

Ammonia-N in Drinking and Surface Waters, Domestic and Industrial Wastes

1 SCOPE

This method covers the determination of Ammonia-N in sewage and effluents, raw and finished drinking waters and industrial wastes. This method is not recommended for brackish water or seawater.

This method, equivalent to USEPA Method 350.1 Rev. 2.0, is approved for the Clean Water Act for use in wastewater compliance monitoring, under National Pollutant Discharge Elimination System (NPDES). This method is also equivalent to Standard Methods 4500-NH₃ H (18th Ed.), 4500-NH₃ G (19th, 20th Ed.) and USGS Method 1-4523-85.

2 RANGE OF APPLICATION

Range 0.02 – 2.0 mg N/L

3 METHOD DETECTION LIMIT

BY USEPA PROCEDURE MDL = 0.004 mg N/L

4 METHOD PRINCIPLE

At alkaline pH greater than 12, ammonia in the sample reacts with hypochlorite (ClO⁻) to form chloramine. This species reacts with alkaline phenol in the presence of nitroferricyanide. During static incubation at 40°C, a blue indophenol dye forms, which is measured photometrically at 660 nm.

5 REFERENCE

Methods for the Determination of Inorganic Substances in Environmental Samples, USEPA 600/R 93/100, August 1993: Method 350.1, Revision 2.0.

6 INTERFERENCES

Dissolved calcium and magnesium may form precipitation under the alkaline reaction conditions to cause positive bias. Heavy-metal cations may precipitate similarly. Reagent recipes for this method include disodium EDTA to prevent precipitation.

If samples have been preserved using sulfuric acid, then reagent recipe adjustments are required for test conditions to attain reaction pH greater than 12. Refer to Section 8 for details. Similarly, highly buffered samples, e.g., containing bicarbonate or phosphate, may require pH adjustment.

When required, interferences are removed by manual distillation of samples. Refer to USEPA 350.1 Rev. 2.0 for the distillation procedure and for discussion of potential interferences during distillation.

Bias from sample turbidity or color is correctable, using sample blanking feature of AQ software.

7 REAGENTS: CHEMICALS REQUIRED AND SAFETY INFORMATION

Consult the material safety data sheets (MSDS) for details on safe handling of chemicals.

<u>Chemical</u>	<u>C.A.S. No.</u>	<u>Safety Information</u>
Ammonium chloride, anhydrous	12125-02-9	Irritating to eyes and skin. May cause irritation of respiratory tract.
Disodium EDTA dihydrate	6381-92-6	Irritating to eyes, skin, and respiratory tract.
Phenol, crystalline	108-95-2	Causes burns at all exposure routes. May be fatal if inhaled, absorbed through the skin or swallowed. May cause liver or kidney damage. Potential for irreversible health effects.
Sodium nitroferricyanide dihydrate	13755-38-9	Toxic if inhaled or swallowed. May irritate eyes and respiratory tract. Contact with acid liberates very toxic gas.
Sodium hydroxide	1310-73-2	Causes severe burns. Harmful if inhaled or swallowed.
Sodium hypochlorite	7681-52-9	Irritating to eyes, skin, and respiratory tract. Harmful if swallowed.
Sulfuric acid, concentrated	7664-93-9	Causes severe burns. Toxic if inhaled or swallowed.

8 REAGENT SOLUTIONS

Use reagent-grade chemicals, certified for analytical or general laboratory use. Reagents of technical or commodity grade must be validated by the user. Use high-purity reagent water, distilled or deionized, and free from organic contamination. Grade 1 (ISO Standard 3696) or better than Type II (ASTM Standard D1193) are suitable.

8.1 SULFURIC ACID, 5 normal

Sulfuric acid, concentrated	70 mL
DI water	dilute to 500 mL

Slowly add 70 mL concentrated sulfuric acid to about 400 mL of deionized water. Caution, the container will become very warm. Cool to room temperature and dilute to 500 mL with deionized water. This reagent is available commercially.

8.2 EDTA BUFFER, 75 g/L EDTA, 2 g/L NaOH

Use this recipe when the Calibration Diluent is deionized water.

Disodium ethylenediaminetetraacetic acid dihydrate	37.5 g	18.7	7.0
Sodium hydroxide	1.0 g	0.5	0.2
DI water	dilute to 500 mL	250	160

To a 500 mL Erlenmeyer flask add 37.5 g disodium ethylenediaminetetraacetic acid dihydrate, 1.0 g sodium hydroxide and about 400 mL deionized water. Stir with gentle warming to dissolve, adding deionized water as needed. Transfer to a 500 mL volumetric flask, fill to the mark with deionized water, and mix.

8.3 MODIFIED EDTA BUFFER, 75 g/L EDTA

Use this recipe when the Calibration Diluent is dilute sulfuric acid, as specified in Section 9.2.

Disodium ethylenediaminetetraacetic acid dihydrate	37.5 g
Sodium hydroxide	see table
DI water	dilute to 500 mL

To a 500 mL Erlenmeyer flask add 37.5 g disodium ethylenediaminetetraacetic acid dihydrate and about 400 mL deionized water. Add sodium hydroxide pellets according to the following table:

Calibration Diluent	grams sodium hydroxide per 500 mL*
Deionized water	1.0 g
0.004 N (finished distillate)	1.8 g
1.0 mL sulfuric acid per liter	9.1 g
1.5 mL sulfuric acid per liter	13.1 g
2.0 mL sulfuric acid per liter	17.2 g

* grams NaOH per 500 mL = $(A \times 1.442 \times (\text{sample vol., } \mu\text{L} / \text{R1-EDTA vol., } \mu\text{L}) + 2.0) / 2$
where A = acid level in samples (mL sulfuric acid per liter)

Stir with gentle warming to dissolve, adding deionized water as needed. Transfer to a 500 mL volumetric flask, fill to the mark with deionized water, and mix.

8.4 ALKALINE PHENATE

Sodium hydroxide	10 g	5g	4
Crystalline phenol	25 g	12.5	10
DI water	dilute to 250 mL	12.5	100

To a 500 mL Erlenmeyer flask add 10 g sodium hydroxide and about 150 mL deionized water. Swirl to dissolve and cool to near room temperature. In the fume hood, add 25 g crystalline phenol and swirl to dissolve. Transfer to a 250 mL volumetric flask, fill to the mark with deionized water and mix. Allow to stand overnight. This reagent may be stored in the refrigerator at least 1 month or until its color becomes dark brown. Crystalline phenol is preferred to liquefied phenol, 90% w/w.

12.5

8.5 SODIUM HYPOCHLORITE, 5% or 5.7% active chlorine, 5.25% or 6.0% NaOCl (w/w)

In the USA, SEAL recommends freshly purchased house-brand bleach from a grocer. The bleach should not contain thickeners, fragrances or additives, other than sodium hypochlorite. Refrigerate this reagent. Alternately, an analytical sodium hypochlorite reagent (5.65 to 6% w/w NaOCl, i.e., 5.4 to 5.7% active chlorine), e.g., from Fischer Scientific (cat. no. SS290-1), may be used. Because this solution slowly decomposes, replace 8 weeks after opening. Refer to Section 11.1.

12.5

8.6 NITROFERRICYANIDE

Sodium nitroferricyanide dihydrate	1.0 g	5
DI water	dilute to 500 mL	250

Dissolve 1.0 g sodium nitroferricyanide dihydrate in 500 mL deionized water. This solution can be stored at least 1 month in a dark bottle. Discard if the reagent becomes bluish in color.

9 STANDARD SOLUTIONS

9.1 AMMONIA STOCK STANDARD SOLUTION (1000 mg N/L)

Ammonium chloride, anhydrous	3.819 g
Sulfuric acid, 5 normal (above)	up to 3 mL
DI water	dilute to 1 L

Dissolve 3.819 g ammonium chloride, anhydrous (dried at 105°C) in about 800 mL deionized water in a 1 Liter volumetric flask. Up to 3 mL sulfuric acid, 5 normal, may be added for preservation. Dilute to the mark with deionized water. Prepare fresh monthly.

9.2 CALIBRATION DILUENT (for acid-preserved samples)

Sulfuric acid, 5 normal (above)	see table
DI water	dilute to 2 L

Use this solution as the diluent for automated and manual dilutions and also as the blank and diluent for auto-calibrations.

Choose the level of sulfuric acid closest to the level of acid added to field samples for preservation. Transfer the volume of 5 normal sulfuric acid, shown in the table (± 0.3 mL), to a 2 liter bottle. Dilute to volume with deionized water.

Sulfuric acid, preservation level	none	0.004 N	1.0 mL/L	1.5 mL/L	2.0 mL/L
Sulfuric acid, 5 normal volume added per 2 L	--	1.6 mL	14.3 mL	21.4 mL	28.6 mL

9.3 AMMONIA TOP STANDARD SOLUTION (2 mg N/L)

Ammonia stock standard (1000 mg N/L, above)	1 mL
Calibration Diluent (above)	dilute to 500 mL

To a 500 mL volumetric flask pipet 1 mL Ammonia stock standard solution (1000 mg N/L) and dilute to the mark with Calibration Diluent. Prepare every two weeks.

This standard is used for auto-standardization through AQ software according to user-defined parameters.

9.4 AMMONIA WORKING STANDARD SOLUTIONS (prepare weekly)

Concentration, mg N/L	0	0.02	0.05	0.1	0.4	0.8	1.6	2
Volume (mL) Ammonia top standard solution diluted as indicated with Calibration Diluent	--	2	5	5	20	40	80	100
Final Volume, mL	100	200	200	100	100	100	100	100

Standard concentrations are suggested concentrations for manual preparation of calibration standards.

10 SAMPLE PRESERVATION AND STORAGE

Samples may be collected in plastic or glass containers.

Samples should be analyzed as soon as practical after collection.

Sample preservation and holding time requirements are as follows, for samples to be reported for wastewater compliance monitoring under the Clean Water Act:

- When samples are held longer than 15 minutes, samples must be preserved with concentrated sulfuric acid (H_2SO_4) to a pH < 2 and cooled to 4°C at the time of collection.
- Samples should not be frozen unless validation data are on file to prove integrity of the sample.
- If storage is required, preserved samples must be maintained at 4°C and must be analyzed within 28 days.

11 PROCEDURE

For NPDES compliance monitoring, samples must be distilled prior to colorimetric analysis. Preliminary distillation is not required if comparability data are on file for representative classes of samples, showing that distillation is not necessary. However, distillation will be required to resolve any controversies.

Prepare standards and reagents as described in Sections 8 and 9. The laboratory must determine standard concentrations that are most suitable to their needs for calibration within the range of application for this method. The concentrations listed within this method are only suggestions.

Any reagents having visible particulates should be filtered.

Pour calibration standards, quality control solutions and samples into sample cups and 45 mL reagent wedges, according to the user-defined worklist.

Before running the analysis batch, check the analyzer for sufficient reagent water, fresh reaction segments, correct location of reagent vessels and a waste collection bottle in place.

11.1 CHEMISTRY TROUBLESHOOTING TIPS

The concentration of active chlorine in the hypochlorite reagent is crucial for obtaining full color development and low values for method detection limit. Outside the United States, users may need to purchase technical grades of sodium hypochlorite, for example, having nominal active chlorine content 10% to 14%.

When first evaluating method performance or if color development and linearity of the calibration line become unsatisfactory, check reaction pH. Measure the pH, after dispensing scaled-up test volumes of samples and reagents into a beaker. The optimum pH range of the mixture is 12.15 to 12.4. Also, check the reaction pH if the purchased bleach is suspect, or when using technical grades of sodium hypochlorite solution. The table below shows the test volumes as scaled up 50-fold.

REAGENT	Test vol., μL	pH test vol., mL
Sample matrix (as preserved)	360	18
Reagent 1 (EDTA)	32	1.6
Reagent 2 (Phenate)	62	3.1
Reagent 3 (Bleach, 6% NaOCl)	35	1.8
Reagent 4 (Nitroferricyanide)	20	1.0

Measured pH of the ammonia reaction = _____

REFERENCES, supplementary

- 1) U.S. EPA, 40 CFR, Part 136, Appendix B and Part 136.3 Tables IB and II, and Part 141.23.
- 2) 2003 NELAC Standard, National Environmental Laboratory Accreditation Conference, June 5, 2003, EPA-600/R-04/003, <http://www.epa.gov/nerlesd1/land-sci/nelac/index.html>
- 3) "Methods Update Rule", Vol. 72 Federal Register No. 47 (12 March 2007) Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; National Primary Drinking Water Regulations and National Secondary Drinking Water Regulations.
- 4) Manual for the Certification of Laboratories Analyzing Drinking Water, 4th ed., USEPA 815/B-97/001, March 1997.

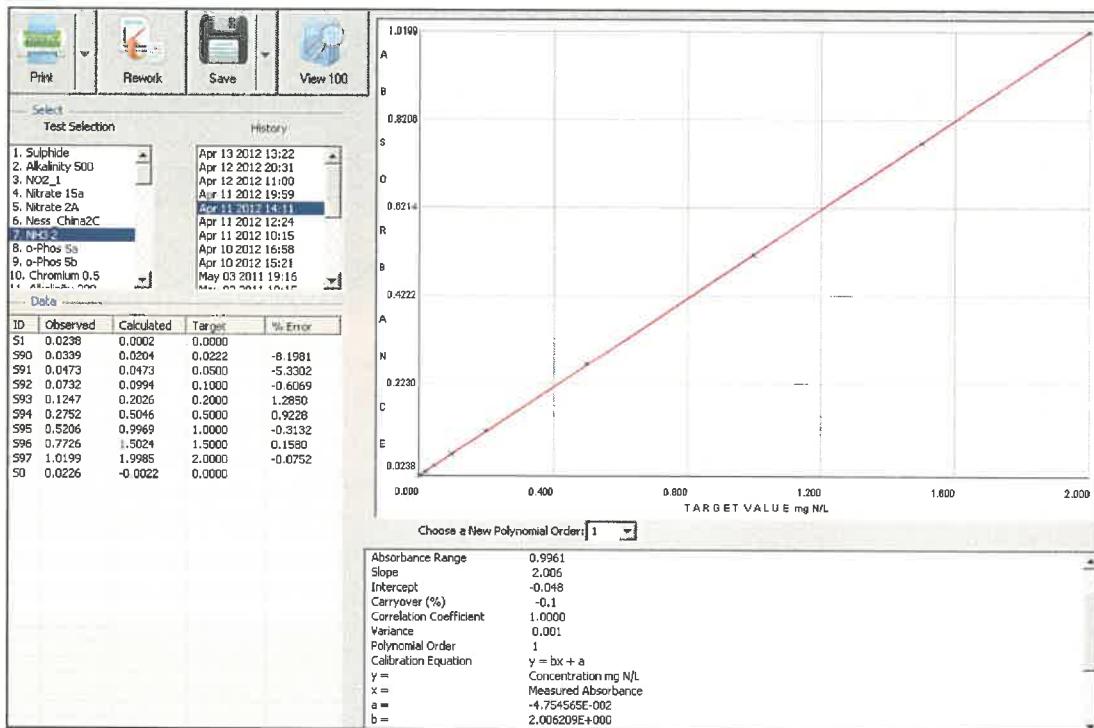
12 TEST PARAMETERS

PARAMETER	AQ2 SETTING
Test name	Ammonia 2
Units	mg N/L
Decimals	4
Test type	End point
Sample volume (μL)	360
Water volume (μL)	0
Number of mixes	1
Cuvette primes	2
Cuvette washes	2
Reaction time (seconds)	600
Wavelength (nm)	660
Polynomial order	1
Number of reagents	4
1. EDTA buffer (μL)	32
2. Phenate (μL)	62
3. Hypochlorite (μL)	35 (6.0% NaOCl) or 42 (5.25% NaOCl) or 26 (8.25% NaOCl)
4. Nitroferricyanide (μL)	20
Advanced Test Parameters	<input checked="" type="checkbox"/> Eliminate Air from Test Transfer <input checked="" type="checkbox"/> Extra Debubbling Action

DEFINITIONS:

- Test Name: Name which appears on the final report
 Decimals: Number of decimal places in the reported result
 Units: Concentration units appearing against the reported result
 Number of mixes: Number of mixes of the sample + reagent in the reaction well
 Reaction time: Time (in seconds) between addition of sample + reagent and measurement of the reaction mixture
 Sample volume: Volume (in microliters) of the sample used in the test
 Number of reagents: Number of reagents defined for the test
 Reagent volume: Volume (in microliters) of the reagent used in the test
 Wavelength: Interference filter wavelength to be used in the test
 Cuvette primes: Number of times the cuvette is flushed with reagent (no sample) prior to sample analysis
 Cuvette wash: Number of washes of the cuvette between aspirations of finished reaction liquid
 Baseline on Wash: Water baseline measured between aspirations to provide drift correction
 Polynomial order: Polynomial order to be used when fitting the standard points to the standard graph (1 = linear)

13 STANDARDIZATION



DEFINITIONS:

Abs. Range: Difference between the absorbance of the highest standard and the absorbance of the blank

Variance: Calculated as follows:

$$\text{Variance} = \frac{\sum (\text{Deviations, i.e., errors})^2}{\sum (\text{Observed values})^2} \times 100$$

Carryover: Calculated as follows:

$$\text{Carryover} = \frac{(A - B)}{C}$$

Where, A = absorbance of carryover blank (S0)

B = absorbance of blank (S1)

C = calibration absorbance range

14 PERFORMANCE VALIDATION DATA

14.1 DETECTION LIMIT STUDY

Replicates of 0.02 mg N/L ammonia standard in reagent water***

	Concentration (mg N/L)				
	0.014	0.011	0.014	0.014	0.015
	0.015	0.009	0.014	0.015	0.013
	0.015	0.010	0.013	0.015	0.016
	0.015	0.011	0.013	0.015	0.014
	0.016	0.010	0.014	0.014	0.015
	0.014	0.010	0.013	0.016	0.012
	0.013	0.008	0.016	0.017	0.013
	0.013	0.011	0.014	0.014	0.015
	0.011	0.009	0.013	0.015	0.012
	0.012	0.011	0.012	0.012	0.013
	0.014	0.008	0.013	0.013	0.012
	0.012	0.009	0.013	0.011	0.014
	0.014	0.008	0.014	0.017	0.013
	0.014	0.011	0.015	0.012	0.013
	0.011	0.009	0.011	0.014	0.015
Statistics					
Mean	0.013	0.010	0.014	0.014	0.014
Std. Deviation	0.002	0.001	0.001	0.002	0.001
MDL*	0.004	0.003	0.003	0.004	0.004

14.2 PERFORMANCE STUDY

Replicates of 0.1 mg N/L ammonia standard in reagent water***

	Concentration (mg N/L)			
	0.114	0.101	0.114	0.115
	0.108	0.106	0.111	0.107
	0.111	0.108	0.116	0.126
	0.103	0.114	0.105	0.104
	0.116	0.104	0.112	0.112
	0.104	0.108	0.105	0.104
	0.113	0.108	0.111	0.114
	0.103	0.111	0.104	0.104
	0.109	0.104	0.113	0.109
	0.108	0.103	0.104	0.106
Statistics			4.5	
Mean	0.109	0.107	0.110	0.111
Std. Deviation	0.005	0.004	0.005	0.006
% RSD**	4.3	4.1	4.5	5.8
% Recovery	109.2	107.2	110.3	110.5

* MDL is calculated by multiplying the Standard Deviation by the Student-t value for the number of replicates run.

** %RSD is calculated by dividing the Standard Deviation by the mean, then multiplying by 100.

*** To prepare these standards, refer to Section 9.

15 REVISIONS

August 23, 2004 Rev 1	Method format changed to meet EPA standards Document tracking information added Equivalent methods listed Sample preservation corrected Method number changed from 2-012-1-L to EPA-103-A Range modified as 0.01 to 2 mg N/L to 0.02 mg N/L. Applicable to drinking and surface waters, domestic and industrial wastes only. Saline matrices excluded Modified EDTA Buffer and acidic calibration diluents for varying sample acidities were added Sample neutralization solutions were added
August 31, 2004 Rev 2	Reagent and sample volumes increased
October 7, 2004 Rev 3	Front page reformatted
June 1, 2005 Rev 4	Method Supplement 1 document created and implemented Method reworked to improve linear calibration fits. Reagent recipes rewritten to accommodate both 10-mm Cuvettes (P/N 1-0031 and P/N 5670).
June 17, 2008 Rev 5	Editorial revisions
May 1, 2009 Rev 6	Minor editorial revisions
April 16, 2012 Rev 7	Method document reformatted New detection limit data collected
October 10, 2012 Rev 8	Minor editorial revisions
November 8, 2012 Rev 9	Added descriptive information regarding standard preparation
December 17, 2012 Rev 10	Test parameter revision