CENTER FOR RESERVOIR AND AQUATIC SYSTEMS RESEARCH (CRASR)

STANDARD OPERATING PROCEDURE #7.0 – Determination of Total N in Water
Analytical Range: 1 to 1000 μg N/L

Revision 1
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1. Method Description

Nitrate is measured in digested samples for determination of TN. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone also can be determined by removing the cadmium column.

2. Equipment and Supplies

2.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

2.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

2.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

2.3.1. Autosampler

2.3.2. Multichannel proportioning pump

2.3.3. Reaction unit or manifold

2.3.4. Colorimetric detector

2.3.5. Data system

2.3.6. Acid washed glassware: All glassware used in the determination of phosphate should be washed with 10% muriatic acid and triple rinsed with distilled water. Preferable, this glassware should be used only for the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again.

2.4. Special Appartus:

2.4.1. Autoclave or Hot Plate

3. Sample Collection and Preservation – See CRASR SOP # 2 for details.

4. Standards

a. Nitrate-N Stock solution (1000 mg P/L) – Add 500 ml deionized water to a 1000 ml volumetric flask. Carefully weigh out 6.0677 g of ACS grade sodium nitrate ($NaNO_3$; FW=84.99) and add it to the flask. Dissolve material, dilute to 1000 ml, and mix well. Solution may be stored indefinitely in refrigerator if also using a laboratory control standard (LCS) as a calibration verification in analyses. If not using LCS calibration verification, the Nitrate-N stock solution should be prepared fresh monthly.

b. Total Nitrogen standard (1.0 mg N / L) – The Total Nitrogen standard is used for the determination of NO2-N+NO3-N in preserved and digested water samples. To prepare, add 500 ml of water to a 1000 ml volumetric flask. Carefully pipette 1.0 ml of Nitrate-N Stock Solution into the flask. Dilute to 1000 ml and mix well. The DNP Mixed standard may be stored in the refrigerator for up to 48 hours.

c. Working standards – Working standards are used as calibration and continuing calibration verification (CCVs) standards in the determination of PO4-P in digested samples. Prepare mixed working standards at concentrations (high range) of 5, 10, 25, 50, 100, 250, 500, and 1000 ppb (µg/L) PO_4-P, or (low range) 5, 10, 25, 50, 75, 100, 150, 200 and 250 ppb or (µg/L) PO_4-P. The following table outlines preparation, use pipet:

HIGH RANGE

Target Concentration (µg/L)	Total volume (ml)	DNP Mixed Standard Concentration (µg/L)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	49.75	0.25
10	50	1000	49.50	0.50
25	50	1000	48.75	1.25
50	50	1000	47.50	2.50
100	50	1000	45.00	5.00
250	50	1000	37.50	12.50
500	50	1000	25.00	25.00
1000	50	1000	0.00	50.00

LOW RANGE

Target Concentration (µg/L)	Total volume (ml)	DNP Mixed Standard Concentration (µg/L)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	49.75	0.25
10	50	1000	49.50	0.50
25	50	1000	48.75	1.25
50	50	1000	47.50	2.50
75	50	1000	46.25	3.75
100	50	1000	45.00	5.00
150	50	1000	42.50	7.50
200	50	1000	40.00	10.00
250	50	1000	37.50	12.50

d. Mixed working standards may be stored in the refrigerator for up to 48 hours.

e. Laboratory Control Standard – A laboratory control standard of 150 ppb should be prepared from a purchased certified aqueous nitrate-nitrogen standard. This standard should be certified by the external source from which it was purchased.

5. Reagents

Digestion Solution – Dissolve 20.1 g ACS grade (low nitrogen) potassium persulfate ($K_2S_2O_8$) and 3.0 g NaOH in 1000ml DI water.

Borate Buffer Solution – Dissolve 61.8 g boric acid (H_3BO_3) and 8.0 g NaOH in 1000 ml DI water.

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube (Lachat Part No. 50100.) Bubble He through the solution for one minute.

Reagent 1. 15 N Sodium Hydroxide

By Volume: Add **150 g NaOH** very slowly to **250 mL or g of water**. **CAUTION:** The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

Reagent 2. Ammonium Chloride buffer, pH 8.5

By Volume: In a **1 L** volumetric flask, dissolve **85.0 g ammonium chloride** (NH_4Cl) and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) in about **800 mL water**. Dilute to the mark and invert to mix. Adjust the **pH to 8.5 with 15 N sodium hydroxide solution.**

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination. An alternative recipe for the ammonium chloride buffer is:

By Volume: CAUTION: Fumes!!! In a hood, to a **1 L** volumetric flask, add **500 mL water**, **105 mL concentrated hydrochloric acid** (HCl), **95 mL ammonium hydroxide** (NH_4OH), and **1.0 g disodium EDTA**. Dissolve and dilute to the mark. Invert to mix. Adjust the **pH to 8.5 with HCl or 15 N NaOH solution.**

Reagent 3. Sulfanilamide color reagent

By Volume: To a **1 L** volumetric flask, add about **600 mL water**. Then add **100 mL of 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake to wet, and stir for 30 min. to dissolve. Dilute to the mark, and invert to mix. Store in a dark bottle. This solution is stable for one month.

6. Procedure

6.1. Prepare reagent and standards as described in sections 4 and 5 of this document.

6.2. Add 10 ml of sample (or standard; all standards and blanks should be digested along with samples) to a digestion tube (borosilicate glass scintillation vial with open-top cap).

6.3. Neutralize all acid preserved blanks, standards, and samples by adding the equivalent amount NaOH necessary. For example, if 2 drops of concentrated (36N) H2SO4 were used to preserve a 45 ml sample, then one drop of 15N NaOH must be used to neutralize a 10 ml aliquot of that sample.

6.4. Add 5 ml digestion solution to each vial, cap tightly, and mix. Autoclave samples for 60 minutes using program 3 on the large autoclave on BSB 3rd floor. Program for autoclave should not include any drying time. After cycle has finished, remove samples from autoclave and allow them to cool to room temperature.

6.5. Set up nitrate manifold as shown in section 9 of this document (manifold diagram is on the last page of the SOP).

6.6. Make sure power is on to all portions of the instrument (autosampler, manifold pump, and main instrument) then open the Omnion software. On the main screen click on the “Configuration” pull down menu then click on “Autosamplers”. When the pop up menu opens, click the button that says “Initialize Autosampler”. This should cause the autosampler to re-center itself over the rinse tube then move permanently down into the rinse tube.

6.7. After you have initialized the autosampler, open the NO2-N+NO3-N and PO4-P template by going to the “Run” pull down menu and clicking on “Open”. Navigate to the folder Omnion/templates and click on the file named NO2-N plus NO3-N and PO4-P.omn. When the file opens, the software will ask if you would like to change the setpoints of the relevant heaters. Click yes. Click again on the “Run” pull down menu, then click “Save As”. Navigate to the folder Omnion/Data/Inorganic Nutrients. Save the template as the batch ID

(yyyymmddPRJmANL; code on Chain of Custody Form) number associated with the sample set you are running. After the template has been saved, click the "Preview" button on the toolbar. This will allow you to view the baseline signal from the flowcell.

6.8. Secure the pump tubes to the pump by clicking down the tubing shafts. Turn on the pump by pressing the manual flow button on the top left of the pump (blue button). Make sure that the probe rinse pump line is submerged in DI water and that the probe is down in the rinse tube on the autosampler. Put all reagent lines into DI water. Pump DI water through all reagent lines and check for leaks and smooth flow. After any air in the system has passed through the flow cell, the baseline in the preview screen should be completely flat. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. This will probably take at least 15 minutes for all chemicals to come into equilibrium in the mixture.

6.9. Place standards in the sampler according to the slot locations in the method template. Fill in samples with the appropriate duplicates and spikes. All information on duplicate and spike samples has already been set up in the template for the analytical method, the only thing that needs to change are the sample IDs.

6.10. Once all sample data has been entered into the sample sheet, save the run again as described earlier. Once saved, check the baseline to insure that reagents have come through and that the baseline is stable. If stable, press the "Run" button on the tool bar.

6.11. The software will check that the LCS, and that all CCVs, duplicates, and spikes meet appropriate QA/QC criteria. If the LCS fails to meet QA/QC criteria, the run will automatically terminate. However, if the LCS passes and one of the CCVs, duplicates, or spikes fails to meet QA/QC criteria, the run will continue. It is imperative that the analyst check the QA/QC results for all CCVs, duplicates, and spikes and rerun any and all sample sets that do not adhere to QA/QC requirements outlined in CRASR SOP #8.

7. Quality Control/Quality Assurance – See CRASR SOP # 8 (Quality Assurance and Quality Control) for details on QA/QC criteria.

8. References

Lachat Procedures #10-107-04-1-C. Determination of nitrate/nitrite in surface and wastewaters by flow injection analysis.

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 353.2.

Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey.

9. Instrument Information

DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and ***will need to be optimized*** using graphical events programming.

Sample throughput:	55 samples/h, 65 s/sample
Pump Speed:	35
Cycle Period:	65

Analyte Data:

Concentration Units:	mg N/L
Peak Base Width:	25 s
% Width Tolerance:	100
Threshold:	5000
Inject to Peak Start:	22 s
Chemistry:	Direct

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg N/L	2.00	0.80	0.20	0.05	0.02	0.01	0.00

Calibration Rep Handling:	Average
Calibration Fit Type:	1st Order Polynomial
Weighting Method:	None
Force through zero:	No

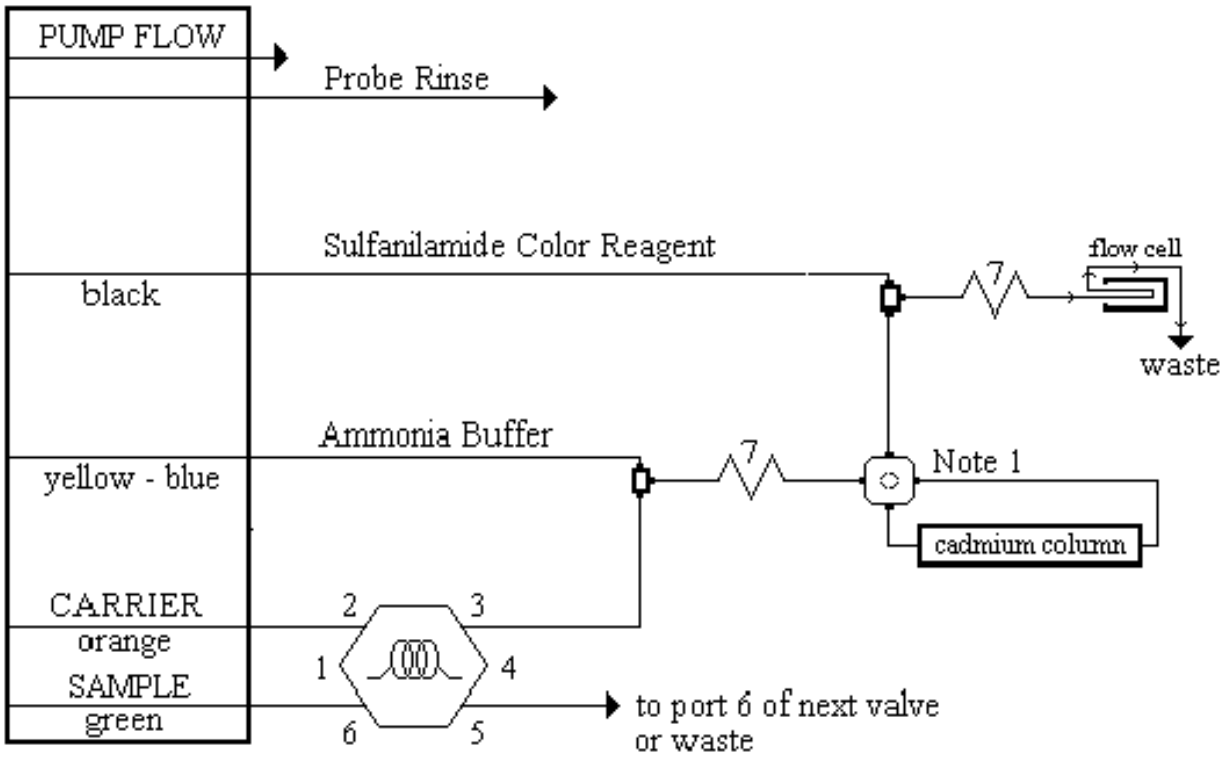
Sampler Timing:

Min. Probe in Wash Period:	12 s
Probe in Sample Period:	32 s

Valve Timing:

Load Time:	0 s
Load Period:	28 s
Inject Period:	37 s

NITRATE/NITRITE MANIFOLD DIAGRAM



Carrier: Helium Degassed DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
AE Sample Loop: 17 cm x 0.8 mm i.d.
QC8000 Sample Loop: 22.5 cm x 0.8 mm i.d.
Interference Filter: 520 nm

Apparatus: An injection valve, a 10 mm path length flow cell , and a colorimetric detector module is required.

7: 135 cm of tubing on a 7 cm coil support

Note 1: This a 2 state switching valve used to place the cadmium column in-line with the manifold