SOP-C-105

Determination of
Nitrate/Nitrite as Nitrogen

Revision 16

Approval:

_____   3-26-24
Laboratory Manager   Date

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Concurrence   Date

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Texas Institute for Applied Environmental Research

i. **Identification of the method**
   a. SM 4500-NO$_3$F (automated) or E (manual) (approved 2018).
   b. *Modifications from published method*: None of the following modifications affect the chemistry of this analysis. The deviation from SM 4500-NO$_3$E for low level (manual) determination is the use of the 10 cm cell instead of a 1 cm cell. A modification in this procedure not described in SM 4500-F (automated) is the use of EDTA in the buffer instead of polyoxethylene laurel ether. Preservation temperature comes from the TNI standard and not the method. Due to the low volumes of sample aliquot, dilute HCl and NaOH are used instead of concentrated to neutralize samples before running. The pH of samples is checked with pH paper, not a meter. Samples are neutralized with NaOH instead of NH$_4$OH to prevent ammonia contamination in the lab.

ii. **Applicable matrix or matrices**
   a. Water samples or aqueous extracts.

iii. **Limits of detection and quantitation**
   a. Determined annually; refer to Laboratory Manager's most recent memorandum; Range LOD to 4 mg/L undiluted. The low range may be extended using the manual method and larger photometric cell; upper range is extended by dilution.
   b. *Routine LOD about 0.017 mg/L*
   c. *Low level LOD about 0.0044 mg/L*
   d. *TCEQ set LOQ = 0.05 mg/L*

iv. **Scope and application, including parameters to be analyzed**
   a. This procedure applies to sample received for combined nitrate + nitrate-nitrogen (NO$_{2+3}$N) analysis by the laboratory at the Texas Institute for Applied Environmental Research (TIAER), at Tarleton State University, Stephenville, TX.
   b. This procedure establishes routine guidelines for determining NO$_{2+3}$N to obtain comparable results from one analyst to another.
   c. Samples that are suspected to be above the range of the manual method should be initially analyzed by the

Autoanalyzer method. Samples that will require a dilution with a factor above times 100 will not yield a dependable result by the manual method. For these samples the sample result from the Autoanalyzer method should be used. If the project or client requires the manual method for high level samples, samples are diluted for the manual method after running by autoanalyzer.

v. **Summary of the method**

    a. Spectrophotometric analysis of water samples using a Cadmium column. Normally, only the combined analysis for nitrate + nitrite nitrogen is done.

    b. This procedure provides a method for the determination of $NO_{2+3}N$ that uses either a flow injection analysis or manual colorimetric reading that both employ a spectrophotometer detector system.

    c. The principle is that nitrate is reduced quantitatively to nitrite using a cadmium catalyst treated with copper sulfate, and is then measured colorimetrically.

vi. **Definitions**

    a. Refer to QAM-Q-101, "Laboratory Quality Control," for definitions of **DI** water, **AWRL, LOQ, LOD, LCS, CCV, CCB, ICV, ICV, MB, MS, MSD, IBV, QCB, PB, ARB** and sample **dup**licate.

    b. **Cadmium column**: commercial or manually packed column filled with cadmium metal, which reduces NO_3-N to NO_2-N.

    c. **Carrier solution**: Solution used to wash out the sample line between samples and to prevent carryover.

    d. **Column storage solution**: Solution used to preserve the cadmium column.

    e. **Flow Injection Analysis (FIA):** Method that introduces the sample into a reagent stream for analysis.

    f. **QAM**s- chapters of the **Quality Assurance Manual**.

vii. **Interferences**

    a. Turbidity is removed by filtering the sample- dissolved $NO_{2+3}N$ is filtered prior to acid preservation and within 15 minutes of sampling; Total $NO_{2+3}N$ is filtered after acid preservation and within holding time.

    b. Concentrations of iron, copper, or other metals above several milligrams per liter lower reduction efficiency.

EDTA may be added to samples to eliminate this interference.

c. Oil and grease will coat the Cd surface. It may be removed by pre-extraction with an organic solvent.

d. Residual chlorine can interfere by oxidizing the Cd column, reducing its efficiency. Suspected chlorinated samples may be checked for residual chlorine. Residual chlorine may be removed by adding sodium thiosulfate ($Na_2S_2O_3$) solution.

e. Sample color that absorbs at about 540 nm interferes.

f. Hydrogen sulfide in water or wastewater samples from anoxic waterbodies may de-activate the column; treat acidified odorous samples by bubbling with air for 15 minutes.

viii. Safety

a. All aspects of this procedure are performed in accordance with QAM-S-101, "Laboratory Safety".

b. Cadmium is toxic and carcinogenic. Collect and store all waste Cd. When handling Cd, wear gloves and follow precautions described on Cd's safety data sheet.

ix. Equipment and supplies

a. Equipment common to both methods

   i. Assorted glassware, Class A grade and general.

   ii. Balance capable of accurately weighing to the nearest 0.01 g.

b. Autoanalyzer method

   i. Autoanalyzer system (FIA). See QAM-I-102, "Operation and Calibration of the Autoanalyzer".

   ii. Cadmium column prepared by manufacturer

c. Manual method

   i. Visible light spectrophotometer at 543 nm with 10 cm cell; See QAM-I-103, "Operation and Calibration of the UV-Vis Spectrophotometer".

   ii. Cadmium column prepared as described below

x. Reagents and standards

a. Reagents common to both methods- Type II ASTM deionized water (DI)

b. Autoanalyzer method

   i. All chemicals are ACS grade or equivalent.

 ii. FIA Manifold solution is DI water.

 iii. Carrier solution is DI water obtained fresh daily.

 iv. Copper sulfate solution: add 2 g of $CuSO_4$ to 100 ml DI.

 v. Column storage solution: a proportion of 50/25/25 NH_4Cl buffer solution, 100 mg/l KNO_2-N and 100 mg/l KNO_3-N respectively.

 vi. Ammonium chloride-EDTA buffer solution:

  1. Add 85g of NH_4Cl to about 800 mL of DI.

  2. Add 0.1g of ethylenediaminetetraacetic acid disodium salt

  3. Adjust pH of solution to 8.5 with concentrated NH_4OH and bring volume to 1liter. Filter the buffer through a 0.45 µm filter, if necessary.

 vii. Autoanalyzer color reagent

  1. Add 50 ml of phosphoric acid (H_3PO_4) to about 400 ml of DI in a 500-ml volumetric flask while stirring.

  2. Add 20 g of sulfanilamide ($C_6H_8N_2O_2S$).

  3. Add 1g of n-1-napthylethylenediamine dihydrochloride. ($C_{12}H_{14}N_2$*HCl) and stir until all components are dissolved.

  4. Bring to final volume with DI.

  5. Filter the reagent through a 0.45-µm filter, if necessary.

  6. Store in brown bottle in the dark when not in use. May be refrigerated at >0-≤6ºC.

c. Manual method

 i. Hydrochloric acid, HCl, 6N. Carefully add 500 mL conc. HCl to 500 mL DI.

 ii. Copper-cadmium granules: Wash 25 g new or used 20 to 100 mesh Cd granules with 6N HCl (1 to 1 HCL) and rinse with DI. Swirl Cd with 100 mL 2% CuSO4 solution for 5 min or until blue color partially fades. Decant and repeat with fresh CuSO4 until a brown colloidal precipitate begins to develop. Gently flush with DI to remove all precipitated Cu. Store activated Cd covered with dilute ammonium chloride-EDTA solution.

   iii. Manual color reagent: To about 800 mL DI add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with DI. Solution is stable for about a month when stored in a dark bottle in refrigerator at >0-≤6ºC. Discard if the solution is highly colored or if a precipitate forms.

   iv. Ammonium chloride-EDTA solution: Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL DI. Adjust to pH 8.5 with conc NH_4OH and dilute to 1 L.

   v. Dilute ammonium chloride-EDTA solution: Dilute 300 mL NH_4Cl-EDTA solution to 500 mL with DI.

   vi. Copper sulfate solution, 2%: Dissolve 20 g CuSO4.5H2O in 500 mL DI and dilute to 1 L.

   vii. Sodium thiosulfate solution-dissolve 3.5 g Na2S2O3-5H2O in DI water and dilute to 1 L. Prepare fresh weekly. Use 1 mL to remove 1 mg/L residual chlorine in 500 mL sample.

  d. Standards

    i. Autoanalyzer method

      1. Label each prepared standard with the following date stamp description format: (NO2, NO3, or NO23) mmddyy-a where "a" is the sequence letter of the next standard prepared that day, "a" being the first. Follow the same naming protocol for the LCS/LCSD, but insert LCS or LCSD in the name also. Other names such as "STOCK" or "WS" for working standard should also be used as a prefix where appropriate. Also label standard bottles with the initials of the preparer, the solution concentration and expiration date.

      2. Record all standards in the Standards E-Log.

    ii. Stock nitrate solution (1000 mg/l NO_3-N): from potassium nitrate crystals (KNO_3) or purchased commercially prepared

1. Dry approximately 10 g of KNO$_3$ at 104°C for 2 hours and store in the desiccator until needed.
2. Dissolve 7.218 g of KNO$_3$ and dilute to 1000 mL in a volumetric with DI.
3. Preserve with 2 ml of chloroform and store at >0-≤6°C. Discard after 6 months or after expiration date of commercial standard.

iii. Stock nitrite solution (1000 mg/L NO$_2$-N) potassium nitrite crystals (KNO$_2$) or purchased commercially.
   1. Dry approximately 10 g of KNO$_2$ at 104°C for 2 hours and store in the desiccator until needed.
   2. Dissolve 6.072 g KNO$_2$ and dilute to 1000 mL in a volumetric flask with DI water.
   3. Preserve with 2 mL of chloroform.
   4. The stock solution should be stored at >0-≤6°C. Discard after 3 months or after expiration date of commercial standard.

iv. Working Solution: (100 mg/L NO$_2$-N/100 mg/L NO$_3$-N).
   1. Dilute 25 mL of each stock solution to 250 mL with DI in separate volumetric flasks. Preserve each with 0.5 mL of chloroform. This may also be purchased commercially.
   2. Discard Working Solutions after 3 months or after expiration date of commercial standard.
   3. The following calibration standards (mg/L) may be made from the 100 mg/L working solutions: (0.05 LOQ, 0.2, 0.5, 1.0, and 2.0 for NO$_2$-N and NO$_3$-N, additionally 3.0 and 4.0 for NO$_3$-N). Calibration standards are good for 28 days from time of preparation, when preserved with sulfuric acid in the same manner as samples.

v. Prepare a 2.0 mg/L nitrite nitrogen standard for a column efficiency check standard when analyzing nitrate nitrogen only.

e. Manual method

      i. Using the WS solutions in the autoanalyzer section above, prepare at least 5 standards in the range 0.01 to 0.20 mg NO_3-N/L. Carry out reduction of standards and blanks exactly as described for samples. Compare a 0.1 mg/L NO_2-N standard to a reduced NO_3-N standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described in above when efficiency of reduction exceeds 110% or falls below 90%.

xi. **Sample collection, preservation, shipment and storage**

  a. Holding Time: 28 days when preserved with sulfuric acid, otherwise the holding time is 48 hours for waters for NO_3-N or NO_2-N separately or together.

  b. Solids are stable for 6 months before extraction.

  c. Preservation: Refrigerate sample to >0-≤6° C, pH < 2 with concentrated H_2SO_4 (about 3 drops of conc. H_2SO_4 to 50 mL for most samples depending on complexity of matrix.)

  d. For dissolved nitrate, nitrite or combined NO_{2+3} as nitrogen, grab samples should be field filtered and preserved in accordance with field collection protocols. For total nitrate, nitrite or combined $NO_{2+3}N$, sample is acid preserved, then filtered immediately prior to analysis to protect from instrument damage by suspended matter.

  e. If not field filtered and preserved, filter samples for dissolved N in the laboratory through 0.45µm filter membrane filters in accordance with QAM-Q-111, "Aliquot Preparation and Sample Preservation".

xii. **Quality control**

  a. Analyze a matrix spike, matrix spike duplicate, field split (if collected) or sample duplicate for every 10 samples or less (QCB), or more frequently in accordance with project requirements. Analyze a method blank and LCS/LCSD for every twenty samples (PB). Immediately after calibration, analyze the ICV and IBV to confirm successful calibration (ARB).

  b. Refer to QAM-Q-101 for acceptance criteria.

  c. All data shall be documented and maintained in accordance with QAM-A-102, "Document and Data Control".

   d. Record the prepared Stock solutions, Working solutions, and calibration standards in the Standards Logbook. Record reagents in the Reagents Logbook.

   e. Quality control requirements, including field splits, method blanks, spikes and calibration verifications, are covered in QAM-Q-101 "Laboratory Quality Control".

   f. If any spike, standard, dup, or LCS does not pass acceptance criteria as described in QAM-Q-101 "Laboratory Quality Control", then initiate corrective action. Complete a Corrective Action Report in accordance with QAM-Q-105, "Corrective Actions".

xiii. Calibration and standardization

   a. See QAM-I-102, *"The Operation and Calibration of the Autoanalyzer"* for the automated method, QAM-I-103, *"Operation and Calibration of the UV-Vis Spectrophotometer"* for the manual method, and in the Procedure section below.

xiv. Procedure

   a. Autoanalyzer method (normally for routine levels)

      i. Neutralize any preserved sample or standard to between pH 5-9 with dilute NaOH dropwise. Unpreserved samples may also require addition of dilute HCl or NaOH for neutralization.

      ii. The LCS and the LCSD should be 2.0 mg/L (high range) or 0.10 mg/L (low range) for both NO_2-N and NO_3-N, and may be combined for convenience with the NH_3-N standard from SOP-C-104, "Determination of Ammonia as Nitrogen". The LCS/LCSD are prepared from sources other than the calibration standard.

      iii. Spiking should be made at 1.0 mg/L. Add 0.01 mL of a 1000 mg/L NO_3-N solution to 10 mL of sample. Some projects may require other spiking levels.

      iv. Preparation of the cadmium column

         1. Remove the cadmium metal granules from the glass column.

         2. Clean the cadmium granules as follows:

            a. Wash granules with 6 N HCl and rinse with DI.

   b. Swirl cadmium in 100 mL 2% $CuSO_4$ solution for 5 minutes. Repeat until brown colloidal precipitate begins to develop.
   c. Gently flush with DI at least ten times to remove all precipitate.
   d. Reintroduce the cadmium granules into the glass column and replace the caps.
   e. Prepared cadmium columns may be purchased from the manufacturer.
3. Cadmium column efficiency check
   a. Analyze a 2.0 mg/L nitrite nitrogen standard at the beginning of an analytical run to check the efficiency of the column with each batch. Values should be 90-110% of the value of a nitrate nitrogen standard at the same level. Record results in the maintenance logbook for the instrument. Initiate corrective action if not passing.

   v. Sample Table
      1. FIA – See QAM-I-102, "Operation and Calibration of the Autoanalyzers."
   vi. Calibration and Initiation of Analysis
      1. FIA – See QAM -I-102
   vii. Data Collection and Analysis
      1. FIA – See QAM -I-102
b. Manual method (normally for low level detection)
   i. See QAM-I-103, "Operation and Calibration of the UV-Vis Spectrophotometer".
   ii. Preparation of reduction column: Insert a glass wool plug into bottom of reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column 18.5 cm long or of equivalent size to provide sufficient surface area of exposure on the granules. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute NH_4Cl-EDTA solution. Activate column by passing through it, at 7 to 10 mL/min, at least 100

mL of a solution composed of 25% 1.0 mg NO_3-N/L standard and 75% NH_4Cl-EDTA solution.

iii. Turbidity removal-For turbid samples, filter through a 0.45 µm nitrate free filter.

iv. pH adjustment-Adjust pH to between 7 and 9, as necessary, using a pH meter or paper and dilute HCl or NaOH. This ensures a pH of 8.5 after adding NH_4Cl- EDTA solution.

v. If chlorine is suspected in the sample, screen for residual chlorine per SOP-C-121, "Determination of Residual Chlorine," and neutralize with sodium thiosulfate solution.

vi. Sample reduction-To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL NH_4Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/min. Discard first 25 mL. Collect the rest in a clean sample flask. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour 50 mL dilute NH_4Cl-EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never let it dry.

vii. Color development and measurement- As soon as possible, and not more than 15 min after reduction, add 2.0 mL color reagent to 50 mL sample and mix. Between 10 min and 2 hr afterward, measure absorbance in a 10 cm cell at 543 nm after zeroing with the ICB. NOTE: If NO_3 concentration exceeds the standard curve range (about 0.2 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.

viii. Obtain a standard curve by plotting absorbance of standards against NO_3-N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized NO_{2+3}-N per liter (the sum of NO_3-N plus NO_2-N) unless the concentration of NO_2-N is separately determined and subtracted.

ix. Matrix spiking for low level determinations are at 0.1 mg/L. 0.025mL of a 100 mg/L NO_3-N solution is added to 25 mL of sample.

xv. **Data analysis and calculations;**
  a. Linear plot curve of absorbance vs. concentration using Beer's Law and the formula of **y=mx+b** first order polynomial.
  b. The standard curve shall have at least 4 reference points plus ICB.
  c. The range of standards for the automated test should be 0.05 to 2.0 mg/L for NO_2-N and 0.05 to 4.0 mg/L for NO_2+NO_3-N. The range for the manual (low level) test is 0.01 to 0.2 mg/L NO_2+NO_3-N. Dilute samples above these ranges so they are bracketed by the standards.
  d. The baseline shall be within plus or minus the method detection limit (or LOQ) values for NO_2-N and NO_3-N.
  e. Describe all dilution methods in detail.

xvi. **Method performance**
  a. Refer to QAM-Q-101, "Laboratory Quality Control".
  b. Each calibration standard used in the curve at these levels shall be ± 25% of the linear value per QAM-Q-101, but higher level calibration standards may narrow to ± 10% of true value. The LOQ verification shall be ± 30% of the true value (QAPP dependent).

xvii. **Pollution prevention**
  a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste"

xviii. **Data assessment and acceptance criteria for quality control measures**
  a. Refer to QAM-Q-101, "Laboratory Quality Control".

xix. **Corrective actions for out-of-control data**
  a. Refer to QAM-Q-105, "Corrective Actions".

xx. **Contingencies for handling out-of-control or unacceptable data**
  a. Refer to QAM-Q-101 and QAM-Q-105 for failed QC and corrective action measures.

xxi. **Waste management**
  a. Waste management and pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste"
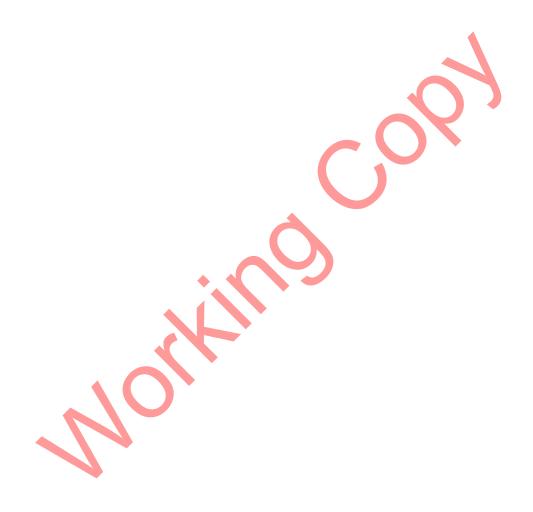
xxii. **References**
  a. LaChat Instruments, QuikChem Methods Manual, Method 10-107-04-1-A, January 1999.

    b. AWWA, Standard Methods For the Examination of Water and Wastewater, latest online edition (Approved <u>2018</u>), Method 4500-NO3 E & F, QA Methods 1020 and 4020.

    c. National Environmental Laboratory Accreditation Conference (NELAC) TNI standard, 2016.

xxiii. Any tables, diagrams, flowcharts and validation data

    a. Attachment 1: Nitrate/Nitrite-Nitrogen Manifold Table, FIA

RANGE	high or low		FIA	
PUMP				
Speed	Percent		35	
Tubes	Buffer		wht/wht	
	Sample		grn/grn	
	Color reagent		org/wht	
	Pull-off		pur/pur	
	Wash/Carrier		wht/wht	
DETECTOR				
505	Filter		520nm	
	Damp			
	Range			
SAMPLER				
	Rate		55/hr	
	Sample Time		20 sec	
	Wash Time		45 sec	
	Pecking			
	Start-up solution		DI water	
	Wash solution		DI water	