

Standard Operating Procedure for Nitrate/Nitrite and Total Nitrogen
By Discrete Analyzer

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Revisions

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1.0 Scope and Application

The SOP covers the determination of total nitrogen (using off-line persulfate digestion), and combined NO₂NO₃ in drinking, surface, waste and sea water. Total nitrogen can also be determined in soils.

2.0 Summary of Method

Nitrate is reduced to nitrite by the passage of the sample through a copperized cadmium coil. The nitrite (reduced nitrate plus any original nitrite present) is measured by diazotizing the sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dichloride resulting in a magenta colored product that is read at 520 nm.

In an offline alkaline digestion, persulfate oxidizes all organic and inorganic forms of nitrogen to nitrate and is measured as Total Nitrogen (Org N + NH₄-N + NO₃-N, NO₂-N). Some nitrogen compounds with triple and double bonds may not be affected by the digestion.

3.0 Acronyms/Definitions

- 3.1 Calibration Blank (CCB) -- A volume of reagent water, the same matrix as the calibration standards, but without the analytes.
- 3.2 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
- 3.4 Laboratory Fortified Sample Matrix (LFM or MS) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

- 3.5 Reagent Blank (BLK) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The BLK is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus. Analyzed after CCV (every 10 injections)
- 3.6 Continuing Calibration Blank (CCV)-- An individual CAL solution which is analyzed after every tenth injection which verifies the previously established calibration curves and confirms accurate analyte quantitation for the previous ten injections analyzed
- 3.7 Method Blank (MB) – Contain all the reagents and in the same volumes as used in processing the samples. Matrix should match that of the samples, i.e. an acid diluent for preserved samples, DI for unpreserved samples. Blanks shall be carried through the complete preparation, and analysis method process.
- 3.8 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.
- 3.9 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. 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.
- 3.10 Stock Standard (SS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source
- 3.11 Instrument Calibration Verification (ICV) -- A solution prepared from a second source to test the performance of the calibration with respect to a defined set of criteria.
- 3.12 Reduction Efficiency (RE) – A nitrite solution prepared to calculate reduction efficiency, compared to a nitrate standard of the same concentration.

- 3.13 Open Tubular Cadmium Reactor Coil (OTCR) – A 14” coiled cadmium tube having a purple cladding. Within the OTCR, nitrate is chemically reduced to nitrite.
- 3.14 Digestion Verification Standard (DVS) – A solution of a nitrogen species, other than NO_2^- or NO_3^- , of known concentration which is digested under the same process as samples. Confirms the complete digestion of nitrogen species.
- 3.15 SOP – Standard Operating Procedure
- 3.16 NO_2 - Nitrate
- 3.17 NO_3 -Nitrite
- 3.18 LSASD – Laboratory Services and Applied Sciences Division
- 3.19 LSB – Laboratory Services Branch
- 3.20 SDS – Safety Data Sheets
- 3.21 EDTA - Ethylenediaminetetraacetic acid
- 3.22 IDC – Initial Demonstration of Capability
- 3.23 MDL – Method Detection Limit
- 3.24 QA/QC – Quality Assurance/Quality Control
- 3.25 H_2SO_4 – Sulfuric Acid
- 3.26 TN – Total Nitrogen
- 3.27 RL – Reporting Limit
- 3.28 RPD – Relative Percent Difference

4.0 Health and Safety Warnings

Sulfuric acid used in this method can cause severe burns and should be handled by an analyst trained to work with this chemical. Gloves and protective clothing must be

worn, and chemicals should be kept under a fume hood. The reagents used are toxic and precautions should be taken when handling them. Safety information is available in the form of SDS sheets and can be obtained on the internet. Links are available on the Region 1 Intranet site.

5.0 Interferences

- 5.1 Buildup of suspended matter in the reduction coil will restrict sample flow. Since nitrate and nitrite are found in a soluble state, samples may be pre-filtered. Total nitrogen can be filtered *after* digestion.
- 5.2 Samples containing strong oxidants or reductants, such as chlorine or sodium thiosulfate, may cause low results by decreasing nitrate reduction efficiency.
- 5.3 Low results will be obtained for samples that contain high concentrations of iron, copper, or other metals. In this method EDTA is added to the buffer to reduce this interference.
- 5.4 Highly alkaline or overly-acidified samples may give low results due to a shift in pH. Preserved samples should be neutralized to pH between 5 and 9.
- 5.5 Samples containing large concentrations of oil and grease will coat the surface of the cadmium. This interference may be eliminated by sample pretreatment with activated carbon or by extracting the sample with an organic solvent.
- 5.6 Bias from sample turbidity or color is correctable, using sample blanking feature of AQ software.
- 5.7 Organic carbon reacts with the persulfate oxidation reagent to form carbon dioxide. Concentrations over 150 mg/L of C may deplete the persulfate before all nitrogen compounds are oxidized and cause a low bias for total nitrogen.
- 5.8 A major interference can come from ammonia contamination of glassware or reagents. To prevent this, glassware and utensils should be washed in 1 N hydrochloric acid and stored in an ammonia-free environment. Use only high purity potassium persulfate and store in a dry ammonia-free environment.

6.0 Personnel Qualifications

The analyst should have at least a 4-year degree in physical science. The analyst must have a satisfactory IDC/MDL in place before analyzing samples. All personnel shall be responsible for complying with all QA/QC requirements that pertain to their organizational/technical function.

7.0 Equipment and Supplies

- 7.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001g
- 7.2 Glassware – Class A volumetric flask and pipettes or plastic containers as required
- 7.3 SEAL AQ300 Discrete analyzer
- 7.4 Autoclave (for total nitrogen off-line digestion)
- 7.5 pH Meter

8.0 Procedures

8.1 Sample Collection and Preservation

Samples should be collected in either plastic or Pyrex glass containers. The sample should be preserved using H_2SO_4 to a $\text{pH} < 2$ and cooled to 4°C at the time of collection. Sample analysis should be performed as soon as possible, but no later than 28 days of sample collection.

8.2 Reagents Preparation

All reagents and standards should be stored in the appropriate bottles and labeled with the following information: manufacturer, lot number, date of preparation, date of expiration, concentration, initials of preparer.

NOTE: All dry chemicals used in this analysis must be low in nitrogen, ACS grade reagent.

8.2.1 Reagent 1. Triton® X-100 7.5% (w/v)

Tare a 100 to 200 mL beaker on a scale. Add 3.75 g of Triton® X-100 neat to the beaker. Stir to dissolve (solution will initially thicken). Dilute to 50 mL. Store at 4°C . Stable for 1 month. Discard if solids are seen.

8.2.2 Reagent 2. Ammonium Chloride Buffer Stock, $\text{pH } 8.55 \pm 0.05$

In a 100 mL volumetric flask, dissolve 18 g ammonium chloride (NH_4Cl) and 0.2 g disodium ethylenediamine tetraacetic acid dihydrate ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$) in about 80 mL of DI water. *Let the solution get to room temperature.* Then, under a fume hood, adjust the pH to 8.55 ± 0.05 using concentrated ammonium hydroxide. Dilute to 200 mL. Prepare as needed. If needed, the final pH of this stock can be increased to a maximum of 9.1 ± 0.05 to accommodate preserved or otherwise acidic samples.

8.2.3 Reagent 3. Working Buffer, 0.0375% surfactant

Add 0.25 mL of Reagent 1 to 50 mL of Reagent 2. Mix thoroughly. Prepare fresh every 2 weeks.

8.2.4 Reagent 4. Sulfanilamide Color Reagent

Reagent can be purchased from Hach Company. Certificates of analysis are required. Prepare the Hach Reagent as follows:

Add the entirety of Sulfanilamide Reagent 2 (glass vial) to Sulfanilamide Reagent 1 (brown bottle). Cap and stir for half an hour. Store at 4°C. Prepare as needed. Filter an aliquot into the reagent wedge before analysis.

Reagent can be prepared in the lab as follows:

In a 500mL volumetric flask, add approximately 250 mL DI water. Dissolve 1.0 g of sodium hydroxide. Then add 20 mL of concentrated phosphoric acid (H_3PO_4) and stir to dissolve. Add 7.5g sulfanilamide and 0.375 g N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Stir to dissolve. Dilute to 500 mL with DI water and filter. As the reagent slowly turns pink, re-filter as needed. Store at 4°C in the dark. This solution is stable for a month. Discard if background absorbance of analyzed blanks exceeds 0.04 AU.

8.2.5 Reagent 5. H_2SO_4 Diluent

In a 2 L volumetric flask, add approximately 1600mL of DI water. Then add 4 mL of concentrated H_2SO_4 and dilute to the mark.

8.2.6 Reagent 6. Digestion Solution (Total Nitrogen Only)

In a 1 L volumetric flask, add approximately 60 mL DI water. Then add 40 g potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), 18 g boric acid (H_3BO_3) and 9 g sodium hydroxide (NaOH). Dilute to the mark and shake to mix. Store at room temperature for up to seven days. *NOTE: Potassium persulfate must be low in nitrogen, ultrapure, ACS reagent grade. If an elevated level of nitrogen is discovered in blanks, potassium persulfate may need recrystallization. Boric acid must also be low in nitrogen.*

8.2.7 Reagent 7. Borate Buffer, 1.0 M, pH 7.5 (For Soils Only)

In a 200 mL volumetric flask, dissolve 12.4 g boric acid and 1.6 g sodium hydroxide in about 150 mL of reagent water. Mix the solution on a magnetic stirrer and dilute to the mark. Expiration of one month from preparation.

8.2.8 Reagent 8. Copper (II) Sulfate 2% (w/v) (for Cd coil preparation)

This solution is commercially available. It can also be prepared as follows: In a 100 mL volumetric flask, dissolve 3.13 g of copper (II) sulfate pentahydrate in about 75 mL of DI water. Dilute to mark and mix. Store at room temperature. This solution is stable for 6 months.

8.2.9 Reagent 9. 6 N Hydrochloric Acid (for Cd coil preparation)

This solution is commercially available. It can also be prepared as follows: Place a 500 mL volumetric flask in an ice bath in a fume hood and add about 125 mL of DI water. Slowly add 250 mL of concentrated hydrochloric acid. **Caution: This solution will become hot.** Cool to room temperature and adjust the final volume to 500 mL with DI. Store at room temperature. Stable for 6 months.

8.3 Preparation of Standards

All standards must be traceable back to the original vendor stock and that standard must be identified in detail (vendor, lot number, and certificate).

8.3.1 Calibration Stock Standard:

1000 ppm nitrate as N (NO_3^- -N) that is commercially prepared as purchased. Certificate of analysis is required. Used for calibration curves.

8.3.1.1 Working Standard 1(WS1): 50 ppm NO_3^- -N

To a 25 mL volumetric flask add 1.25 mL of the calibration stock standard and dilute to the mark with DI.

8.3.1.2 Autocalibration Standard: 1000 ppb NO_3^- -N

To a 25 mL volumetric flask, add 0.5 mL of WS1 and dilute to the mark with diluent.

8.3.1.3 CCV: 400 ppb NO_3^- -N

To a 25 mL volumetric flask, add 0.2 mL of WS1 and dilute to the mark with diluent.

8.3.1.4 Autocalibration Curve:

Calibration point	Analysis	1	2	3	4	5	6	7	8	9
Concentration as $\mu\text{g N/L}$	NO_3+NO_2	0	40	70	100	200	350	450	600	1000
	Total Nitrogen	0	68	80						
Percent of auto calibration standard used	NO_3+NO_2	0	4	7	10	20	35	45	60	100
	Total Nitrogen	0	6.8	8						

8.3.2 NO_3^- ICV Standard: 400 ppb NO_2^- -N

To a 25 mL volumetric flask add 0.1 mL of a second source 100 ppm nitrate as N stock standard and dilute to the mark with diluent.

8.3.1 NO_2^- RE Standard: 400 ppb NO_2^- -N

To a 25 mL volumetric flask add 0.1 mL of a 100 ppm nitrite as N stock standard and dilute to the mark with DI water.

8.3.2 Spike Stock Standard:

A third source standard containing 100 ppm NO_3^- -N used for LFB/MS/MSD. Certificate of analysis is required.

8.3.2.1 Working Standard 3 (WS3): 10 ppm NO_3^- -N

To a 50 mL volumetric flask add 5 mL of Spike Stock Standard. Dilute to the mark with DI.

8.3.2.2 LFB: 400 ppb NO_3^- -N

To a 25 mL volumetric flask add 1 mL of WS3. Dilute to the mark with diluent (Reagent 5).

8.3.2.3 MS/MSD

To a 25 mL volumetric flask add 1 mL of WS3. Dilute to the mark with sample water.

8.3.2.4 LFB Low:

Combined $\text{NO}_3^- + \text{NO}_2^-$: 40 ppb NO_3^- -N

To a 25 mL volumetric flask add 0.4 mL of WS 3. Dilute to the mark with diluent (Reagent 5).

Total Nitrogen: 68 ppb NO_3^- -N

To a 25 mL volumetric flask add 0.68 mL of WS 3. Dilute to the mark with diluent (Reagent 5).

8.3.3 DVS:

If using ERA WatR™ Pollution Complex Nutrients (#525) prepare the WS as follows:

8.3.3.1 Working Standard 3:

To a 50 mL volumetric flask, add 0.25 mL of stock solution, dilute to mark with diluent. Final concentration can be found in the CoA for the lot used.

8.3.3.2 Digestion Verification Standard:

Dilute WS3 in diluent to be within calibration range.

If preparing with ammonia:

8.3.3.3 Working Standard 3: 10 ppm standard solution as NH_3 -N

To a 50 mL volumetric flask add 0.5 mL of stock standard 1000 ppm of ammonia (NH_3) as N and dilute to the mark with DI.

8.3.3.4 NH_3 Digestion Verification Standard: 400 ppb NH_3 -N

To a 25 mL volumetric flask, add 1.0 mL of WS3 and dilute to the mark with DI.

8.4 Sample Preparation

8.4.1 Combined Nitrate and Nitrite (preserved, aqueous samples)

Unpreserved samples require no preparation. Preserved samples may be analyzed un-neutralized in pH 9.1 buffer. Should low recovery occur with 9.1

buffer, acidified samples may be neutralized.

Neutralization:

- 8.4.1.1 Prepare LFB Ls, LFBs, DUPs, MS/MSDs as in section 8.3.
- 8.4.1.2 Pour aliquots of samples (including QC), LFB Ls, LFBs, and an MB into labeled plastic cups.
- 8.4.1.3 In the fume hood, place the pH probe into the sample, and begin adding concentrated ammonium hydroxide dropwise.
- 8.4.1.4 Adjust pH to between 5 - 9. If pH exceeds 9, lower using sulfuric acid.

8.4.2 Total Nitrogen (preserved, aqueous samples) Digestion

- 8.4.2.1 Begin heating the autoclave prior to sample preparation.
- 8.4.2.2 Prepare standards as described in section 8.3.
- 8.4.2.3 Transfer 25 mL of samples, standards, and QC samples to digestion tubes.
- 8.4.2.4 Add 12 mL of the digestion solution (Reagent 6) to all digestion tubes.
- 8.4.2.5 Remove plastic liner from caps and place them slightly ajar on all samples to release any pressure.
- 8.4.2.6 Place samples in the autoclave, seal the chamber and start the preloaded method that is set to digest samples for 60 min at 121°C at constant pressure.
- 8.4.2.7 After cycle is finished, release pressure to open doors and remove samples.
- 8.4.2.8 Allow to cool to room temperature and analyze samples.
- 8.4.2.9 In the fume hood, neutralize to a pH between 5 to 9 using concentrated ammonium hydroxide as described in 8.4.1.

8.4.3 Nitrogen Analysis in Soils

- 8.4.3.1 Samples must be prepared for analysis of combined $\text{NO}_3^-/\text{NO}_2^-$ and Total Nitrogen. Weigh 0.5 to 1.0 g of sample and dilute to 25 mL with DI water. For total nitrogen, follow digestion procedure from 8.4.2 apart from adding 1 mL of borate buffer (Reagent 7).

8.5 Data Analysis and Calculation

- Allow at least 40 minutes for the lamp to warm up.
- Perform the appropriate coil regeneration procedure (See Section 12.1). Typically an Air/Water cleaning is sufficient.
- Perform the daily AQ300 daily startup.
- Place samples and standards in the carousel.
- Fill reagent wedges and place in the correct positions and start the analysis.
- AQ300 Analytical Sequence:
 - Auto calibration standard

- ICV NO3
- RE NO2 (Combined NO3+NO2)
- DVS (TN)
- MB
- CCV
- BLK
- LFB(s)
- Samples
- Calibration is completed by injecting standards and plotting absorbance vs standard concentration. Concentration is calculated using a first order polynomial regression equation.
- Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- Report sample results for nitrate/nitrite in µg N/L as N to two significant figures for samples above the reporting limit.
- The coil efficiency can be calculated by running a known concentration of nitrite standard, and a matching concentration of nitrate standard, typically the ICV. If the efficiency is less than 90%, the coil needs to be regenerated. If efficiency does not improve, the coil needs to be replaced. See Section 12.1 for coil maintenance.

$$E = \frac{[\text{NO}_3^-]}{[\text{NO}_2^-]} * 100$$

9.0 Data and Record Management

9.1 Reporting Limits

For aqueous samples, the RL is equal to the lowest calibration standard.

For soil samples: using 25 mL reflux tube:

$$\text{RL (mg/Kg)} = \frac{0.025 \text{ L} \times \text{low cal. std. (ug/L)}}{\text{wt. (g)}}$$

9.2 Data Package

Each data package must include a copy of the following:

- Project Notes
- Calculation Sheet (soils)
- Raw instrument data and analytical sequence (reports from SEAL instrument).
- Bench sheet
- Certificate(s) of Analysis

All of the above-mentioned files, excluding raw data files, are available in Y:\LSB\Wetchem in \Forms or in \Certificates of Analysis.

9.3 Project Review

Upon completion of a project a project review form should be filled out and accompany the final report in the report folder. The first section (requested analysis and data folder completeness check) should be completed by the analyst. The last two sections (data evaluation and final report) should be completed by two different chemists that have knowledge of the method. Project Review forms are available in Y:\LSB\Wetchem\Forms

10.0 Quality Control and Quality Assurance

10.1 Method Detection Limit (MDL):

A low LFB of a concentration two to three times the last calculated MDL is run with each analysis. At least once every thirteen months the MDL_s is calculated from low LFBs and the MDL_b is calculated from the blanks as described in 40 CFR 136 Appendix B. The verified MDL is the greater of the two values.

10.2 Initial Calibration Verification (ICV)

An ICV is prepared and analyzed immediately after the system has been calibrated to verify the accuracy of the initial calibration. The source standard is different from the source of the calibration standard. If not within $\pm 10\%$ of stated value, terminate analysis and recalibrate instrument.

10.3 Reduction Efficiency Check (RE)

A RE is prepared and analyzed before the first CCV/BLK pair in a combined nitrate/nitrite analysis to verify the coil reduction efficiency. If reduction efficiency is not above 90%, perform coil maintenance as described in Section 12.1.

10.4 Continuing Calibration Verification (CCV)

Analyze CCV after the ICV, after every ten injections, and at completion of analysis. Recoveries must be within 10% of the true value. If recoveries fall outside of this range the cause of the failure needs to be determined, corrected, and the instrument may need to be recalibrated.

10.5 Reagent Blank (BLK)

A water blank (DI water) needs to be analyzed immediately after every initial and continuing calibration verification standard and at the end of the sequence. Analyzed once per batch of 20 samples. Values that exceed the detection limit indicate laboratory or reagent contamination and corrective action must be taken.

10.6 Laboratory Fortified Blank (LFB)

An LFB is prepared and analyzed once per batch of 20 samples. It is analyzed after the continuing calibration and blank are run. Recovery needs to be within 90 - 110%. When sufficient performance data becomes available, control limits can be developed, and upper/lower control limits can be calculated.

10.7 Laboratory Fortified Matrix Samples (MS/MSD)

MS/MSD should be prepared and analyzed every 10 samples or less. Recoveries must be within $\pm 20\%$ of true value. MSD is not required for Total Nitrogen.

10.8 Digestion Verification Standard (DVS)

For total nitrogen, prepared and analyzed immediately before or after the ICV to verify the completion of the digestion. The stock source of nitrogen cannot be NO_3^- or NO_2^- , as NO_3^- would not be digested, and NO_2^- can be detected even with incomplete digestion by this method. A complex nutrient mix containing organic nitrogen, such as ERA's WatR™ Pollution Complex Nutrients, or ammonia are recommended for this standard. Recoveries must be within 10% of the true value.

11.0 Waste Management and Pollution Prevention

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner.

The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques found in method 353.2-12 to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

The quantity of chemicals purchased should be based on expected usage during its

shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202) 872-4477.

12.0 Preventative Maintenance

Daily maintenance includes emptying and replacing the rinse water, replacing used reaction wells, and running the "Daily Startup" function in the software to rinse the cuvette and perform the daily water baseline check. Verify smooth movement and good aspiration.

Weekly maintenance includes cleaning the sampler and aspiration wash baths, inspecting the probes to make sure they are straight/clean/not dripping, and verifying that the baseline voltages don't indicate that the lamp needs changing.

Monthly maintenance includes replacing and lubricating the pump tubes, bleaching the wash container with a 2% bleach solution, and removing and washing the fan air filter.

Six-month maintenance includes replacing the syringe assembly/o-ring, cleaning and lubricating the syringe screw drive, replacing the lamp, replacing the reagent wedges, replacing the probe wash assembly, and inspecting the cuvette tubing and probes.

All tubing and connectors should be checked during each run for leaks and blockages. Replace as necessary.

If results are inconsistent, and the cadmium coil has been properly maintained, verify the reaction pH by combining 5 mL acidified sample/standard and 2.65 mL working buffer. A reaction pH is ideal over 8.

Special care is required for the upkeep of the cadmium coil depending on the frequency at which the coil is used.

In general, if the coil is being used every two weeks one only needs to acid strip once a month and do a water/air cleaning every other week. If a coil has reduced efficiency and regeneration fails, a depleted coil should be disposed per health and safety SOP in LSASD.

12.1 Cadmium Coil Maintenance Order

12.1.1 New Coil

Perform an **Acid Strip** and **Install** the coil. In the Maintenance menu, prime the syringe and **Regenerate** according to the Acid Strip specifications.

12.1.2 Daily Start-up

If the coil has been used within 3 days, in the Maintenance menu, prime the syringe and **Regenerate** once with **water**.

12.1.3 Idle coil

If it has been 3 to 7 days since the coil as used, perform a **Water/Air Cleaning**, and **Install** the coil. **Regenerate** according to the Water/Air Cleaning instructions.

12.1.4 Low Reduction Efficiency

If coil reduction efficiency has fallen below 90% follow **New Coil** instructions.

12.2 Cadmium Coil Maintenance Instructions

12.2.1 Installation

Avoid making crimps or kinks to the 1/16" o.d. tubes.

12.2.1.1 The exposed ends are sleeved with Tygon® tubing, p/n 5719.

The tee connector on the rotating sampler arm connects to a 15 cm piece of 1/16" o.d. tubing.

12.2.1.2 Moisten the free end of this tube and insert it into the OTCR inlet sleeve, leaving 1 mm gap from the cadmium coil end.

12.2.1.3 Locate the 1/16" o.d. dilutor transfer line, which passes beneath the sampler arm cosmetic cover. Insert the end of this tube into the other sleeve at the outlet of the OTCR.

12.2.1.4 Prime the syringe twice to expel any air.

12.2.2 Water/Air Cleaning

12.2.2.1 Remove the coil by gently sliding the soft sleeve tubes off the cadmium ends.

12.2.2.2 Use a 6" piece of Tygon® tubing to connect the male luer of a 50 mL syringe to the coil. SEAL recommends tubing p/n 116-0536-18, having 0.11" i.d.

12.2.2.3 Using the syringe, rapidly draw deionized water into the coil, while purposely drawing air in alternation. Done vigorously, this technique expels the previous activated copper coating, seen as fine black flakes.

12.2.2.4 Regenerate once with copper sulfate and once with water.

12.2.3 Acid Strip

- 12.2.3.1** Remove the coil by gently sliding the soft sleeve tubes off the cadmium ends and attach the coil to the syringe as described above (12.1.2)
- 12.2.3.2** Draw 6 N HCl into the coil and stand for 2–5 minutes.
- 12.2.3.3** Perform Water/Air Cleaning (See 12.1.2)
- 12.2.3.4** Regenerate twice with copper sulfate.

12.2.4 Regenerate

- 12.2.4.1** In MAINTENANCE menu, select the Cadmium coil icon.
- 12.2.4.2** Place copper sulfate (or water) in position 18, 50 or 100 ppm high standard nitrate in position 17 and working buffer in position 16.
- 12.2.4.3** Click “continue” to run regeneration.

12.3 Cadmium Flush Volume

- 12.3.1** Log in to the software with the username “seal” and the password “toledo.” Enter into the maintenance menu and navigate to cadmium coil.
- 12.3.2** Place DI water in position 15 and click “Start” for the cadmium volume checker. In the line between the sample probe and the Y block there will be an air bubble. Click “More” until the air bubble enters into the cadmium coil and record the number displayed. Then click “Finish.”
- 12.3.3** In any cadmium coil method, adjust the “Cadmium Flush Volume” parameter to match the recorded number.

13.0 References

- 13.1** Standard Methods for the Examination of Water and Wastewater, 23rd Edition, Method 4500-NO3 F (1992)
- 13.2** U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes Method 353.2
- 13.3** AQ Method EPA-127-D Rev 2A Nitrate-N + Nitrite-N in Drinking and Surface Waters, Domestic and Industrial Waters
- 13.4** QuikChem Method 10-107-04-4-A. Determination Total Nitrogen in Manual Persulfate Digests, 11 December 2010

14.0 Appendix 1: Suggested Timing Parameter Table

PARAMETER	AQ SETTING
Test name	NO3_NO2 2
Units	mg N/L
Decimals	3
Test type	Cadmium reduction
Sample volume (µL)	500
Water volume (µL)	0
Number of mixes	2
Cuvette primes	1
Cuvette washes	2
Reduction time (seconds)	25
Cadmium flush volume*	470
Reaction time (seconds)	480
Wavelength (nm)	520
Polynomial order	1
Number of reagents	2
1. Working Buffer (µL)	265
2. Sulfanilamide – NEDD (µL)	350
Advanced Test Parameters	<input checked="" type="checkbox"/> Extra Debubbling Action

*Cadmium Flush Volume subject to slight changes, contact SEAL Tech Support for more information

15.0 Appendix 2: Acceptance Criteria

QA/QC Sample	Frequency	Acceptance Criteria	Corrective Action
Blanks: Digested for TN. Not digested for Nitrate/nitrite/combined	1 per batch (up to 20 samples) After calibration, continuing calibration, at the end of the run	< MDL	Recalibrate, prepare new blank
Initial Cal. Verification (ICV)	After calibration	$\pm 10\%$ of True value	Recalibrate, prepare new ICV
Continuing Calibration Verification (CCV)	After the ICV, every 10 samples and at the end of the run	$\pm 10\%$ of True value	Recalibrate
Laboratory Fortified Blank (LFB)	1 per batch (up to 20 samples)	$\pm 10\%$ of True value	Re-prep and if issue persist qualify data (J)
LFM or MS/MSD - Laboratory Fortified Matrix/ Matrix Spike	1 per 10 samples	$\pm 20\%$ of True value	Re-run analytical spike, if out of spec but LFB is within range, qualify data (J) with explanation.
Laboratory Duplicate	1 per batch (up to 10 samples)	$\pm 20\%$ RPD	Qualify data (J) for duplicate sample
Digestion Verification Standard	After calibration	$\pm 10\%$ of True value	Re-prep and if issue persist qualify data (J)
Sample /Holding Time	Section 8	Samples must be analyzed within holding times	If re-sampling is not available, samples are qualified (J).
IDC	Annually or when there is a change in the method or equipment	4 replicates of 2-5 times of MRL (lowest calibration point). 80-120% recovery <20% RSD	Investigate problems and repeat.
MDL	Initially and at least once every thirteen months	< half MRL, 80-120% recovery, <20% RSD	Investigate Problems and repeat.
QCS commercially prepared	Recommended run each time	Manufacturer certificate	Investigate problems and repeat the run