

EPA New England Region 1

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

The Office of Environmental Measurement and Evaluation
EPA Region 1 New England
11 Technology Drive
North Chelmsford, MA 01863

Prepared by: Bhavita Patel 3-19-2015
Bhavita Patel, Chemist
Investigations and Analysis Unit, OEME
Date

Approved by: E - W - 3/19/2015
Ernest Waterman, Chief
Acting Laboratory QA Officer
Investigations and Analysis Unit, OEME
Effective
Date

Approved by: E - W - 3/19/2015
Ernest Waterman, Chief
Investigations and Analysis Unit, OEME
Effective
Date

The controlled version of this document is the electronic version viewed on-line only. If this is a printed copy of the document, it is an uncontrolled version and may not be the version currently in use.

This document contains direction developed solely to provide internal guidance to U.S. Environmental Protection Agency (EPA) personnel. EPA retains the discretion to adopt approaches that differ from these procedures on a case by case basis. The procedures set forth do not create any rights, substantive or procedural, enforceable at law by a party to litigation with EPA or the United States.

TABLE OF CONTENTS

1.0	Scope and Application	3
2.0	Summary	3
3.0	Health and Safety Warnings	3
4.0	Personnel qualifications.....	3
5.0	Interferences	3
6.0	Equipment and Supplies	4
7.0	Sample collection and preservation.....	4
8.0	Reagents and Standards	5
9.0	Quality control	8
10.0	Calibration and standardization	9
11.0	Procedure.....	9
12.0	Data Analysis and Calculations	12
13.0	Troubleshooting	12
14.0	Pollution Prevention	13
15.0	Waste management.....	14
16.0	Table, Diagrams, Flow charts	15
17.0	References	17
	Appendix 1: Acceptance Criteria	18
	Appendix 2: Reagent Preparation	19
	Appendix 3: Standard Preparation Sheet.....	21
	Appendix 4: Standard Preparation Sheet.....	22
	Appendix 5: Comparison Table.....	23

1.0 Scope and Application

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

2.0 Summary

Nitrate is reduced to nitrite by the passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus any original nitrite present) is measured by diazotizing the sulfanilamide followed by coupling N-(1-naphthyl) ethylenediamine dichloride. The resulting magenta colored product is read at 520 nm. Nitrite alone can also be determined by removing the cadmium column.

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 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 similar precautions should be taken when handling them. Safety information is available in the form of MSDS sheets and can be obtained from the health and safety officer.

4.0 Personnel qualifications

The analyst should have at least 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.

5.0 Interferences

- 5.1. Buildup of suspended matter in the reduction column will restrict sample flow. Since nitrate and nitrite are found in a soluble state, samples may be pre-filtered.
- 5.2. Residual chlorine can interfere by oxidizing the cadmium reduction column. (see section 16, Troubleshooting)
- 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. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.
- 5.5. Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45µm pore diameter membrane filter prior to analysis.
- 5.6. 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.
- 5.7. 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 Equipment and Supplies

- 6.1. Balance- analytical, capable of accurately weighing to the nearest 0.0001g.
- 6.2. Glassware- Class A volumetric flask and pipettes or plastic containers as required.
- 6.3. Automated Ion Analyzer- Lachat QuikChem AE
 - Autosampler
 - Multichannel proportioning pump
 - Manifold (reaction unit) with heated module
 - Auto dilutor
 - Colorimetric Detector
 - Data System
 - 10mm, 80uL, glass flow cell
 - 520 nm interference filter
- 6.4. Hot block (only for Total Nitrogen off-line digestion)

7.0 Sample collection and preservation

- 7.1. Samples should be collected in either plastic or Pyrex glass containers.
- 7.2. For total nitrogen: if the analysis cannot be performed the day of collection the sample should be preserved by addition of 1 mL concentrated H₂SO₄ per liter preferably in the field and refrigerated at 4°C. Sample analysis should be performed within 28 days of sample collection.

- 7.3. For nitrate/nitrite (individually): Samples need to be refrigerated at 4°C and analyzed within 48 hours. Samples cannot be preserved.

8.0 Reagents and Standards

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

Use DI water to prepare solutions, except when analyzing seawater (See note for Reagent 4, b). To prevent bubble formation, degas all solutions *except* standards with helium. Bubble He through the solution for approximately 2 minutes.

8.1. Reagent 1. 15N Sodium Hydroxide

To a 250 mL volumetric flask add 100mL of DI water and slowly add 150g of NaOH. Add DI water and bring to the mark. CAUTION: The solution will get very hot! Swirl until dissolved. Dilute to the mark. Cool and store. Prepare as needed.

8.2. Reagent 2. Ammonium Chloride, pH 8.5

Buffer solutions can be purchased from HACH Company. Certificates of analysis are required. Buffer can also be prepared in the lab as follow;

In a 1L volumetric flask, dissolve 85.0g ammonium chloride (NH₄Cl) and 1.0g disodium ethylenediamine tetraacetic acid dihydrate (Na₂EDTA·2H₂O) in about 800mL of water. Dilute to the mark and invert to mix. Adjust pH to 8.5 with reagent 1 (15N Sodium hydroxide). Prepare as needed.

8.3. Reagent 3. Sulfanilamide Color Reagent

Stock standard can be purchased from HACH Company. Certificates of analysis are required. Reagent can be prepared in the lab as follows;

In a 1L volumetric flask, add approximately 600mL DI water. Then add 100mL of 85% phosphoric acid (H₃PO₄), 40.0g sulfanilamide and 1.0g N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Shake until wet and stir with stir bar for 30 minutes or until dissolved. This solution is stable for a month, store in the dark.

8.4. Reagent 4.

- a. **Carrier: DI water** -carrier for Nitrate/ Nitrite and total Nitrogen in all matrices except sea water samples.
- b. **Carrier: Synthetic sea water** -carrier for Nitrate/ Nitrite in sea water samples.

8.5. Reagent 5. Digestion Solution (Total Nitrogen Only)

In a 1L volumetric flask, add approximately 60mL DI water. Then add 40g Potassium persulfate ($K_2S_2O_8$), 18g Boric acid (H_3BO_3) and 9g sodium hydroxide (NaOH). Dilute to the mark and shake to mix. Store at room temperature for up to seven days.

8.6. Reagent 6. Borate Buffer, 1.0 M, pH 7.5 (For Soils only)

In a 200-mL volumetric flask, dissolve 12.4 g boric acid, 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.

8.7. Reagent 7. Control Standard (for soil samples only)

1000 ppm as N glycine standards commercially prepared or 2.67grams of glycine dried for 1 hour at 75°C dissolved in 500 ml of DI water.

8.8. Reagent 8. Concentrated hydrochloric acid (HCl)

Adjusting pH of the sample prior to analysis if found to be below 5 and or above 9.

8.9. Reagent 9. Concentrated Ammonium hydroxide (NH₄OH)

Adjusting pH of the sample prior to analysis if found to be below 5 and or above 9.

8.10. Sulfuric Acid, 5.6M (For persulfate digestion)

In a 1 liter volumetric flask add 500 mL DI water and 310 mL concentrated sulfuric acid. Dilute to the mark and shake to mix. Prepare as needed.

8.11. Preparation of standards

All standards used to analyze drinking, surface, and waste water samples need to be prepared using DI water. For sea water samples, synthetic sea water needs to be used for standard preparation and for the eluent.

1. Stock standard solution(s) of 1000 ppm as Nitrate (NO_3^-) and as Nitrite (NO_2^-) that are commercially prepared are purchased. Certificate of analysis is required. Used for **calibration curves**.
2. A stock standard (second source) of 100ppm as Nitrate (NO_3^-) and as Nitrite (NO_2^-) that is commercially prepared and used for **ICV**. Certificates of analysis are required.
3. A Dionex standard containing both Nitrate (NO_3^-) and Nitrite (NO_2^-) is used for **LFB** and **MS/MSD** (when analyzing for Nitrate and Total Nitrogen only) Certificate of analysis is required.

8.10.1. Working standard : 50ppm (ug/ml) as NO_3^-

To a 200mL volumetric flask add 10mL of 1000 ppm stock standard and dilute to the mark. Eight calibration standards and a blank are prepared for calibration.

Prepare fresh before every run.

Calibration point	10.0 ppm	5.0 ppm	3.0 ppm	1.0 ppm	0.5 ppm	0.2 ppm	0.1 ppm	0.04 ppm	0 ppm
Concentration as NO_3^-	10	5	3	1	0.5	0.2	1	0.04	0
Volume(mL) of std 8.10.1 Diluted to the 25ml with DI water	5	2.5	1.5	500uL	250uL	100uL	50uL	20uL	-

8.10.2. Working Standard: 50ppm (ug/ml) as NO_2^-

To a 200mL volumetric flask add 10mL of 1000 ppm stock standard and dilute to the mark. Eight calibration standards and a blank are prepared for calibration.

Prepare fresh before every run.

Calibration point	10.0 ppm	5.0 ppm	3.0 ppm	1.0 ppm	0.5 ppm	0.2 ppm	0.1 ppm	0.04 ppm	0 ppm
Concentration as NO_2^-	10	5	3	1	0.5	0.2	1	0.04	0
Volume(mL) of std 8.10.2 Diluted to the 25ml with DI water	5	2.5	1.5	500uL	250uL	100uL	50uL	20uL	-

8.10.3. ICV Preparation: 250uL of 100ppm stock standard (NO_2^- and or NO_3^-) to 25mL with DI water. Final concentration 1.0ppm as NO_2^- and or NO_3^-

8.10.4. LFB /LFM Preparation: as follows

LFB For NO_2^- and NO_3^- (analyzed through Cadmium column) 250uL of Dionex standard to 25mL with DI water. Final concentration 2.35ppm as NO_2^-

LFB For NO_2^- (analyzed without Cadmium column) 250uL of Dionex standard to 25mL with DI water. Final concentration 1.35ppm as NO_2^-

MS/MSD: For NO_2^- and NO_3^- (analyzed through Cadmium column) 250uL of Dionex standard to 25mL with sample.

MS/MSD: For NO_2 (analyzed without Cadmium column) 250uL of Dionex standard to 25mL with sample.

9.0 Quality control

Method Detection Limit (MDL) must be established:

- Seven replicates of reagent water fortified with two to four times the reporting limit need to be prepared and analyzed.
- The mean accuracy should be 80-120% and the RSD should be less than 20%. If these criteria are not met action must be taken and analyses repeated.

Initial Calibration Verification (ICV): prepared and analyzed immediately after system has been calibrated. The accuracy of the initial calibration shall be verified by the analysis of an ICV Standard (source different from the source of calibration standard). If not within $\pm 10\%$ of stated value, terminate analysis and re calibrate instrument.

Continuing calibration verification (CCV) and Calibration blank (CCB): need to be analyzed immediately after ICV, after every ten injections and at the end of the run. Prior to running samples one must verify that the instrument is within $\pm 10\%$ of calibration.

Reagent blank (BLK): A Water Blank (DI water) shall be analyzed immediately after every initial and continuing calibration verification standard,

and at the end of the sequence. Analyzed one per batch. Values that exceed the reporting limit indicate laboratory or reagent contamination and corrective action must be taken.

Laboratory Fortified Blank (LFB): prepared and analyze one LFB per batch. Analyzed after continuing calibration and blank are run. Recovery need to be within 90-110 %. When sufficient performance data became available control limits can be developed and upper and lower control limits can be calculated.

Laboratory Fortified Matrix Sample (MS/MSD): prepared and analyzed every 10 samples. The added analyte concentration should be the same as that used for LFB.

10.0 Calibration and standardization

- 10.1. Prepare reagents and standards as described in Section 8.
- 10.2. Set up manifold as required by the Lachat method.
- 10.3. Input data system parameters as required by Lachat method
- 10.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Make sure that the cadmium column is switched OFF.
- 10.5. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.
- 10.6. Place standards in the auto sampler tray. Set up sequence as data system requires.
- 10.7. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve.
- 10.8. Verify calibration using a midrange calibration standard (CCV) every ten samples. If % recovery exceeds +/-10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

11.0 Procedure

11.1 Nitrate & Nitrite analysis, for aqueous samples (unpreserved).

- Prepare reagents and standards as described in Section 8.
- Set up manifold as shown in section 16.2
 - a. For nitrite (NO_2^-) analysis only: Calibrate using nitrite standards. (Refer to section 16.2 for manifold setup Note 2)
 - b. For nitrite and converted nitrate ($\text{NO}_2^- + \text{converted NO}_3^-$)

analysis: Calibrate using nitrate standards(Refer to section 16.2 for manifold setup Note 1)

- Input the data system parameters as shown in section 16.1
- Pump DI water through all the reagent lines and check for leaks and smooth flow. Note: Ensure the cadmium column is installed as described in section 13.1. Important: never run DI water through installed cadmium column. Switch to reagents and allow bubbles to disappear before turning the valve to allow reagents to flow through the cadmium column.
- Allow the system to equilibrate as required, obtain a stable base line with all reagents.
- No preparation is required to analyze these samples. Place standards and samples in the auto sampler.
- Input the sample identification required by the data system.
- Start the run.
- Data generated from manifold without cadmium column will not require further calculation. Instrument results are read directly as NO₂ concentrations representative of the value of nitrite as NO₂ present in sample.
- Data from manifold with cadmium column will require calculations. Data degenerated will be the sum value of all nitrite (NO₂) and all converted nitrate (converted NO₃⁻) present in sample. Refer to calculation described in section 12.

11.2 Total Nitrogen analysis (aqueous samples):

Samples must be digested for analysis of combined Nitrate & Nitrite.

Digestion Procedure as follows:

1. Begin heating the hot block prior to sample preparation.
2. Prepare standards as described in section 8.10.
3. Take 25 ml of sample, standards and QC samples and carefully transfer to digestion tubes.
4. Add 0.5 mL of 5.6 M H₂SO₄ along with 15 mL of the digestion solution (Reagent 8.5) to all digestion tubes.
5. Put samples in the hot block for 60 minutes at 110°C.
6. Allow to cool to room temperature and adjust volume to 25 ml with DI water.
7. Check pH: Check if the pH range after digestion is between 5-8 using pH stripes. A successful digest need to have pH in the range of 5-8. If pH is

out of range, samples need to be re-digested.

Analysis procedure

1. Digest all standards as described above.
2. Set up manifold as shown in section 16.2
 - a. For combined Nitrate-Nitrite analysis: Calibrate using digested nitrate standards (Refer to section 16.2 for manifold setup Note 1)

11.3 Total Nitrogen Analysis (Soils)

Samples must be prepared for analysis of combined Nitrate & Nitrite. For soil samples use 0.5 to 1.0 g of sample and dilute to 25mL with DI water.

A **control standard (Reagent 7)** prepared from 1000 ppm glycine is treated as a sample and analyzed with each batch. Take 25 uL of 1000 ppm standard to 25 mL with DI water. Acceptance criteria for this QC samples is $\pm 20\%$ of the true value.

Digestion Procedure as follows:

1. Begin heating the hot block prior to sample preparation.
2. Prepare standards as described in section 8.10.
3. Take 25 ml of standards and QC samples and carefully transfer to digestion tubes.
4. Take 0.5-1.0grams of sample and carefully transferred to digestion tubes.
5. Add 0.5 mL of 5.6 M H_2SO_4 (reagent 8.10) along with 15 mL of the digestion solution (Reagent 8.5) to all digestion tubes.
6. 1 mL of borate buffer (See reference 8.6) to all soil samples.
7. Prepare control standard (QC samples) as described above.
8. Put samples in the hot block for 60 minutes at 110°C.
9. Allow to cool to room temperature and adjust volume to 25 ml with DI water.
10. Check pH: Check if the pH range after digestion is between 5-8 using pH stripes. A successful digest need to have pH in the range of 5-8. If pH is out of range, samples need to be re-digested.

Analysis procedure

1. Digest all standards as described above.
2. Set up manifold as shown in section 16.2
 - b. For combined Nitrate-Nitrite analysis: Calibrate using digested nitrate standards (Refer to section 16.2 for manifold setup Note 1)

12.0 Data Analysis and Calculations

- 12.1 Calibration is completed by injecting standards and plotting area vs. standard concentration by data system. Concentration is calculated using 2nd polynomial regression equation.
- 12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
- 12.3 Report sample results for nitrate/nitrite in mg N/L as NO_3^- or NO_2^- to two significant figures for samples above the RL.
- 12.4 Data from manifold Channel 2 will represent value of all nitrite (NO_2^-) and all converted nitrate (converted NO_3^-), present in sample.
 - $[\text{NO}_2 + \text{converted NO}_3] = \text{Data generated from channel 2}$
 - $[\text{NO}_2] = \text{Data generated from channel 1}$

Calculation is as follows; $\text{NO}_2 = 1.35 \text{ NO}_3$

$(\text{Data from channel 2} - \text{Data from channel 1}) \div 1.35 = \text{NO}_3^-$

13.0 Troubleshooting

The most common problem is deactivation of the cadmium column which results in artificially low values and non-linear calibration curves. The deactivation of the column is quantified by a column having a less than 90% efficiency factor. The only solution is replacement of the column. This procedure is outlined in the following section

Note: Pre-packed cadmium columns are available from Hach Company.

13.1 Cadmium Column Installation:

- Visually inspect for air gaps in the column, changes in cadmium granule color
- Disconnect the tubing from one end of the switching valve
- Disconnect the tubing from the one end of the column tubing assembly
- Connect the switching valve and the column tube assembly tubing
- Open switching valve until buffer solution fills the tubing assembly and then close the valve
- Remove the column plugs from the column and insert flangeless fitting into the

column

- Install the column on the manifold and turn the switching valve to the open position.
- Condition column by running 1 mg/L standard for 10 minutes if a new reduction column is being used. Subsequently wash the column with reagents for 20 minutes.
- Flow check: To check the flow efficiency of the cadmium column disconnect cadmium column from the manifold and reconnect to a green-green pump tubing. Pump buffer through packed column and collect in graduate cylinder. The flow rate should be greater than 4.0 ml per minute. A low flow rate indicates that the column is partially clogged.
- Column efficiency test: Before running samples column efficiency needs to be determined:
- Run nitrate calibration standards
- Run a known concentration of nitrite standard
- Run a matching concentration of nitrate standard
- Calculate the column efficiency

$$E = \frac{[\text{NO}_3]}{[\text{NO}_2] * 1.35} * 100$$

If the efficiency is less than 90% cadmium column need to be replaced.

13.2 Disposal of a used column: Once the column is depleted remove the flangeless fitting from the both ends of the column cap both side of the column and dispose per health and safety SOP in NERL.

14.0 Pollution Prevention

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.

15.0 Waste management

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.

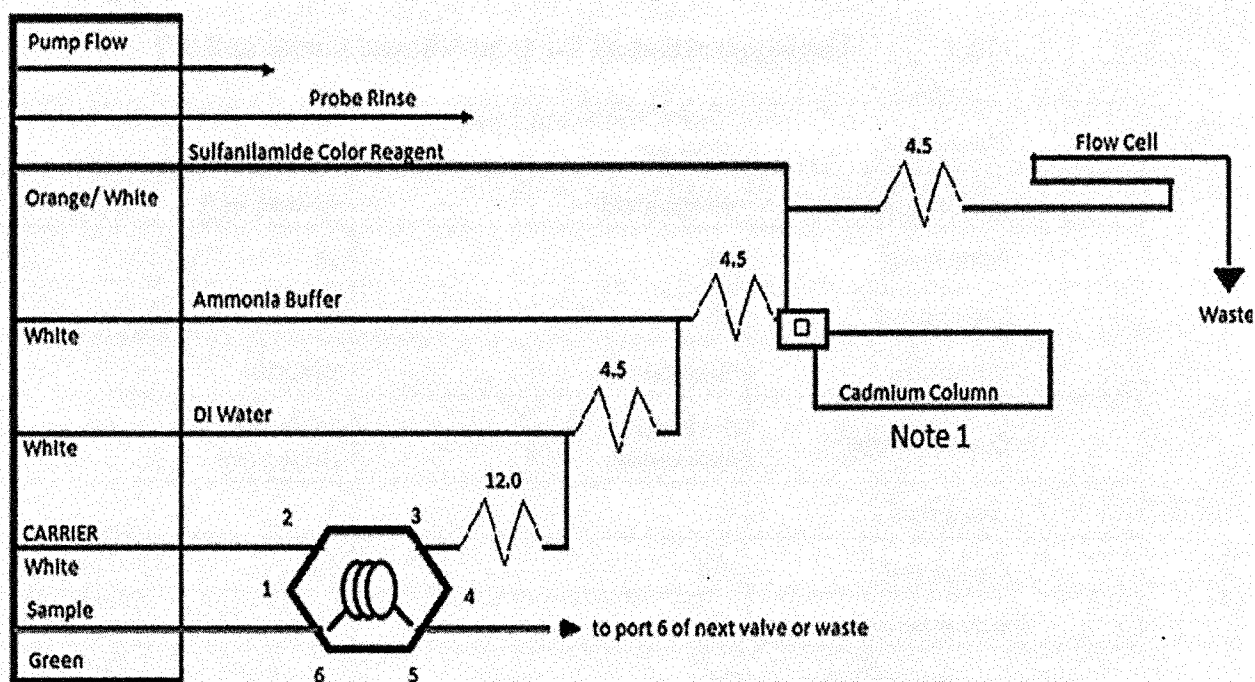
For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Section 14.0.

16.0 Table, Diagrams, Flow charts

16.1 Timing parameters for total Nitrate/Nitrite and TN

Parameters	Nitrate/ Nitrite and Total Nitrogen	Nitrate/ Nitrite in Sea Water
Sample throughput	60 samples/h	60 samples/h
Pump speed	35	35
Cycle period	65	65
Chemistry	Brackish	Brackish
Calibration fit type	2 nd Order Polynomial	2 nd Order Polynomial
Load time	0	0
Load period	10	10
Injection period	55	55
Sample reaches first valve	32	32
Min. probe wash	5	5
Loop (cm)	13	40

16.2 Total nitrogen Manifold Diagram



Note: This is a module with 2 state switching valve used to place the cadmium column in and off-line.

Note 1:

Channel 2: Nitrate + Nitrite

Solution flow is through the
Cadmium column.

Note 2:

Channel 1: Nitrite

Solution flow by-passes the cadmium

17.0 References

1. Standard Methods for the Examination of Water and Wastewater, 17th Edition, pp. 4-91, Method 4500-NO3 F (1992)
2. U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes Method 353.2
3. QuikChem Method 10-107-04-1-A. Determination of Nitrate/Nitrite in surface and wastewater by flow injection analysis(low- flow method) , 28 August 2000

Appendix 1: Acceptance Criteria

QA/QC Sample	Frequency	Acceptance Criteria	Corrective Action
Blanks: Distilled for TN. Not distilled for Nitrate/nitrite	1 per batch (up to 20 samples) After calibration, continuing calibration, at the end of the run	< RL	Recalibrate, prepare new blank
Initial Cal. Verification (ICV)	After calibration	$\pm 10\%$ of True value	Recalibrate, prepare new ICV
Continuing Calibration Verification (CCV)	after 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 persists qualify data (J)
MS/MSD - Laboratory Fortified Matrix/ Laboratory Fortified Matrix Duplicate	1 per 10 samples	$\pm 20\%$ of True value, and RPD $\pm 20\%$	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
Sample /Holding Time	Section 7	Samples must be analyzed within holding times	If re-sampling is not available, samples are qualified (J).
IDC	Annually or when there is the change in the method or equipment	4 replicates of LFB 80-120% recovery <20% RSD	Investigate problems and repeat.
MDL	Initially or if there is the change in the method or equipment	2 to 4 times low calibration point.	Investigate Problems and repeat.
QCS commercially prepared	Recommended run at minimum quarterly	Manufacturer certificate	Investigate problems and repeat the run

Appendix 2: Reagent Preparation

Date:

Analyst:

PN:

Survey:

1. Preparation of 15N Sodium Hydroxide (SOP Section 8.1, Reagent 1)

Sodium Hydroxide:

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

_____ grams Sodium hydroxide +
to _____ L with DI water

2. Preparation of Ammonium Chloride Buffer, pH 8.5 (SOP Section 8.2, Reagent 2) adjust pH using 15N NaOH (Reagent 1)

Ammonium Chloride:

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

_____ grams ammonium chloride +
_____ disodium ethylenediamine
tetra acetic acid dehydrate
($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$)
to _____ L with DI water

Disodium ethylenediamine tetra acetic acid dehydrate ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$):

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

3. Preparation of Sulfanilamide Color Reagent (SOP Section 8.3, Reagent 3)

Sulfanilamide

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

_____ mL conc. H_3PO_4 +
_____ grams sulfanilamide +
_____ grams N-(1-
naphthyl)ethylenediamine
dihydrochloride (NED)
to _____ L with DI water

N-(1-naphthyl)ethylenediamine dihydrochloride Phosphoric Acid
(NED) 85% phosphoric acid (SOP Section 8.3, Reagent 3)

Barcode: _____ Lot#: _____ Date Opened: _____

Mfg.: _____ Date Rec'd: _____ Date Expire: _____

4. Preparation of Digestion Solution (Total Nitrogen Only) (SOP Section 8.5, Reagent for persulfate digestion)

Potassium persulfate ($K_2S_2O_8$)

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

_____ grams Potassium persulfate +

_____ grams Boric acid (H_3BO_3) +

_____ grams sodium hydroxide

to _____ L with DI water

5. Borate Buffer, 1.0M pH 7.5 (SOP Section 8.6) (soils only)

Boric Acid

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

_____ grams of boric acid

_____ grams Sodium hydroxide

to _____ L with DI water

6. Glycine standard

Glycine

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

_____ grams of Glycine

to _____ L with DI water

7. Concentrated hydrochloric acid (HCl)

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

8. Conc. Ammonium hydroxide
(NH_4OH)

Barcode: _____

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expire: _____

9. Preparation of Sulfuric acid, 5.6M (SOP Section 8.10) - for persulfate digestion

Barcode: _____

Mfg.: _____

Lot#: _____

Date Opened: _____

Date Rec'd: _____

Date Expire: _____

_____ mL conc. H_2SO_4 diluted

to _____ L with DI water

Appendix 3: Standard Preparation Sheet

Date:

Analyst:

PN:

Survey:

Working standards Nitrate -50ppm

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date opened: _____

Date Expires: _____

50ppm as NO_3^- working standard: _____ mL of 1000 ppm stock to 200mL DI water = 50ppm

Calibration Standard Table

Calibration point <u>Nitrate or Total Nitrogen</u>	10.0 ppm	5.0 ppm	3.0 ppm	1.0 ppm	0.5 ppm	0.2 ppm	0.1 ppm	0.04 ppm	0 ppm
Concentration as NO_3^-	10	5	3	1	0.5	0.2	1	0.04	0
Volume(mL) of std 8.10.1 Diluted to the 25ml with DI water	5	2.5	1.5	500uL	250uL	100uL	50uL	20uL	-

ICV Std 100ppm (NO_3^-): 250uL of Standard to
25mL with RO H_2O

FC= 1.00ppm NO_3^-

Mfg _____

Lot# _____

Date Rec'd _____

Date Opened _____

Exp. Date _____

Bar Code _____

Code _____

ICV Std 100ppm (NO_2^-): 250uL of
Standard to 25mL with RO H_2O

FC= 1.00ppm NO_2^-

Mfg _____

Lot# _____

Date Rec'd _____

Date Opened _____

Exp. Date _____

Bar

LFB Std: 250uL of Dionex Standard to 25mL with RO H_2O FC= 2.35ppm as NO_2

Mfg _____

Lot# _____

Date Rec'd _____

Date Opened _____

Exp. Date _____

Bar Code _____

MS/MSD sample: 100ppm std 250uL of Dionex std to 25 mL with sample _____

Duplicate sample: _____

Appendix 4: Standard Preparation Sheet

Date:

Analyst:

PN:

Survey:

Working standard Nitrite -50ppm

Mfg.: _____

Lot#: _____

Date Rec'd: _____

Date Opened: _____

Date Expires: _____

50ppm as NO_2^- working standard: _____ mL of 1000 ppm stock to 200mL DI water = 50ppm

Calibration Standard Table

Calibration point (Nitrite Only)	10.0 ppm	5.0 ppm	3.0 ppm	1.0 ppm	0.5 ppm	0.2 ppm	0.1 ppm	0.04 ppm	0 ppm
Concentration as NO_2^-	10	5	3	1	0.5	0.2	1	0.04	0
Volume(mL) of std 8.10.2 Diluted to the 25ml with DI water	5	2.5	1.5	500uL	250uL	100uL	50uL	20uL	-

ICV Std 100ppm (NO_2^-): 250uL of Standard to 25mL with RO H_2O FC= 1.00ppm NO_2^-

Mfg. _____

Lot# _____

Date Rec'd _____

Date Opened _____

Exp. Date _____

Bar Code _____

Appendix 5: Comparison Table

Method	EPA Series Method 353.2	EIASOP-INGNO2NO3
Parameter		
Applicability	Drinking and surface waters, domestic and industrial wastes.	Drinking, surface, domestic, soils, sea water and industrial wastes
Analytes	Nitrate, nitrite	Nitrate, nitrite, total nitrogen
Method Validation	Initial demonstration of performance: The Linear Calibration Range (LCR) must be determined initially and verified every 6 months. The verification of linearity must use a minimum of blank and 3 standards.	Initial demonstration of performance: The Calibration Range (CR) must be determined initially and verified every run.
	A Quality Control sample (QCS), an independent standard, is prepared and analyzed at least quarterly to verify the calibration standards and instrument performance. If not within $\pm 10\%$ of stated value, determine source of problem and correct before continuing with analyses.	A Quality Control sample, an independent standard, recommended analyzing at least quarterly to verify the calibration standards and instrument performance. Acceptance criteria limits by manufacturer certificate
	Determine MDLs by analyzing seven replicates of Laboratory fortified blanks at concentration of 2 to 3 times the estimated detection limit. MDLs must be determined every six months.	Determine MDLs by analyzing seven replicates of Laboratory fortified blanks at concentration of 2 to 4 times the reporting limit. MDLs need to be determined for new instrument and or after major repair to an instrument.
QC Check Standards/ Samples	After calibration is completed, verify by analyzing QCS. If not within $\pm 10\%$ of stated value, terminate analysis and re calibrate instrument.	After calibration is completed, verify by analyzing ICV. If not within $\pm 10\%$ of stated value, terminate analysis and re calibrate instrument.
QC Check Standards/ Samples	Prepare and analyze a Laboratory Fortified Blank (LFB) with each batch of samples by fortifying laboratory reagent water with the QCS. If the recovery of the analyte is not within 90-110%, the analyte is judged out of control. Determine source of problem and correct before continuing with analyses.	Prepare and analyze a Laboratory Fortified Blank (LFB) with each batch of samples by fortifying laboratory reagent water with the standard. If the recovery of the analyte is not within 90-110%, the analyte is judged out of control. Determine source of problem and correct before continuing with analyses or qualify (J) entire batch.
Standard Solution Expiration	Stock standard: Not specified.	Stock standard and Working standard solution: Requirement on expiration in section 8.

Initial Calibration	Minimum of 3 levels and a blank. Range: 0.01-1.0 mg N/L	6 levels and a blank. Range: 0.04-10 mg/L as NO ₂
Continuing Calibration	Analyze Instrument performance check (IPC) solution (mid-range check standard) immediately following calibration, after every 10 samples and at the end of the run. If not within $\pm 10\%$ of stated value, reanalyze IPC.	Analyze Instrument performance check (CCV) solution (mid-range check standard) immediately following calibration, after every 10 injection and at the end of the run. If not within $\pm 10\%$ of stated value, reanalyze CCV
	If second analysis of IPC is not within $\pm 10\%$ of stated value, discontinue analysis, determine the cause and/or in the case of drift recalibrate instrument. Reanalyze all samples since last compliant IPC.	If second analysis of CCV is not within $\pm 10\%$ of stated value, discontinue analysis, determine the cause and/or in the case of drift recalibrate instrument. Reanalyze all samples since last compliant CCV
Accuracy/Precision	Spike and analyze one sample out of every 10 (Laboratory Fortified Matrix: LFM). The added analyte concentration should be the same as that used in the LFB. %R = 90-110	Spike and analyze one sample out of every 10 (MS/MSD). The added analyte concentration should be the same as that used in the LFB. %R = 85-115
Blanks	A Laboratory reagent blank (LRB) is carried through the entire sample preparation and analysis scheme with each batch of samples. Values that exceed the MDL indicate contamination should be suspected and corrective actions must be taken before continuing analysis. A Calibration blank (CCB) is to be analyzed after each IPC solution.	A Laboratory reagent blank (LRB) is carried through the entire sample preparation and analysis scheme with each batch of samples. Values that exceed the reporting limits indicate contamination and corrective actions must be taken before continuing analysis. A Calibration blank (CCB) is to be analyzed after each CCV solution.
Preservation/Storage Conditions	pH <2 with H ₂ SO ₄ for TN and not preserved for nitrate, nitrite 4°C storage required	pH <2 with H ₂ SO ₄ for total nitrogen and unpreserved for nitrate, nitrite. 4°C storage required
Holding Time	28 days for TN. As soon as possible for nitrate, nitrite	28 days for total nitrogen. As soon as possible for nitrate, nitrite