 50 | 0.7 | 1.0 | |
| | Orn/Yel resample | 75 | 1.05 | 1.5 | |
| | Std Cal 3.0 | 100 | 1.4 | 2.0 | |
| | | 150 | 2.1 | 3.0 | |
| | | 200 | 2.8 | 4.0 | |

Appendix 1

