slowly add 32 g NaOH. Cool and invert to mix thoroughly. Do not degas.

c. Hypochlorite: To a tared 500-mL container add 250 g 5.25% sodium hypochlorite (NaOCl) bleach solution and 250 g water. Stir or shake to mix.

d. Nitroprusside: To a tared 1-L container, add 3.50 g sodium nitroprusside (sodium nitroferricyanide), $Na_2Fe(CN)_5NO \cdot 2H_2O$, and 1000 g water. Invert to mix.

e. Stock ammonia standard, 1000 mg/L N: In a 1-L volumetric flask, dissolve 3.819 g ammonium chloride (NH_4Cl) that has been dried for 2 h at 110 °C, in about 800 mL water. Dilute to mark and invert to mix.

f. Standard ammonia solutions: Prepare ammonia standards in the desired concentration range, using the stock standard (¶ *e* above), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NH_3:2 and follow the method supplied by the manufacturer or laboratory standard operating procedure for this method.

5. Calculations

Prepare standard curves by plotting the absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-NH_3:4.

b. MDL: A 650-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 0.020-mg/L N standard. These gave a mean of 0.0204 mg/L N, a standard deviation of 0.0007 mg/L N, and an MDL of 0.002 mg/L N.

Reference

1. U.S. Environmental Protection Agency. 1989. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-NO_3^-

Nitrogen (Nitrate)

Methods A–F, H, I: Approved by Standard Methods Committee, 2016. Editorial update, 2019. Joint Task Group: Thomas R. Holm (chair), Jack Bennett, Roger Blakesley, Gayle K. Gleichauf, Patrick K. Jagessar, William C. Lipps, Nadejda Vilissova, Melissa A. Woodall.

Method J: Approved by Standard Methods Committee, 2018. Joint Task Group: Charles J. Patton (chair), Ellen R. Campbell, Wilbur H. Campbell, William Lipps.

4500-NO_3^- A. Introduction

1. Selection of Method

Nitrate (NO_3^-) determination can be difficult because of the high probability that interfering constituents are present in various matrices. This section includes numerous methods that can be used to detect NO_3^-. Consider the advantages and limitations of each method when selecting a test strategy by reviewing the sample matrix, concentration range, and data needs for a particular application.

The ultraviolet (UV) light technique (4500-NO_3^- B), which measures NO_3^- absorbance at 220 nm, is suitable for screening uncontaminated water (low in organic matter).

Screen a sample if necessary, then select a method suitable for its concentration range and probable interferences. Nitrate can be determined by ion chromatography (Section 4110), capillary ion electrophoresis (Section 4140), or the methods shown here. Applicable ranges for the methods in this section are:

- 0.2 to 11 mg/L nitrate-nitrogen (NO_3-N); Ultraviolet Spectrophotometric Method (4500-NO_3^- B)
- 0.5 to 2.5 mg/L NO_3-N; Second Derivative Ultraviolet Screening Method (4500-NO_3^- C)
- 1 to 50 mg/L NO_3-N; Nitrate Electrode Method (4500-NO_3^- D)
- 0.05 to 1.0 mg/L nitrate + nitrite-nitrogen (NO_3 + NO_2-N); Cadmium Reduction Method (4500-NO_3^- E)
- 0.05 to 10 mg/L nitrate + nitrite-nitrogen (NO_3 + NO_2-N); Automated Cadmium Reduction Method (4500-NO_3^- F)
- 0.05 to 10 mg/L nitrate + nitrite nitrogen (NO_3 + NO_2-N); Automated Hydrazine Reduction Method (4500-NO_3^- H)
- 0.01 to 2.0 mg/L or 0.05 to 5.0 mg nitrate + nitrite (NO_3 + NO_2-N); Cadmium Reduction Flow Injection Method (4500-NO_3^- I)
- 0.05 to 10 mg/L nitrate + nitrite-nitrogen (NO_3 + NO_2-N); Enzymatic Reduction Manual Method (4500-NO_3^- J)

Other ranges may be possible for any of the above referenced methods. Refer to manufacturer's instructions.

For higher NO_3^--N concentrations, dilute to the range of the selected method. See Sections 4110 B and 4110 C for the working range of ion chromatography methods.

Filter turbid samples. Test filters for NO_3^- contamination.

2. Collection and Storage of Samples

Collect samples in polyethylene, fluoropolymer, or glass containers. If possible, start NO_3^- determinations promptly after sampling. Samples can be stored un-acidified for up to 48 h below 6 ° C without freezing. Acidification converts any nitrite (NO_2^-) to NO_3^-. As a result, NO_3^- values are the sum of NO_3^- and NO_2^- levels. If samples

must be stored for more than 48 h, acidify to pH <2 with sulfuric or hydrochloric acid (depending on the method) and store from 0 to 6 °C without freezing (or 2–6 °C for Safe Drinking Water Act [SDWA] compliance samples) for up to 28 d. Chlorinated samples are stable for at least 14 d without acid preservation.

3. Quality Control

Quality control (QC) practices and acceptance criteria are described in Sections 1020 and 4020. The following section applies to all NO_3^--N methods; however, some methods have additional QC measures. Complete the initial QC tasks, including initial demonstration of capability for each analyst, estimation of the method detection limit (MDL), and determination of the dynamic range, before analyzing any samples and at least annually thereafter. Apply the rest of these measures whenever samples are analyzed. If the acceptance criteria are not satisfied, stop and correct the problem. Regulators may specify different acceptance criteria than those given here.

Calibrate or verify the calibration of each instrument daily. Using a calculator, electronic spreadsheet, or instrument software, calculate the slope, intercept, and correlation coefficient (r) or coefficient of determination (r^2) of the calibration curve. The r value must be at least 0.995 ($r^2 \geq 0.99$). Back-calculate the apparent concentrations of the standards. For standards more than 10 times the MDL, the measured values must be 90% to 110% of the true values. The calibration requirements are different for the method described in 4500-NO_3^- D; see instructions in 4500-NO_3^- D.4*b* or 4500-NO_3^- D.4*c*.

Prepare a calibration-verification standard (CVS) from a stock solution separate from that used to prepare the calibration standards. The CVS's NO_3^--N concentration should be 30% to 70% of the highest calibration standard; however, some QA/QC programs may require different concentrations. Run the CVS immediately after calibration; the result must be 90% to 110% of the expected value.

Run an initial calibration blank (ICB) immediately after the CVS to check for contamination. The ICB reading must be less than one-half of the minimum reporting level (MRL).

Run a midpoint calibration standard as continuing calibration verification (CCV) and a continuing calibration blank (CCB) after every 10 samples and after the last sample. If the measured NO_3^--N concentration in the CCV is not 90% to 110% of the expected value, recalibrate and rerun all samples read since the last good CCV reading. The CCB must be less than one-half of the MRL.

Bibliography

Pfaff JD, Hautman DF, Munch DJ. Determination of inorganic anions in drinking water by ion chromatography; Method 300.1. Cincinnati (OH): National Exposure Research Laboratory, Office of Research & Development, U.S. Environmental Protection Agency, 1997.

U.S. Environmental Protection Agency. Guidelines establishing test procedures for the analysis of pollutants under the Clean Water Act; National primary drinking water regulations; National secondary drinking water regulations; and Analysis and sampling procedures. 40 C.F.R. Sections 122, 136, 141, 143, 430, 455, and 465 (2007).

4500-NO_3^- B. Ultraviolet Spectrophotometric Screening Method

1. General Discussion

a. Principle: Use this technique only to screen samples low in organic matter (i.e., uncontaminated natural waters and potable water supplies). The NO_3^- calibration curve follows the Beer–Lambert law up to 11 mg NO_3^--N per liter.

Measuring UV absorption at 220 nm enables analysts to determine NO_3^- rapidly. Be aware that dissolved organic matter also may absorb at 220 nm but NO_3^- does not absorb at 275 nm, so a second measurement can be made at 275 nm and used to correct the NO_3^- value, if needed. The extent of this empirical correction is related to the nature and concentration of the organic matter and may vary from one water to another, so this method is not recommended if a significant correction is required. Nevertheless, measuring UV absorption may be useful in monitoring NO_3^- levels in a waterbody with a constant type of organic matter.

b. Interferences: Potential interferences include dissolved organic matter, surfactants, NO_2^-, hexavalent chromium [Cr(VI)], and various inorganic ions not normally found in natural water, such as chlorite and chlorate. Correction factors for organic matter absorbance can be established by the method of additions combined with analysis of the original NO_3^- content via another method. Sample filtration eliminates interference caused by suspended particles. Acidification with 1 M hydrochloric acid (HCl) to pH <2 prevents interference from hydroxide or carbonate concentrations up to 1000 mg calcium carbonate ($CaCO_3$) per liter. Chloride does not affect the determination.

Compensate for inorganic substances by independently analyzing their concentrations and preparing individual correction curves. Filter turbid samples. Test filters for NO_3^- contamination.

2. Apparatus

Spectrophotometer, for use at 220 and 275 nm with matched silica cells of 1-cm or longer light path.

3. Reagents

a. Reagent water: Use reagent water as defined in Section 1080 to prepare all solutions and dilutions.

b. Stock nitrate solution: Dry potassium nitrate (KNO_3) in an oven at 103 to 105 °C for 24 h. Dissolve 0.7218 g ±0.0005 g in water and dilute to 1000 mL; 1.00 mL = 100 µg NO_3^--N. Preserve with 2 mL chloroform ($CHCl_3$)/L. Solution is stable for at least 6 months. Alternatively, use a commercial NO_3^--N stock solution.

c. Intermediate nitrate solution: Dilute 100 mL stock NO_3^--N solution to 1000 mL with water; 1.00 mL = 10.0 µg NO_3^--N. Preserve with 2 mL $CHCl_3$/L. Solution is stable for 6 months.

d. Hydrochloric acid solution, (~1 M): Dilute 83 mL concentrated HCl to 1 L with reagent water. Store in a glass or high-density polyethylene (HDPE) bottle. Solution is stable for 1 year if kept closed.

4. Procedure

a. Treatment of sample: To 50 mL clear sample (filtered if necessary), add 1 mL 1 M HCl solution and mix thoroughly.

b. Standards: Prepare NO_3^- calibration standards in the range 0 to 7 mg/L NO_3^--N by diluting to 50 mL the following volumes of intermediate NO_3^- solution: 0, 1.00, 2.00, 4.00, 7.00 mL. Other standard concentrations may also be used. Treat NO_3^--N standards in same manner as samples.

c. Spectrophotometric measurement: Read absorbance or transmittance against reagent water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain NO_3^--N reading and a wavelength of 275 nm to determine any interference due to dissolved organic matter.

5. Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3^--N. If the correction value is greater than 10% of the reading at 220 nm for a particular sample, then the NO_3^--N concentration is considered a rough estimate. Use an electronic spreadsheet, a calculator, or instrument software to find the slope and intercept of the calibration curve by least squares linear regression. Calculate the NO_3^--N concentration from the following equation:

$$C = \frac{A - I}{S}$$

where:

C = concentration,
A = absorbance,
I = intercept of the regression line, and
S = slope of the regression line.

Bibliography

Hoather RC, Rackman RF. Oxidized nitrogen and sewage effluents observed by ultraviolet spectrophotometry. Analyst. 1959;84(1002):549–551.

Goldman E, Jacobs R. Determination of nitrates by ultraviolet absorption. J Amer Water Works Assoc. 1961;53(2):187–191.

Armstrong FAJ. Determination of nitrate in water by ultraviolet spectrophotometry. Anal. Chem. 1963;35(9):1292–1294.

Navone R. Proposed method for nitrates in potable waters. J Amer Water Works Assoc. 1964;56(6):781–783.

4500-NO_3^- C. Second-Derivative Ultraviolet Spectrophotometric Method

1. General Discussion

a. Principle: Even though NO_3^- strongly absorbs UV light, determining NO_3^- by measuring the absorbance at one wavelength may not be feasible because natural organic matter (NOM) and other solutes also absorb UV light. The spectra of NOM from a variety of water sources usually are different, so if NOM were present the UV NO_3^- screening procedure (4500-NO_3^-.B) would not yield reliable results. The UV spectrum of NO_3^- is quite different from that of NOM. In the NO_3^- spectrum, the absorbance increases rapidly from 230 to 210 nm, whereas in NOM spectra, the absorbance increases gradually in the same wavelength range. Computing the second derivative of a sample spectrum effectively eliminates the background NOM contribution.

b. Interferences: The NO_2^- and NO_3^- UV spectra are similar, but NO_2^- concentrations usually are much lower than NO_3^- concentrations. Bicarbonate absorbs weakly at wavelengths <210 nm but does not affect the second-derivative signal of NO_3^-. Bromide (Br^-) interferes at seawater concentrations (68 mg/L Br^-, salinity 35%), so this method cannot be used to determine NO_3^- in seawater or samples containing high concentrations of Br^-. Neither iron (Fe) nor copper (Cu) interferes at 2 mg/L, but both metals highly interfere at 20 mg/L.[1] For NO_3^--N concentrations up to 3 mg/L, the UV absorbance spectrum has a peak at 203 nm. For higher concentrations, the peak gradually shifts to 207 nm and the second-derivative maximum also shifts from 224 nm to higher wavelengths. The shape of the UV and second-derivative spectra may be affected by the spectrophotometer slit width. The method has been tested for potable water but not for wastewater.

2. Apparatus

a. Spectrophotometer capable of scanning from 250 to 200 nm. Some spectrophotometers can compute the second derivative of a spectrum; if the instrument does not, a computer interface is needed (see ¶ *d* below).

b. Cuvettes, matched 1-cm quartz.

c. Pipets: If adjustable pipets are used, calibrate according to manufacturer's directions.

d. Computer, cable, and software to transfer data from spectrophotometer and compute second-derivative spectra. Cable and software may be available from the spectrophotometer manufacturer. These items are needed only if the spectrophotometer cannot compute second-derivative spectra.

3. Reagents

a. Nitrate stock solution A, 100 mg/L NO_3^--N: Prepare as directed in 4500-NO_3^- B.3*b* or obtain from a commercial source.

b. Nitrate stock solution B, 100 mg/L NO_3^--N: Purchase from a commercial source different from stock nitrate solution A or prepare as directed in 4500-NO_3^- B.3*b* using KNO_3 from a commercial source different from that used to prepare stock nitrate solution A.

c. Reagent water (see 4500-NO_3^- B.3*a*): Use to prepare standards and dilute samples if necessary.

4. Procedure

a. Sample treatment: Filter samples if turbid.