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Standard Operating Procedure for Determination of Total Dissolved Nitrogen (TDN) and Total Nitrogen (TN) in Fresh/Estuarine/Coastal Waters Using Alkaline Persulfate Digestion of Nitrogen to Nitrate and Measured Using Cadmium Reduction (References EPA 353.2, Standard Methods #4500-N C, 4500-NO3 F)

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Revision 2018-1
Replaces Revision 2017-1
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 9.2.3: Changed MDL procedures to match EPA changes. Added sub sections 9.2.3.1 through 9.2.3.6.

Determination of Total Dissolved Nitrogen (TDN) and Total Nitrogen (TN) in Fresh/Estuarine/Coastal Waters Using Alkaline Persulfate Digestion of Nitrogen to Nitrate and Measured Using Cadmium Reduction

1. SCOPE and APPLICATION

1.1 Potassium Persulfate is used to oxidize organic and inorganic nitrogen to NO_3 under heated alkaline conditions.
1.2 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.
1.3 A Method Detection Limit (MDL) of 0.05 mg TDN as NO_3-N/L 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.
1.4 The Quantitation Limit for TDN as NO_3 was set at 0.15 mg TDN as NO_3-N/L.
1.5 This procedure should be used by analysts experienced in the theory and application of aqueous organic and inorganic analysis. A three month training period with an analyst experienced in the analysis of TDN in aqueous samples by cadmium reduction is required.
1.6 This method can be used for all programs that require analysis of TDN.
1.7 This procedure references Standard Methods #4500-N C, 4500-NO3 F and EPA Method 353.2 (1979).

2. SUMMARY

2.1 An exact amount of filtered samples (whole water for TN) are placed in test tubes where an exact amount of Potassium Persulfate Digestion Reagent is added. Under initially alkaline conditions and heat, nitrate is the sole nitrogen product.

2.2 The now digested samples are buffered, then mixed and passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite, both that which was reduced from nitrate and 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 – There are multiple analytical ranges/standard curves used for determination of TDN. See Appendix 1 for all analytical ranges used.

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 the 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, 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 (CRM) – 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 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, depending on the degree of confidence desired. 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.

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 Metals, highly reduced substances, and excessive amounts of nitrogen have the potential of using up potassium persulfate before all nitrogen products have been oxidized.

4.2 Suspended matter in the sample will restrict flow through the apparatus. All samples must be filtered for TDN. See Section 8.

4.3 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

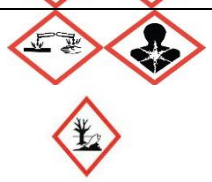
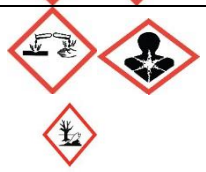
5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes must be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact 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-napthylethylene diamine 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		
Potassium Persulfate	2	0	2	OXY	
Boric Acid	2	0	0		
Glutamic Acid	0	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 ± 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. Test tubes used in this analysis are predigested and rinsed with copious amounts of reagent water.

6.4 Pressure Cooker with pressure regulator and pressure gauge.

6.5 Hot plate with variable heat settings.

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 the 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
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 the 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
Reagent water	up to 1000 mL
Copper Sulfate Reagent, 2%	6 drops
Sodium Hydroxide Pellets	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 the 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 ($C_6H_8N_2\,O_2S$)	10 g
Phosphoric Acid (H_3PO_4), concentrated (80%)	100 mL
N-1-napthylethylenediamine dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)	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-napthylethylenediamine dihydrochloride and dissolve. Dilute to 1000 ml with reagent water and add 5 mL of 30% Brij-35. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number 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	0.253 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 the name of preparer, preparation date, reagent 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	1.0 mL
Reagent water	up to 100 mL

In a volumetric flask, dilute 1.0 mL of Stock Nitrate Standard to 100 mL with reagent water to yield a concentration of 50 µM NO_3 –N/L (0.70 mg N/L). Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 4 weeks.

7.9 Working Nitrate Standard for TDN – See Appendix 1 for all working Nitrate Standards for TDN.
Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh for every digestion batch.

7.10 Stock Nitrite Standard –

Sodium nitrite ($NaNO_2$), primary standard grade, dried at 45ºC	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 µmoles N). Add 1 mL of chloroform as a preservative. Write the name of preparer, preparation date, reagent 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	1.0 mL
Reagent water	up to 100 mL

In a volumetric flask, dilute 1.0 mL of Stock Nitrite Standard to 100 mL with reagent water to yield a concentration of 50 µM NO_2 –N/L (0.70 mg N/L). Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 4 weeks.

7.12 Glutamic Acid Stock Standard, -

Glutamic Acid dried at 45°C	0.3705 g
Reagent water	up to 500 mL
Chloroform ($CHCl_3$)	0.5 mL

In a 500 mL volumetric flask, dissolve 0.3705 g of glutamic acid in about 400 mL of reagent water and dilute to 500 mL with reagent water. Add 0.5 mL of chloroform as a preservative. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book.

7.13 Working Glutamic Acid Standard for TDN – See Appendix 1 for all working Glutamic Acid Standards for TDN.
Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh for every digestion batch.

7.14 Potassium Persulfate Digestion Reagent –

Sodium Hydroxide (NaOH)	3 g
Potassium Persulfate ($K_2S_2O_8$), Low N	20.1 g
Reagent water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 3g of sodium hydroxide and 20.1 g of potassium persulfate in ~800mL of reagent water. Dilute to 1000 mL with reagent water. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Make fresh daily.

7.15 Borate Buffer Solution –

Boric Acid (H_3BO_3)	61.8 g
Sodium Hydroxide (NaOH)	8 g
Reagent water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 61.8 g of boric acid in ~ 300mL reagent water. Add 8g of sodium hydroxide and dilute to 1000mL with reagent water. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Make fresh every 4 months.

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for TDN should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.
8.2 Prior to initial use, capped 30 mL test tubes must be digested with Digestion Reagent, then rinsed thoroughly with reagent water.
8.3 A prescribed amount (typically 10mL) of sample should be added to each sample rinsed, capped 30mL test tube.
8.4 Water collected for TDN should be frozen at ≤-20° C.
8.5 Frozen TDN samples may be stored longer than 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.

8.6 Digested TDN samples may be stored up to three months.
8.7 TDN 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 (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 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed during the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 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.

9.2.3 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.

9.2.3.1 Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

9.2.3.2 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.

9.2.3.3 Compute the MDLs (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,\ 1-\alpha=0.99)} S_S$$

where:
MDLs = 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.

9.2.3.4 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 MDLb 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 MDLb equal to the highest method blank result. If more than 100 method blanks are available, set MDLb 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 x 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 MDLb as:

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

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.

9.2.3.5 The verified MDL is the greater of the MDLs or MDLb. 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.)

9.2.3.6 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 (TDN) 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 end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 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 sample 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 digested reagent blanks 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. 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 18-23 samples (CCV), two calibration verifications are analyzed to assess instrument performance. The CCVs are made from the different material than the calibration standards (KNO_3), and are to be within the expected value ± 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. Specific CCV's can be found in Appendix 1.

9.3.6 Reduction Efficiency Verification (REV) – The REVs are made from $NaNO_2$, 50 µM NO_2 (0.70 mg N/L) and are to be within the expected value ± 3*s* of the equivalent, 50 µM NO_3 (0.70 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. Analyte recovery is also assessed through the percent recovery of an organic standard that was digested with each batch 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 = (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 (LRB) and CCV are tracked daily in the raw data file, copied to Reagent Blank (LRB) and CCV Control Charts.

Table 2

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Correlation Coefficient	≥ 0.998	If <0.998, 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)	± 10%	If QCS value is outside ± 10% of the target value reject the run, correct the problem and rerun samples.	Beginning of run and at end of run.
Initial Calibration Verification (ICV)	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Continuing Calibration Verification (CCV)	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 18-23 samples.
Method Blank/Laboratory Reagent Blank (LRB)	≤ 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 18-23 samples following 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.	1/10 (spike OR duplicate)
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.	1/10 (spike OR duplicate)

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Four point calibrations are used with the Technicon Bran & Luebbe AutoAnalyzer II in replicates of three. ASTM Type I digested water (reagent water) is used as the zero point in the calibration.

10.2 Working TDN Standards – See Appendix 1 for all working TDN Standards.

10.3 Prepare standard curve by plotting response on recorder of standards processed through the manifold against TDN as NO_3 –N/L concentration in standards.

Compute sample mg TDN/L concentration by comparing sample response on recorder with standard curve. 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 0.998.

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 l 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 ($CuSO_4$ $5H_2O$). 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 reagent water.

11.7 Set up Manifold, following general procedure of the manufacturer in the following described 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 ≤ ~1mm.

11.9 Ammonium Chloride Reagent initiates analytical sample stream from 1.40 mL/min Yellow/Blue pump tube.

11.10 Air is injected from 0.32 mL/min Black/Black pump tube.

11.11 Sample is added from 0.16 mL/min Orange/Yellow pump tube.

11.12 Mixing occurs in five turn coil.

11.13 Air bubbles are removed 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 1.50, analyze Secondary Nitrate Standard (50 µM NO_3 –N/L (0.70 mg N/L)) and Secondary Nitrite Standard (50 µM NO_2 –N/L (0.70 mg N/L)). If peak height of Secondary Nitrate Standard is <90% of peak height of Secondary Nitrite Standard, prepare a new cadmium reduction column.

11.26 Analyze Inorganic Nitrate Standards for column assessment. (See Appendix 1 and use the same standards as used for Inorganic Linearity Check.)

11.27 Prepare standard curve by plotting response on recorder of standards processed through the manifold against NO3-N/L concentration in standards.

11.28 At the end of the run, 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.

11.29 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).

12.2 Turn on Colorimeter and Recorder. Set Colorimeter Standard Calibration setting to 1.00. Let liquid pump through the Manifold and Colorimeter for 15 minutes.

12.3 At the conclusion of the 15 minutes, turn Baseline Knob on Colorimeter to obtain 5 chart units deflection on Recorder.

12.4 Attach Color Reagent line to the Color Reagent. Open the valve to allow Ammonium Chloride Reagent Stream to flow through the column. 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 desired Standard Calibration (See Appendix 1), analyze inorganic linearity. Repeat the top standard to check for good replication. If replicates are not within ± 10%, repack the column and repeat. If repeating fails a second time, prepare a new cadmium reduction column. 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 Working TDN Standards using the NAP Software Program. (For NAP Software Program procedures, see Appendix 2.) The NAP Software Program will prepare standard curve by plotting response on recorder of standards processed through the manifold against TDN as NO_3 –N/L concentration in standards.

12.7 Analyze CRM sample at the beginning of the first group of samples.

12.8 Analyze samples. The NAP Software Program will compute sample TDN as NO_3 –mg N/L concentration by comparing sample response on Recorder with standard curve.

12.9 Change the Standard Calibration if a sample peak is larger than 100%. Standard Calibration of 1.5 and 2.0 can both be turned down to 1.0. Calculate the Change in Gain by multiplying the peak height times 100/79.9 for correcting a Standard Calibration of 1.5 to 1.0 and 100/68.0 for correctingo a Standard Calibration of 2.0 to 1.0. This will give a corrected peak height. Use the corrected peak height with the daily regression in order to calculate the sample concentration in mg/L.

12.10 At the end of the sample run, analyze CRM sample.

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 tubes from end rails.

13 **PROCEDURE – SAMPLE DIGESTION**

13.1 TDN/TDP samples are digested simultaneously in the same ampule. In our procedures, this ampule is a 30 mL screw cap test tube.

13.2 Prepare working standards, QCS (CRM), and CCV in labeled 100 mL volumetric flasks:

13.2.1 Select concentration range for both TDN/TDP that best fits the sample batch from Appendix 1.

13.2.2 Fill 100 mL volumetric flasks with 80 mL reagent water.

13.2.3 Add appropriate amount of KNO_3 and KH_2PO_4 to each labeled working standard volumetric flask from Appendix 1.
13.2.4 Add appropriate amount of glutamic/glycerophosphate to each labeled CCV and % recovery volumetric flask from Appendix 1.
13.2.5 Bring up to 100 mL volume with reagent water.
13.2.6 Mix each 100 mL labeled volumetric flask thoroughly

13.3 Sub-sample working standards into 30mL screw cap test tubes:

- 13.3.1 Prepare 3, 30mL labeled test tubes for each working standard concentration.
- 13.3.2 Sample rinse each test tube with the appropriate working standard.
- 13.3.3 Add exactly 10mL of each working standard to each test tube.
- 13.3.4 Prepare 3 labeled test tubes with exactly 10 mL reagent water for the zero point in the calibration curve.
- 13.3.5 Set aside 3 empty labeled test tubes to be digested with the batch with digestion reagent only (labeled RB).
- 13.3.6 Prepare 2, 30mL labeled test tubes for glutamic/glycerophosphate for percent recovery by adding exactly 10mL to each test tube.
- 13.3.7 Prepare 2, 30mL labeled test tubes for glutamic/glycerophosphate for CCV by adding exactly 10mL of the designated CCV solution to each test tube.
- 13.3.8 Thaw a Quality Control Sample (CRM) sample stored in freezer and sub-sample exactly 10mL into a labeled 30mL test tube to be used for QCS.

13.4 Prepare Digestion Reagent by dissolving 20.1 g Potassium persulfate and 3 g Sodium hydroxide in a 1000 mL volumetric flask:

- 13.4.1 Rinse volumetric flask with reagent water.
- 13.4.2 Add 20.1 g Potassium persulfate directly to the volumetric flask.
- 13.4.3 Add reagent water until the meniscus is slightly below full volume.
- 13.4.4 Add 3 g Sodium Hydroxide to the persulfate and water solution, cap immediately and mix thoroughly.
- 13.4.5 Bring to volume with reagent water.
- 13.4.6 Make fresh daily.
- 13.4.7 Digestion Reagent has a shelf life of about 4 hours.

13.5 When ready to digest, thaw frozen samples at room temperature.
13.6 Rinse dispensing vessel with reagent water and sample rinse with digestion reagent.
13.7 Add thoroughly mixed digestion reagent.
13.8 Check dispensing vessel for desired dispensing amount.
13.9 Add desired amount of digestion reagent (Typically 5mL), cap tube, shake for mixing and add test tube to pressure cooker.

13.10 Add desired amount of digestion reagent (5mL) to the standards at the beginning, middle and end of the sequence of loading the samples.

13.11 When all samples and standards have received digestion reagent and have been loaded into the pressure cooker, place pressure cooker on hot plate, add reagent water until tubes are 75% immersed, wet the gasket on the lid with a few drops of water and place lid on the pressure cooker.

13.12 Turn the hot plate on maximum temperature and have the pressure cooker come up to full steam. (This takes about 1 hour.)

13.13 When full steam is achieved, place the pressure regulator on the steam vent. Maintain heat for the cooker containing samples and standards at 3-4 psi for 55 minutes.

13.14 Turn off pressure cooker and unplug the hot plate when finished. Keep the lid on the pressure cooker.

13.15 After samples have cooled, usually the next day, remove the pressure cooker lid, add 1 mL Borate Buffer to each tube, cap, and shake.

13.16 Sample batch is now ready to analyze and is stable for 3 months.

14 DATA ANALYSIS AND CALCULATIONS

14.1 Upon completion of all analysis, results are saved to a Lotus 123 daily report file. The file is named by the run date. The daily report file for analytical batch of January 1, 2017 would be named 010117tdnp. The instrument software has calculated final sample concentration from the designated standard curve in a program called New Analyzer Program (NAP) Software. Dilution by the analyst is noted and recalculated by multiplying the original peak height times the dilution factor to calculate a corrected peak height. Use the corrected peak height with the daily regression to calculate the sample concentration in mg/L. The analyst examines each peak height and peak marker within the NAP Software and compares it to the peak height from the chart recorder. Results are eliminated that are outside the limits of the calibration range.

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.

Range	Pump Tubes	umoles NO3/L	mg N/L	ml 1 NO3 std/100ml	Spike Conc.	Inorganic Check For Linearity	Glutamic/Glycerphosphate for % Recovery	Nap File Created	CCV	Correction Coefficient
		0	0	DI H2O						
Low	Orn/Yel sample	25	0.35	0.5	200 umole NO3	50 NO3	1 ml Glutamic	Low TDN (group)	0.5 mL Glutamic	50 umoles
10 ml sample	Yel/Blu NH4Cl	50	0.70	1.0	12 umole PO4	50 NO2		lown2 (samp. Appendix)	0.31 mgN/L	NO3
5 ml persulfate	Std Cal. 1.5	75	1.05	1.5		35 NO3				
						10 NO3				
						3.5 NO3				
		0	0	DI H2O						
High	Orn/Grn sample	25	0.35	0.5	200 umole NO3	75 NO3	1 ml Glutamic	High TDN (group)	0.5 mL Glutamic	75 umoles
10 ml sample	Yel/Blu NH4Cl	50	0.70	1.0	12 umole PO4	50 NO3		lown2 (samp. table)	0.31 mgN/L	NO3
5 ml persulfate	Std Cal. 1.5	100	1.40	2.0		50 NO2				
						35 NO3				
						10 NO3				
		0	0	DI H2O						
XHigh	Orn/Wht sample	50	0.70	1.0	400 umole NO3	100 NO3	2 ml Glutamic	XHigh TDN (group)	1.0 mL Glutamic	100 umoles
10 ml sample	Yel/Yel NH4Cl	100	1.40	2.0	12 umole PO4	50 NO3		Xhigh TDN (samp. table)	0.72 mgN/L	NO3
5 ml persulfate	Yel/Yel DI	200	2.80	4.0		50 NO2				
	Blk/Blk resample					35 NO3				
	Std Cal 2.0					10 NO3				
		0	0	DI H2O						
TWS TDN	Blk/Blk sample	150	2.1	3.0	2.5 ml of	100 NO3	4 ml Glutamic	TWS TDN (group)	4 ml Glutamic	100 umoles
5 ml sample	Yel/Yel NH4Cl	300	4.2	6.0	400 umole NO3 &	50 NO3		TWS TDN (samp. table)	2.88 mgN/L	NO3
15 ml persulfate	Yel/Yel DI	400	5.6	8.0	12 umole PO4	50 NO2				
	Blk/Blk resample				Added to 2.5 ml	35 NO3				
	Std Cal 2.0				sample prior to digestion	10 NO3				

Appendix 1. Methods and Standards Used for TDN Cadmium Reduction