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 NO_3+NO_2-N/L was determined using the Student's t value (3.14, n=7) times the standard deviation of a minimum of 7 replicates.

1.3 The Quantitation Limit for NO_3+NO_2 was set at 0.0035 mg NO_3+NO_2-N/L.

1.4 The method is suitable NO_3+NO_2 concentrations 0.0007 to .056 mg NO_3+NO_2-N/L.

1.5 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.

1.6 This method can be used for all programs that require analysis of dissolved inorganic nitrate plus nitrite.

1.7 This procedure conforms to 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-napthylethylenediamine 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 – 0.0007 to 0.056 mg NO_3+NO_2-N/L, using black/black sample pump tube and yellow/yellow ammonium chloride diluent pump tube at a Standard Calibration setting of 9.00.

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. A **preparation batch** is composed of one to 300 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 10 hours. 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, distinct from the Initial Calibration Standards (STD), analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.12.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 18-23 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)

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 (KNO_3)) 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 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to 3.14 times 7 replicates that make up the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.25 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.

3.26 Laboratory Reagent Blank (LRB) – A blank matrix (i.e., DI 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.

3.27 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)

3.28 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. (ACS)

3.29 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, depending on the degree of confidence desired.

3.30 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).

3.31 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.

3.32 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.

3.33 May – Denotes permitted action, but not required action. (NELAC)

3.34 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)

3.35 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.36 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)

3.37 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.

3.38 Proportioning Pump – A peristaltic pump that mixes and advances samples and reagents through proscribed precision pump tubes proportionately for the reactions to take place and for the concentration to be measured.

3.39 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.

3.40 Recorder – A graphic recorder used to record electronic output from the colorimeter.

3.41 Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.

3.42 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 deionized water-filled wash receptacle, where deionized 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.

3.43 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.44 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.45 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.46 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.

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 should 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 Business Manager of the incident. Contact the CBL Business Manager 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).

Chemical	Health	Flammability	Reactivity	Contact	Storage
Sodium Hydroxide	3	0	2	4	White Stripe
Copper Sulfate	2	0	0	2	Green
Ammonium Chloride	2	0	2	2	Green
Sulfanilamide	0	1	1	1	Green
N-1-napthylethylenediamine dihydrochloride	2	1	1	2	Green
Brij-35	1	0	0	1	Green
Phosphoric Acid	3	0	2	4	White
Hydrochloric Acid	3	0	2	4	White
Acetone	1	4	2	1	Red
Cadmium	3	2	1	4	Red
Potassium nitrate	2	0	3	2	Yellow
Sodium nitrite	2	0	3	2	Yellow
Chloroform	3	1	1	3	Blue

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)
STORAGE
Red – Flammability Hazard: Store in a flammable liquid storage area.
Blue – Health Hazard: Store in a secure poison area.
Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
White – Contact Hazard: Store in a corrosion-proof area.
Green – Use general chemical storage (On older labels, this category was orange).
Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.

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 ± 5° 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
Deionized water	up to 1000 mL

Add 0.20 g of sodium hydroxide pellets to 1000 mL of deionized 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 ($CuSO_4$ $5H_2O$)	2 g
Deionized water	up to 100 ml

In a 100 mL volumetric flask, dissolve 2 g of copper sulfate in ~80 mL of deionized water. Dilute to 100 mL with deionized 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 (NH_4Cl)	10 g
Deionized 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 Deionized Water. Dilute to 1000 mL with Deionized 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 ($C_6H_8N_2$ O_2S)	20 g
Phosphoric Acid (H_3PO_4), concentrated (80%)	200 mL
N-1-napthylethylenediamine dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)	1 g
Deionized water	up to 2000 mL
Brij-35, 30%	1 mL

In a 2000 mL volumetric flask, add 200 mL concentrated phosphoric acid and 20 g of sulfanilamide to ~1500 mL deionized water. Dissolve completely. Add 1 g of N-1-napthylethylenediamine dihydrochloride and dissolve. Dilute to 2000 ml with deionized water and add 1 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 4ºC.

7.7 Nitrate Stock Standard, 5000 µM –

Potassium nitrate (KNO_3), primary standard grade, dried at 45ºC 0.5055 g

Deionized water up to 1000 mL

In a 1000 mL volumetric flask, dissolve 0.5055 g of potassium nitrate in ~800 mL of deionized water. Dilute to 1000 mL with deionized 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 4 months or when < 20% remains in bottle.

7.8 Secondary Nitrate Standard –

Stock Nitrate Standard	0.80 mL
Deionized water	up to 100 mL

In a volumetric flask, dilute 0.80 mL of Stock Nitrate Standard to 100 mL with deionized water to yield a concentration of 40 µM NO_3 –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 deionized 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 (.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 45ºC 0.345 g

Deionized water up to 1000 mL

In a 1000 mL volumetric flask, dissolve 0.345 g of sodium nitrite in ~800 mL of deionized water. Dilute to 1000 mL with deionized water (1 mL contains 5 µmoles 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 4 months or when < 20% remains in bottle.

7.11Secondary Nitrite Standard –

Stock Nitrite Standard	0.80 mL
Deionized water	up to 100 mL

In a volumetric flask, dilute 0.70 mL of Stock Nitrite Standard to 100 mL with deionized water to yield a concentration of 35 µM NO_2 –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 frozen at -20° C. The AutoAnalyzer vial container should be clean and sample rinsed.
8.3 Frozen NO_3+NO_2 samples may be stored up to 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.
8.4 NO_3+NO_2 samples may be refrigerated at 4° C for no longer than one day.

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 (NO_3+NO_2) – 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 and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% 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) – MDLs should be established for NO_3+NO_2 using a low level ambient water sample, typically three to five times higher than the estimated MDL. To determine the MDL values, analyze seven replicate aliquots of water. Perform all calculations defined in the procedure (Section 10) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = St_{(n-1,1-\alpha=0.99)}$$

Where,

S = Standard deviation of the replicate analyses.
n=number of replicates
$t_{(n-1,1-\alpha=0.99)}$ = Student's *t* value for the 99% confidence level with n-1 degrees of freedom (*t*=3.14 for 7 replicates.)

MDLs should be determined annually, whenever there is a significant change in instrumental response, change of operator, or a new matrix is encountered.

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 Nanopure 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. LRB above the MDL requires that the source of the problem must be identified and corrected before proceeding with analyses.

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 end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ 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 is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). 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. Set up an accuracy chart by using percent recovery since the concentration of the QCS/SRM varies. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5 Continuing Calibration Verification (CCV) – Following every 18-23 samples, one CCV of 4.0 µM NO_3 (.056 mg N/L) is analyzed to assess instrument performance. The CCVs are made from the same

material as calibration standards (KNO_3), and are to be within TV ± 3σ. 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.

9.3.6 Reduction Efficiency Verification (REV) – The REVs are made from $NaNO_2$, 35 µM NO_2 (0.49 mg N/L) and are to be within TV ± 3*s* of the equivalent CCV, 35 µM NO_3 (0.49 mg N/L). Failure to meet the criteria requires correcting the problem.

9.3.7 Reagent Blank – The Reagent Blank Control Chart for Reagent Blank samples is constructed from the average and standard deviation of the 20 most recent Reagent Blank measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter Reagent Blank results on the chart each time the Reagent Blank is analyzed.

9.4 Assessing Analyte Recovery - % Recovery

9.4.1 Analyte recovery is assessed through percent recoveries of laboratory spikes of samples.

9.4.2 Percent Recovery for each spiked sample should fall within 80-120%. Where:
%SR = (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 = (Laboratory Duplicate Result 1 – Laboratory Duplicate Result 2)/[(Laboratory Duplicate Result 1 + Laboratory Duplicate Result 2)/2] X 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.

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Six point calibrations are used with the Technicon Bran & Luebbe AutoAnalyzer II. Type I water is used as the "zero point" in the calibration.

10.2 Working Nitrate Standards – Dilute Dilute 1, 2.5, 5, 7.5 and 10 mL of Secondary Standard to 100 mL with deionized 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 NO_3 –N/L concentration in standards.

10.4 Compute sample NO_3 +NO_2 –N/L concentration by comparing sample response on recorder with standard curve. If NO_3 –N/L concentration is required, subtract NO_2 –N/L concentration from NO_3 +NO_2 –N/L concentration.

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 deionized 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 l minute.

11.3 Decant 1 N Hydrochloric Acid and wash the cadmium several times with deionized water.

11.4 Decant deionized water and add 20 mL of 2% (w/v) Copper Sulfate ($CuSO_4$ $5H_2O$). 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 ($CuSO_4$ $5H_2O$). 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 deionized 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 Ammonium Chloride Reagent 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 ≤ ~1mm.

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 deionized 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 deionized 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 (40 µM NO_3 –N/L (0.56 mg N/L)) and Secondary Nitrite Standard (40 µM NO_2 –N/L (0.56 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 NO_3 –N/L concentration in standards.

11.27 Analyze samples. Compute sample NO_3 –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 (40 µM NO_3 –N/L (0.56 mg N/L)) and Secondary Nitrite Standard (40 µM NO_2 –N/L (0.56 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 deionized water to flow through the sample line for 10 minutes. Close the valve to the column, diverting flow. Allow deionized 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 deionized water to run through the sample line, Ammonium Chloride Reagent to run through its line and deionized 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 one hour.

12.3 At the conclusion of that hour, 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 –N/L (0.49 mg N/L)) and Secondary Nitrite Standard (35 µM NO_2 –N/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 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 –N/L concentration in standards in Excel.

12.7 Analyze samples. Compute sample NO_3 –N/L concentration by comparing sample response on Recorder with standard curve in Excel.

12.8 At the end of the run, at a Colorimeter Standard Calibration setting of 1.00, analyze Secondary Nitrate Standard (35 µM NO_3 –N/L (0.49 mg N/L)) and Secondary Nitrite Standard (35 µM NO_2 –N/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.

12.9 Allow deionized water to flow through the sample line for 10 minutes. Close the valve to the column, diverting flow. Allow deionized 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.

12.10 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 METHOD PERFORMANCE

14.1 On 29 separate dates from January through December 2008, 29 replicate analyses of SPEX® Corporation QC 6-42 NUT 1 were performed by Cadmium Reduction. This produced a mean value of 0.194 mg NO_3-N/L, SD 0.0177, Relative Percent Difference of 4.1% from the expected value of 0.202 ± 10%. This is a mean recovery of 96%.

14.2 For some estuarine samples analyzed by Cadmium Reduction in 2008, the mean difference in concentration between 87 duplicates analyzed on 29 separate dates was 0.00044 mg NO_3-N/L. The standard deviation of the difference between duplicates was 0.00043 NO_3-N/L.

15 REFERENCES

15.1 Technicon Industrial Method No. 158-71 W/A Tentative. 1977. Technicon Industrial Systems. Tarrytown, New York, 10591.

15.2 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.