Standard Operating Procedure for the Determination of Total Nitrogen

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STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF TOTAL NITROGEN

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This document is effective upon final approval

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1.0 Identification of Test Method

1.1 This method is the Standard Operating Procedure for the determination of Total Nitrogen (TN) and Total Dissolved Nitrogen (TDN) in the laboratory of the South Florida Water Management District. It is identified by the method number SFWMD-LAB-SOP-3090-003 and is effective upon final approval.

1.2 TN/TDN is analyzed on a highly automated flow injection instrument called a Flow Injection Analyzer (FIA).

1.3 TN/TDN analysis is based on SM4500-NC. This method has been modified by optimizing the concentration and ratio of the digestion reagents ((potassium peroxidisulfate ($K_2S_2O_8$) and sodium hydroxide (NaOH)) for analyzing TN/TDN in acid preserved water samples (pH < 2 but > 1.3). The pH range of the digested TN/TDN samples is the same as the acid preserved water samples, and therefore the digested TN/TDN sample can be analyzed using the optimized automated cadmium reduction method that is based on QuikChem method 31-107-04-1-E (Lachat) and SM4500-NO3F omitting borate buffer. The ammonium chloride (NH_4Cl) / disodium ethylenediaminetetraacetic acid ($Na_2EDTA*2H_2O$) buffer and sulfanilamide ($C_6H_8N_2O_2S$) color reagents have different concentrations than those prescribed in the instrument manufacturer's recommendations and Standard Methods.

1.4 This revision optimized the SOP for laboratory production and minor edits.

2.0 Applicable Matrix or Matrices

2.1 This method can be applied to the determination of TN/TDN in surface water, groundwater and pore water.

3.0 Detection Limit

3.1 The detection limit for this method is determined using the procedure outlined in 40 CFR Part 136, Appendix B, using at a minimum seven replicates of a spiked sample. The prepared spiked sample value is preferably in the range of three to five times the expected detection limit, but not more than 10 times the determined detection limit.

3.2 The applicable method detection limit for TN/TDN is 0.02 mg/L. The MDL is reviewed annually by the QA officer and is subject to change. The current MDL is noted on the Test Control Parameters sheet and is available from the QA officer.

4.0 Scope and Application

4.1 This method is useful for determining TN/TDN in surface water, groundwater and pore water.

4.2 This method is applicable for ranges from MDL to 5.00 mg/L. Samples with concentrations greater than 5 mg/L (highest calibration standard) are diluted with reagent blank digestate and re-analyzed.

5.0 Summary of the Test Method

5.1 All forms of nitrogen are converted to nitrate nitrogen during the alkaline-persulfate digestion. NO_3-N in the digestate is reduced to NO_2-N by passage of the sample through a granular cadmium reduction column. The NO_2-N produced is determined by the diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2$*2HCl) to form an azo dye that is measured at 540nm on the Lachat FIA.

5.2 The recovery of organic N is verified using the acetanilide standard solution.

6.0 Definitions

6.1 A comprehensive listing of terms and definitions in common use in the SFWMD laboratory can be found in the Laboratory Quality Manual glossary. All acronyms are defined upon first use in this document. Only terms specific to this procedure are defined in this section.

7.0 Interferences

7.1 All samples are preserved with sulfuric acid to pH < 2 but > 1.3. Samples with pH < 1.3 will interfere with the conversion of nitrogen forms to nitrate nitrogen during digestion, NO_3-N reduction to NO_2-N in the cadmium reduction column, and/or the color development during analysis.

7.2 Low nitrogen content potassium persulfate must be used as digestion reagent. The high nitrogen content potassium persulfate will not only increase the MDL but also decrease the cadmium reduction column life.

7.3 High concentrations of iron, copper and other metals could interfere with the NO_3–N reduction in the cadmium column. EDTA is added to the buffer solution to reduce the interference. However, EDTA may precipitate in flow cell and waste line due to low solubility of EDTA in acid solutions, which makes the FIA baseline drift and noisy when it's mixed with acidified color reagent. The optimum EDTA concentration, that is high enough to complex iron and copper ions in water sample and low enough to prevent the precipitation in the flow cell and the waste line, should be prepared for the buffer solution to prevent the interferences.

7.4 High salinity samples must have the caps loose in order to vent chlorine gas during digestion; the chlorine gas could interfere with the NO_3-N reduction in the cadmium column.

7.5 TN and TDN samples should be digested separately from Total Phosphorous (TPO4) and Total Dissolved Phosphorous (TDPO4) samples to prevent potential nitrogen contamination from the TPO4/TDPO4 digestion reagent ammonium persulfate ($(NH_4)_2S_2O_8$).

8.0 Safety

8.1 Wear safety glasses and a full-length, long-sleeved laboratory coat.

8.2 Latex or polyethylene gloves (non-powdered) may be worn when handling the samples.

8.3 Preparation of all reagents containing sulfuric acid (H_2SO_4), sodium hydroxide or ammonium hydroxide (NH_4OH) should be conducted in a fume hood. Use Nitrobutyl gloves when handling concentrated acids and bases. Glass containers of concentrated sulfuric acid should be transferred inside an acid resistant rubber container.

8.4 Before starting any run, all lines connecting the instrument to the reagents should be checked and tightened if necessary. In case of a leak onto an electrical system, the power should be disconnected before conducting any repairs.

8.5 The electrical power should be disconnected before conducting any repairs inside the instrument, on controllers, electrical wiring or any other components near sources of electricity.

8.6 In case of a concentrated sulfuric acid spill, the spill should be first neutralized with an appropriate spill kit and the contaminated absorbent should be collected and placed into adequate storage containers for disposal.

8.7 The disposal of samples can be done in the sink with ample flushing using tap water.

8.8 All personnel conducting this method should be familiar with the SFWMD Chemical Hygiene Plan and should have reviewed any pertinent Material Safety Data Sheets.

9.0 Equipment and Supplies

9.1 Lachat Flow Injection Analyzer (FIA), Model 8500 consisting of:
- 9.1.1 Lachat autosampler (XYZ)
- 9.1.2 Lachat peristaltic pump
- 9.1.3 Lachat detector
- 9.1.4 Lachat TN manifold

9.2 Personal Computer equipped with Lachat Omnion software.

9.3 Class A volumetric glassware (pipettes and volumetric flasks).

9.4 Eppendorf 100 µL adjustable pippetor or equivalent.

9.5 Adjustable macropipetter.

9.6 Eppendorf repipettor with 50 mL capacity tip.

9.7 Autoclave unit.

9.8 Autoclavable tube racks.

9.9 Fisherbrand Borosilicate glass tubes with cap, 16×100 mm (Catalog #:14-962-26F) or equivalent.

9.10 Top loading balance.

9.11 Copperized Cadmium Column (Lachat catalog #50237) or manually prepared copperized cadmium column. See section 10.17.

10.0 Reagents and Standards

Note: The volumes of all following reagents and standards can be increased or decreased. Reagent ratios must be kept the same and all variances must be recorded in the reagent log book.

10.1 **50% Sodium hydroxide (Certified grade with N content ≤5 mg/L):** 50% sodium hydroxide solutions can be purchased commercially. If a 50% sodium hydroxide stock solution in not available it can be prepared as follows: Slowly add 100 g of sodium hydroxide to 100 ml of laboratory reagent water in a 500 ml beaker (**caution**: this will get very hot). Once this solution cools, store in a high density polyethyline (HDPE) bottle. This is stable for one year.

10.2 **TN/TDN Digestion Reagent:** Mix 814 ml of laboratory reagent water, 42 g of low nitrogen potassium persulfate and 12 ml of 50% sodium hydroxide in a 1 L HDPE bottle. Place on stir plate and stir until all potassium persulfate has been dissolved. **This solution must be prepared fresh <u>daily</u>.**

10.3 **Buffer** - In a 2 L volumetric flask, dissolve 140 g of ammonium chloride and 0.4 g of disodium ethylenediamine tetraacetic acid dihydrate in approximately 1500 ml of laboratory reagent water. Adjust solution to pH of 9 using 16 ml of concentrated ammonium hydroxide. Dilute to the mark with laboratory reagent water and mix. This reagent is stable for one year. **This reagent must be prepared in a hood.**

10.4 **Color reagent** - In a 500 ml volumetric flask, add 50 mL of 85% phosphoric acid (H_3PO_4), 20 g of sulfanilamide and 0.5 g of N-1 Naphthylethylenediamine dihydrochloride in approximately 300 ml of laboratory reagent water. Stir for 30 minutes on stir plate, dilute to mark with laboratory reagent water, and mix. Store the reagent in a light-resistant HDPE bottle. This reagent is stable for one month.

10.5 **Carrier solution** – The carrier solution is digested in the same process as the samples. In a 500 ml autoclaveable screw top container containing 230 ml of standard 7, add 230 ml of the digestion reagent (10.2). Cap the bottle and mix. Loosen the cap ¼ turn and autoclave this solution with the samples (14.2).

10.6 Stock TN (as NO_3-N) standard (N.I.S.T. traceable stock TN standards are purchased commercially and come in concentration of 1000 mg/L). Alternately, TN stock can be prepared as follows:

10.6.1 **TN 1000 mg/L** – Dissolve 7.218 g of potassium nitrate (KNO_3) dried at 100°C into a 1 L volumetric flask containing 800 ml of laboratory reagent water acidified with 2 ml of 50% sulfuric acid (H_2SO_4). Dilute to the mark with laboratory reagent water and mix. This standard is stable for one year.

10.6.2 **Organic Check Stock (TN 100 mg/L)** – Dissolve 0.4825 g of acetanilide ($CH_3CONHC_6H_5$) dried at 100°C into a 500 ml volumetric flask containing 350 ml of laboratory reagent water. Dilute to the mark with laboratory reagent water and mix. Store this solution in a brown HDPE bottle and refrigerate. This standard is stable for one year.

10.7 **Sulfuric Acid 36 N**, ACS Plus grade, concentrated.

10.8 **Sulfuric Acid 18 N**: Add 100 mL of 36 N sulfuric acid to 100 mL of laboratory reagent water. **This reagent must be prepared in an ice bath.**

10.9 **All standards must be prepared fresh <u>biweekly</u> using class A volumetric pipettes and flasks.**

10.10 **See table 24.3 for Standard preparation.**

10.11 **Spiking Solution 60 mg/L TN** - To a 100 ml class A volumetric flask containing 50 ml of laboratory reagent water, pipette 6 ml of 1000 mg/L TN stock solution. Dilute to the mark with laboratory reagent water and mix.

10.12 **Organic Check Solution 4 mg/L TN (LCS3) –** To a 100 ml class A volumetric flask containing 50 ml of laboratory reagent water, pipette 0.2 ml of 18N sulfuric acid and 4 ml of organic stock solution. Dilute to the mark with laboratory reagent water and mix.

10.13 **All LCS's must be prepared monthly and are prepared from a separate source or distinct lot number than the source used for the calibration curve.**

10.14 N.I.S.T. – Traceable stock TN standards (as NO_3-N, LCS stock) are purchased commercially and come in concentrations of 1000 mg/L for TN. Alternately, the stock solution can be prepared as follows:

10.14.1 **TN 1000 mg/L** – Dissolve 3.611 g of potassium nitrate (KNO_3) dried at 100°C into a 500 ml volumetric flask containing 350 of ml laboratory reagent water acidified with 1 ml of 18N sulfuric acid. Dilute to the mark with laboratory reagent water and mix. This standard is stable for one year.

10.15 **See table 24.4 for LCS's preparation.**

10.16 **LCS6 (1.00 mg/L TN):** Prepared as a saline matrix check. The concentration is updated with each prepared lot. See LIMS standard log.

10.17 **Copperized Cadmium** – Pre-packed copperized cadmium columns are purchased commercially from Lachat instruments (catalog #50237). If pre-packed copperized cadmium columns cannot be obtained, they can be prepared as follows:

10.17.1 **Copperized Cadmium - Equipment and Supplies**

10.17.1.1 Lachat acrylic column

10.17.1.2 U.S.A. Standard Testing Sieve #18

10.17.1.3 125 mL HDPE bottle

10.17.1.4 250 mL HDPE bottle

10.17.2 **Copperized Cadmium - Reagents and Standards**

10.17.2.1 **Cadmium Granules** (Particle Size 0.3 – 1.6 mm)

10.17.2.2 **2% Cupric Sulfate –** In a 1 L volumetric flask, dissolve 32 g of cupric sulfate pentahydrate ($CuSO_4*5H_2O$) in approximately 700 mL of laboratory reagent water. Dilute to the mark with laboratory reagent water and mix. This reagent is stable for one year.

10.17.2.3 **10% Hydrochloric acid (HCl) –** In a 250 mL HDPE bottle filled with 180 mL of laboratory reagent water, add 20 mL of concentrated hydrochloric acid and mix. This reagent is stable for one year.

10.17.3 **Copperized Cadmium - Procedure**

NOTE: Never allow air to enter the column after liquids have been introduced into the column. Always use gloves when working with cadmium granules.

10.17.3.1 Pour the cadmium granules into the U.S.A. Standard Testing Sieve #1, gently sieve the granules and retain the fraction between 25 and 60 mesh size (0.25 – 0.71 mm). Discard granules that appear like powder into the waste cadmium container, these will plug the column.

10.17.3.2 Pour the sieved granules into a labeled 125 mL HDPE bottle.

10.17.3.3 Attach the outgoing end of the acrylic column to the manifold fitting attachment. Use a small funnel to fill the column with the sieved cadmium granules. When the column is packed to the top, gently tap the column with your finger to allow the granules to become further packed filling empty spaces. Refill the column to the top with granules and attach the other end of the manifold fitting attachment to the column.

10.17.3.4 Place the buffer reagent line and the carrier line in the 250 mL bottle containing 10% hydrochloric acid. Place the color line in a separate container with laboratory reagent water. Turn on the Lachat pump module and allow the manifold to fill with 10% hydrochloric acid and laboratory reagent water until no air remains in the manifold. Open the manifold valve allowing 10% hydrochloric acid to enter the cadmium column. When the column fills with 10% hydrochloric acid, gently tap the column so the granules will further pack. Turn off the manifold valve, remove the manifold fitting attachment to the column where the liquids are introduced and fill the column with granules to the top. Replace the manifold fitting attachment on the column, open the manifold valve to allow 10% hydrochloric acid to flush through the column for two minutes and then close the manifold valve.

10.17.3.5 Place the buffer reagent line and the carrier line in laboratory reagent water and open the manifold valve when all the air has exited the manifold. Allow laboratory reagent water to flush through the column for one minute and then close the manifold valve.

10.17.3.6 Place the buffer reagent line and the carrier line in 2% cupric sulfate and open the manifold valve when all the air has exited the manifold. Allow 2% cupric sulfate to flush through the column for 2 ½ minutes and then close the manifold valve.

10.17.3.7 Carefully detach the manifold fittings from both ends and reverse the column. Re-attach the manifold fittings to the column, open the manifold valve and continue flushing 2% cupric sulfate through the column for 2 ½ minutes and then close the manifold valve.

10.17.3.8 Place the buffer reagent line and the carrier line in laboratory reagent water and open the manifold valve when all the air has exited the manifold. Allow laboratory reagent water to flush through the column for one minute and then close the manifold valve.

10.17.3.9 Place the buffer reagent line in the buffer reagent and the carrier line in the carrier reagent and open the manifold valve when all the air has exited the manifold. Allow the reagents to flush through the column for five minute and then close the manifold valve.

10.18 All Standard stocks and LCS stocks "Certificates of Analysis" or product assays must be scanned into LIMS using the procedure described in SFWMD-LAB-SOP-5510, current version.

10.19 All working standard, reagent and LCS containers must be properly labeled as per Laboratory Quality Manual, current version.

11.0 Sample Collection, Preservation, Shipment and Storage

11.1 Samples are collected using the procedures outlined in the SFWMD Field Sampling Quality Manual, current version.

11.2 All samples are preserved by the addition of sulfuric acid to a $1.3<pH<2$, placed in a HDPE bottle, and stored at ≤6°C and not frozen.

11.3 Samples must be analyzed with 28 days of collection.

11.4 Samples are transported to the lab in coolers containing wet ice or other devices to maintain the temperature of the sample at ≤6°C and not frozen until delivery to the SFWMD laboratory.

11.5 Samples are stored in the laboratory in a cooler that is maintained at ≤6°C and not frozen.

12.0 Quality Control

12.1 The TN standard recoveries are checked by the analyst before conducting sample analyses.

12.2 A LCS1 is rerun every 20 samples and a Continuing Calibration Check (CCV) (Standard 2) is rerun every 20 samples, both are run at the end of the run.

12.3 LCS1, LCS2, LCS3, LCS5, LCS6, and Method Blank are analyzed at beginning of each batch.

12.4 A Method Blank is run at the beginning of each 20 sample bracket.

12.5 A matrix spike (MS) analysis (sample selected at random) should be conducted for every 20 samples analyzed. The matrix spike recovery is determined (using the formula in section 15.0).

12.6 A repeat analysis (sample selected at random) or a matrix spike duplicate (MSD) should be conducted for every 20 samples analyzed. The relative percent difference (RPD) of the duplicate set is determined using formula in section 15.0.

13.0 Calibration and Standardization

13.1 The calibration curve is analyzed in the order of the highest concentration standard to the lowest concentration standard and consists of 7 calibration points.

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13.2 The correlation coefficient must be ≥0.998.

13.3 Matrix spikes are prepared by adding 0.1 ml of **TN Spiking Solution (60 mg/L TN, 10.11)** to a sample digestion tube selected at random. The 5 ml sample is pipetted into the digestion tube with the spike and digested normally.

13.4 All LCS's must be within established limits.

13.5 Laboratory Blanks must be less than the Method Detection Limit.

13.6 TN and TDN quantitation is based on peak areas.

13.7 Peak areas are calculated using the **Omnion** software "brackish" window option. This option forces the software to integrate the refractive dip in the chromatogram that is caused by brackish and saline samples insuring the proper peak integration of low concentration, blank and saline samples.

14.0 Procedure

14.1 To create an analytical batch, refer to SFWMD-LAB-SOP-5000, current version.

14.2 Sample Digestion

14.2.1 Referring to the batch, complete a digestion log (refer to section 24.7 example) with the following information: date and time digestion was started, autoclave temperature and pressure, digestion reagent lot #, and the order in which each sample is processed.

14.2.2 Take the quality control solutions, checks, and samples needed for digestion out of the refrigerator and let them reach room temperature Note: Samples should remain in their original tray during processing.

14.2.3 A set of working standards is digested for each batch up to 100 samples.

14.2.4 Arrange disposable digestion tubes in the digestion rack according to the tray protocol.

14.2.5 Using a calibrated pipetter with disposable tips, pipette 5 ml aliquot of standards, LCS's and samples into their respectively labeled tubes while changing tips between each aliquot. Each solution and sample must be mixed thoroughly before taking an aliquot.

14.2.6 Using a calibrated 100 µL pipetter with disposable tip, pipette 100 µl of spike solution into the MS/MSD tubes.

14.2.7 To each tube, add 5 ml of digestion reagent using a repeater pipettor with 50 ml tip.

14.2.8 When all tubes are filled, cap the tubes tightly. Put a board on the top of the tubes, hold the board and sample rack with hands and invert the tubes 10 times to mix the digestion reagent with the samples.

14.2.9 Prepared samples must be digested as soon as the digestion reagent is added. Loosen the caps ¼ turn, place a sterilization indicator strip on the rack and place the tray in the autoclave. Samples must be autoclaved for 30 minutes. (See autoclave SOP for operating instructions: SFWMD-LAB-SOP-5300).

14.2.10 When the cycle is finished, the autoclave will automatically stop and the pressure will decrease gradually. Note: The autoclave door will not open until the pressure is completely released. **Allow digested samples to sit a minimum of 1 hour prior to analysis.**

14.3 Turn on Lachat 8500 FIA (Autosampler and System Unit).

14.4 Latch platens and turn on Lachat peristaltic pump module.

14.5 Place all the lines in laboratory reagent water. Check for leaks and once baseline is stable, write down the baseline milli-voltage.

14.6 Connect all reagent lines to their perspective containers. See analytical diagrams (Section 24.6) for proper placements of reagent lines with reagents.

14.7 Click on the configuration pull down menu and select autosampler. Make sure the autosampler probe depth in the sample cup is 20 mm less than that in the wash bath. Then click the initialize autosampler.

14.8 After all air bubbles have passed through the system turn the cadmium column bypass valve to the open position so the sample and reagent stream pass through the column.

14.9 Open **Omnion** on the computer by selecting its icon on the Windows desktop and open the TN sample table template.

14.10 To create the TN sample table in **Omnion**, load the TN template and import the batch created in LIMS.

14.11 When the analytical run is started the **Omnion** software will automatically assign a run I.D. using an OM_date_time.OMN format (i.e. OM_8-24-2011_10-03-52.OMN).

14.12 Click on the preview icon in **Omnion.**

14.13 Uncap the digested sample tubes and begin pouring the working standards, LCS's and the first 20 matrix samples. If the digested samples have particles/sediments, the top clear digested solutions should be transferred into the centrifuge tubes using the disposable pipettes.

14.14 When the baseline is stable and smooth click **Start** to begin data collection. Check to make sure that the autosampler probe is at least 1 cm above the bottom of the digestion/sample tube.

14.15 Click **Spectra** icon to monitor data collection. Spikes and noise in the baseline or elevated baseline are caused by air bubbles, clogged lines and/or expired or contaminated reagents.

14.16 Observe that the standards appear linear and that peaks do not have spikes or any unusual shape to them.

14.17 Once the peaks for the calibration curve and LCS samples appear, verify that the correlation coefficient is ≥0.998 and all LCS recoveries fall within QC limits.

14.18 If any samples are over range dilute the sample using digested blank to the middle of the curve and repeat the sample after the over-range sample.

14.19 Pour the next 20 samples, once the matrix spike sample for the 2nd group of 20 samples is analyzed, resume pouring the next group and so on, as per 14.13.

14.20 When the analytical run is complete and the Lachat software stopped collecting samples, turn the cadmium column bypass valve to the closed position.

14.21 Clean analytical manifold by placing the lines in laboratory reagent water for 5 minutes. Follow by placing the lines in 50% HCl solution for 5 minutes, and finish the cleaning process by placing the lines back in laboratory reagent water for 5 more minutes.

14.22 Turn off the Lachat peristaltic pump module and unlatch platens.

14.23 Turn off the Lachat autosampler and system unit.

14.24 Consult your supervisor before making any major changes, adjustments, and/or repairs to the instrumentation.

14.25 To send the data to LIMS see SFWMD-LAB-SOP-5000, current version.

15.0 Calculations

15.1 The recovery of the CCV is calculated as follows:

$$\%\text{Recovery} = \left(\frac{\text{CCV Value}}{2.5 \text{ mg/L}} \right) \times 100$$

15.2 The recovery of the matrix spike is calculated as follows:

$$\%\text{Spike Recovery} = \left(\frac{[\text{Spiked Value} \times 1.02 - \text{Sample Value}]}{1.2} \right) \times 100$$

*The value of 1.02 is the correction for the dilution during the spike preparation and 1.2 is the spike amount with dilution correction.

15.3 The relative percent difference (RPD) is calculated as follows:

$$RPD = \frac{|(Value1 - Value2)|}{\left(\frac{(Value1 + Value2)}{2} \right)} \times 100$$

15.4 The recoveries of the LCS's are calculated as follows:

$$\%\text{Recovery} = \frac{ObservedValue}{TrueValue} \times 100$$

16.0 Method Performance

16.1 This procedure was developed based on the following:

16.1.1 TN recovery using different persulfate and sodium hydroxide concentration in the digestion reagent.

16.1.2 TN recovery of the water/sea water sample with different pH (sulfuric acid concentration.

16.1.3 TN recovery of different organic and inorganic N compounds.

16.1.4 Compared 773 water sample TN results from this method with those calculated from the sum of TKN and NOX. The method validation package is available from the QA officer.

17.0 Pollution Prevention

17.1 All reagents and standards used in this method pose little threat to the environment when disposed down the sink with excess tap water.

17.2 Standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents to be disposed.

18.0 Data Assessment and Acceptance Criteria for Quality Control Measures

18.1 After the analytical run is complete, check each quality control value to be sure it falls within acceptable limits per Laboratory Quality Manual, current version.

18.2 Verify the correctness/acceptability of the results (lab and field QC criteria). Note any discrepancies on the general chemistry checklist.

18.3 Fill out the FIA stability checklist with the date, analyst, batch #, water baseline milli-voltage, the reagents baseline milli-voltage and the peak area of the highest standard.

19.0 Corrective Actions for Out-of-Control Situations

19.1 See the Table 24.1 for corrective actions. The recommended corrective actions in Table 24.1 are intended as general guidelines to initiate the analytical trouble shooting process. In all cases, if the out-of-control condition persists beyond the second step in troubleshooting, notify your supervisor of the condition and request assistance.

20.0 Contingencies for Handling Out-of-Control or Unacceptable Data

20.1 The LIMS system is designed to review for out of control conditions and will apply the appropriate qualifiers upon data review and supervisor approval.

20.2 It may be necessary to obtain technical service to make repairs to instruments that are producing unacceptable data. See your supervisor for assistance in obtaining this service.

21.0 Waste Management

21.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

22.0 Instrument Maintenance

22.1 A maintenance logbook for each piece of instrumentation identified in this procedure shall be maintained by the analyst. The maintenance logbook must include the following items:

22.1.1 The name or laboratory designation of the instrument and the associated software.

22.1.2 The manufacturer's name, type identification, serial number or other Unique identification, and the SFWMD inventory control number.

22.1.3 Initial calibration records or other checks to confirm that the instrument complies with the method requirements.

22.1.4 The current location of the instrument.

22.1.5 Manufacturer's instructions, owner's manuals, or reference to their location.

22.1.6 Dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of the next calibration.

22.1.7 The maintenance plan as appropriate, and documentation of all maintenance carried out to date, including all routine and non-routine maintenance activities and reference material verifications.

22.1.8 Records of any damage, malfunction modification or repair to the instrument.

22.1.9 The date received and condition when first placed into service.

22.2 The following maintenance must be carried out by the analyst analyzing TN/TDN on the Lachat instrument and noted in the maintenance logbook:

22.2.1 The manifold must be rebuilt when the millivolts differ >0.005 on Standard 1 from the beginning of the peak area window to the end of the peak area window.

22.2.2 The pump tubes must be replaced when the peak morphology is being affected.

22.2.3 The cadmium column must be rebuilt when the CCV's and LCS's begin to decline throughout the analytical run. The condition of the cadmium granules in the column should be visually checked with every analytical run. The column should be repacked or replaced when the granules begin to "pack" together of if unusual conditions are observed.

22.2.4 The 540nm filter should be replaced every two years or when the peak area of Standard 1 begins to decline.

23.0 References

23.1 SFWMD-LAB-SOP-5000, current version.

23.2 Standard Methods for the Examination of Water and Wastewater, 21th Edition, SM4500NC.

23.3 Standard Methods for the Examination of Water and Wastewater, 20th Edition SM4500NO3F.

23.4 Chemistry Laboratory, Quality Manual, current version.

23.5 Lachat Omnion tutorial manual 3.0.

23.6 Lachat Omnion QuikChem 8500 Manual.

23.7 Lachat QuikChen method 31-107-04-1-E

23.8 SFWMD-LAB-SOP-5510, current version.

23.9 SFWMD-LAB-SOP-5030, current version.

24.0 Tables, Diagrams, Flowcharts and Validation Data

24.1 Corrective actions for out-of control laboratory quality controls.

LCS Activity	Acceptance Criteria	Recommended Corrective Action
Initial Instrument Blank Method Reagent Blank	<MDL response & value	Trouble shoot the instrument and determine cause of error (reagents, calibration standards, environment, equipment failure, etc.), correct the problem, then reanalyze.
Initial Calibration Standards	Correlation coefficient ≥0.998 for all other analyses	Re-analyze standards. If same response is obtained, trouble shoot the instrument & re-start analysis. If same response is obtained, prepare new standards & re-start analysis.
Quality Control or Check Standards	Accuracy within established limits. See Quality Manual	Re-analyze LCS check standard. If same response is obtained, trouble shoot the instrument & re-analyze. If same response is obtained, prepare new LCS & re-start analysis.
Continuing Calibration Standard	Accuracy within established limits	Re-analyze CCV. If same response is obtained, trouble shoot the instrument & re-start analysis. If same response is obtained, prepare new standards & re-start analysis.
Matrix Spikes	Accuracy within established limits. See Quality Manual	Re-analyze and re-make if needed. If results are not acceptable, spike a different sample. If second sample is not acceptable, spike all samples in that work order to check for matrix interference. If second spike is acceptable notify your supervisor.
Replicate/Duplicate Sample	Precision within established limits. See Quality Manual	Re-analyze sample. If same response is obtained, trouble shoot process. Re-analyze affected samples as appropriate.

Note: The recommended corrective actions in Table 24.1 are intended as general guidelines to initiate the analytical trouble shooting process. In all cases, if the out-of-control condition persists beyond the second step in troubleshooting, notify your supervisor of the condition and request assistance.

24.2 Corrective actions for out-of-control field quality control samples.

LCS Check	Acceptance Criteria	Recommended Corrective Action
Field Duplicates (FD) Replicate Samples (RS) Split Samples (SS)	Precision within limits See Quality Manual (only if values > 5*PQL)	Laboratory should re-analyze failing field QC samples to confirm results.

24.3 Standards Preparation Table:

STANDARD	CONCENTRATION mg/L	ADD SOLUTION	*50% H_2SO_4* mL	*FINAL VOLUME* mL
TN (NO_3-N) STOCK SOLN	1000	-	-	-
Solution A	25.0	5 mL stock		200
Standard 1	5.00	20 mL soln. A	0.2	100
Standard 2	2.50	10 mL soln. A	0.2	100
Standard 3	1.00	4 mL soln. A	0.2	100
Standard 4	0.50	2 mL soln. A	0.2	100
Standard 5	0.15	6 mL STD 2	0.2	100
Standard 6	0.05	2 mL STD 2	0.2	100
Standard 7	0	D.I water	2	1000

24.4 LCS's Preparation Table:

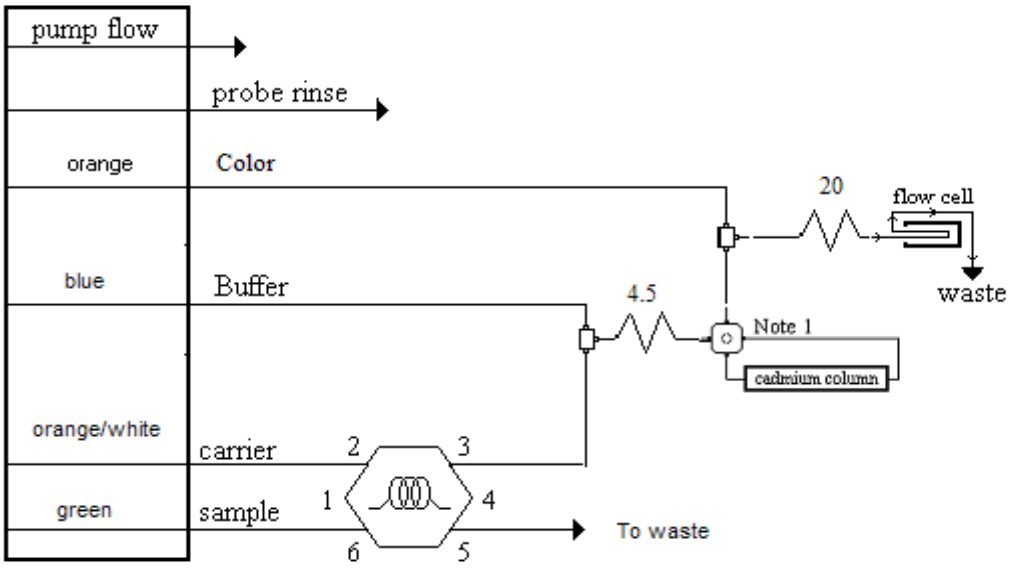
STANDARD	CONCENTRATION mg/L	ADD SOLUTION	*50% H_2SO_4* mL	*FINAL VOLUME* mL
TN (NO_3-N) STOCK SOLN	1000	-	-	-
LCS1	4.00	2 ml Stock	1	500
LCS2	0.40	10 mL LCS1	0.2	100
LCS3	4.00	See Section 10.12	-	-
LCS5	0.05	Use STD 6	-	-
LCS6	Varies (See LIMS for T.V.)	See Section 10.16	-	-

24.5 Configure/Data system parameters (Omnion Software):

(All time and probe depth are approximate and may vary with instrument conditions)

Tab	Item	Setting
Configure Autosampler	Autosampler Type	ASX500
	Tray	ASX500
	Standard Rack	Slot 1
	Standard Rack	Type ASX500
	Probe Depth (mm)	Sample Cup Depth 145
	Probe Depth (mm)	Wash Bath Depth 160
Racks	Racks	AS4X60
Timing - Run	Method cycle period	75
	Sample period	15
	Probe in wash	60
	Pump standby	Off
	Use minutes	Off
	Channel in minutes	Off
	Analyte in minutes	Off
	Pump idol before standby	60
	Pump speed before analysis	30
Timing – Channel 2	Load period	8
	Inject period	20
	Time to valve	35
	Use retention time	Off
Timing – TN	Expected inject peak start	33
	Expected peak base width	80
	Brackish shutter offset	0
	Brackish shutter width	79
Analytes – Channel 2	Channel	Off
	Method	FIA
	Heater Setpoint	Off
Analytes –TN	Analyte name	Total N
	Concentration units	mg/L
	Calibration fit	First order
	Clear calibration	On
	Force through zero	Off
	Calibration weighing	1/X
	Auto dilution trigger	Off
	% high standard	110
	Chemistry	Brackish
	Calibration by height	Off

24.6 Analytical Cartridge Diagram for TN:



Carrier:	Digested standard 8
Manifold Tubing:	0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
Sample Loop:	20 cm
Interference Filter:	540 nm
Apparatus:	An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.
4.5:	70 cm of 0.8 mm tubing on a 4.5 cm coil support
20:	500 cm of 0.8 mm i.d. tubing on a 20 cm coil support
Note 1:	Switching valve for Cd column

24.7 Digestion Log

PAGE 1 OF _2____

TEMP:_____ ° C

TOTAL N DIGESTION LOG SM4500N-C PRESSURE:_____PSI

AUTOCLAVE USED: CONSOLIDATED [ ] MARKET FORGE* [ ]

Prep Batch #	Digested By :	Date/Time Digestion Started:
Analytical Batch #	Analyzed By:	Date/Time Digestion Ended:
Analytical HBN#	Analysis Date:	* No detail tape with the Market Forge
Digestion Reagents: NaOH: Fischer lot# _____		Peroxydisulfate: Fluka lot# _____

RACK 1 ""				RACK 2 ""			
Tube #	**Sample ID**	**Tube #**	**Sample ID**	**Tube #**	**Sample ID**	**Tube #**	**Sample ID**
1	S1	21		1		21	
2	S2	22		2		22	
3	S3	23		3		23	
4	S4	24		4		24	
5	S5	25		5		25	
6	S6	26		6		26	
7	S7	27		7		27	
8	LCS1	28		8		28	
9	LCS2	29		9		29	
10	LCS6	30		10		30	
11	LCS 3	31		11		31	
12		32		12		32	
13	**Spike #1**	33		13		33	
14	**MSD**	**34**		14		34	
15		**35**	**Spike #2**	15		35	
16		**36**	**MSD**	**16**		36	
17		37		**17**	**Spike #3**	37	
18		38		**18**	**MSD**	38	
19		39		19		**39**	**Spike #4**
20		40		20		**40**	**MSD**

PAGE 2 OF _2____

TEMP:_____ °C

TOTAL N DIGESTION LOG SM4500N-C PRESSURE:_____PSI

AUTOCLAVE USED: CONSOLIDATED [ ] MARKET FORGE* [ ]

Prep Batch #	Digested By :	Date/Time Digestion Started:
Analytical Batch #	Analyzed By:	Date/Time Digestion Ended:
Analytical HBN#	Analysis Date:	* No detail tape with the Market Forge
Digestion Reagents: NaOH: Fischer lot# _____	Peroxydisulfate: Fluka lot# _____	

RACK 1 ""				RACK 2 ""			
Tube #	**Sample ID**	**Tube #**	**Sample ID**	**Tube #**	**Sample ID**	**Tube #**	**Sample ID**
1		**21**	**Low Spike #5**	1		**21**	
2		**22**	**MSD#5**	2		**22**	
3		23		**3**	**Low Spike #6**	23	
4		24		**4**	**MSD#6**	24	
5		25		5		25	
6		26		6		26	
7		27		7		27	
8		28		8		28	
9		29		9		29	
10		30		10		30	
11		31		11		31	
12		32		12		32	
13		33		13		33	
14		34		**14**	**Low Spike #7**	34	
15		35		**15**	**MSD#7**	**35**	
16		36		16		**36**	**LCS6**
17		37		17		37	S8
18		38		18		38	S1
19		39		19		39	S2
20		40		**20**		40	LCS1

24.8 Checklist

CHECKLIST

Parameter / SOP #	
Analysis Date	
ANALYSIS HBN #	
PREP HBN #	

Analyst Review	**ANALYST YES**	**ANALYST NO**	**SPVR YES**	**SPVR NO**
Initial Calibration and Quality Control criteria met				
Continuing Quality Control and Calibration criteria met				
Sample Analysis				
Sample holding times met				
Reported Data readings within the calibration range				
Samples analyzed out of designated bottle set (if not note in comments)				
Other				
Required forms completed and submitted				
Above cited SOP read and followed				
All reagents and standards documented as required				
Electronic Content				
Post Run Annotations				
All required documents printed / scanned into LIMS				
All stability infromation entered into the proper spreadsheet				

Comments: _____

Analyst: _____ **Date:** _____

Supervisor: _____ **Date:** _____

QA: _____ **Date:** _____

25.0 **SOP Addendums and Changes**

26.0 Document History

Document Version	Date Issued	Description	Author
3090-001	10/28/13	New SOP	Meifang Zhou SFWMD
3090-002	4/18/14	Clarification of reference method	Meifang Zhou SFWMD
3090-003	5/19/14	New revision of SOP	Chris Janson SFWMD