Method 350.1, Revision 2.0: Determination of Ammonia Nitrogen by Semi-Automated Colorimetry
METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

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METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of ammonia in drinking, ground, surface, and saline waters, domestic and industrial wastes.

1.2 The applicable range is 0.01-2.0 mg/L NH₃ as N. Higher concentrations can be determined by sample dilution. Approximately 60 samples per hour can be analyzed.

1.3 This method is described for macro glassware; however, micro distillation equipment may also be used.

2.0 SUMMARY OF METHOD

2.1 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically.

2.3 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Section 9.0, Quality Control.

3.0 DEFINITIONS

3.1 Calibration Blank (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
3.3 **Instrument Performance Check Solution (IPC)** -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4 **Laboratory Fortified Blank (LFB)** -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 **Laboratory Fortified Sample Matrix (LFM)** -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6 **Laboratory Reagent Blank (LRB)** -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7 **Linear Calibration Range (LCR)** -- The concentration range over which the instrument response is linear.

3.8 **Material Safety Data Sheet (MSDS)** -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 **Method Detection Limit (MDL)** -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 **Quality Control Sample (QCS)** -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11 **Stock Standard Solution (SSS)** -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.0 **INTERFERENCEs**
4.1 Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out.

4.2 Residual chlorine must be removed by pretreatment of the sample with sodium thiosulfate or other reagents before distillation.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sulfuric acid (Section 7.6)

5.3.2 Phenol (Section 7.7)

5.3.3 Sodium nitroprusside (Section 7.10)

6.0 EQUIPMENT AND SUPPLIES

6.1 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2 Glassware - Class A volumetric flasks and pipets as required.

6.3 An all-glass distilling apparatus with an 800-1000 mL flask.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump
6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water - Ammonia free: Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

Note: All solutions must be made with ammonia-free water.

7.2 Boric acid solution (20 g/L): Dissolve 20 g H$_3$BO$_3$ (CASRN 10043-35-3) in reagent water and dilute to 1 L.

7.3 Borate buffer: Add 88 mL of 0.1 N NaOH (CASRN 1310-73-2) solution to 500 mL of 0.025 M sodium tetraborate solution (5.0 g anhydrous Na$_2$B$_4$O$_7$ [CASRN 1330-43-4] or 9.5 g Na$_2$B$_4$O$_7$•5H$_2$O [CASRN 1303-96-4] per L) and dilute to 1 L with reagent water.

7.4 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in reagent water and dilute to 1 L.

7.5 Dechlorinating reagents: A number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

7.5.1 Sodium thiosulfate: Dissolve 3.5 g Na$_2$S$_2$O$_3$•5H$_2$O (CASRN 10102-17-7) in reagent water and dilute to 1 L. One mL of this solution will remove 1 mg/L of residual chlorine in 500 mL of sample.

7.5.2 Sodium sulfite: Dissolve 0.9 g Na$_2$SO$_3$ (CASRN 7757-83-7) in reagent water and dilute to 1 L. One mL removes 1 mg/L Cl per 500 mL of sample.

7.6 Sulfuric acid 5 N: Air scrubber solution. Carefully add 139 mL of conc. sulfuric acid (CASRN 7664-93-9) to approximately 500 mL of reagent water. Cool to room temperature and dilute to 1 L with reagent water.

7.7 Sodium phenolate: Using a 1-L Erlenmeyer flask, dissolve 83 g phenol (CASRN 108-95-2) in 500 mL of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 L with reagent water.

7.8 Sodium hypochlorite solution: Dilute 250 mL of a bleach solution containing 5.25% NaOCl (CASRN 7681-52-9) (such as "Clorox") to 500 mL with reagent water.
water. Available chlorine level should approximate 2-3%. Since “Clorox” is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

7.9 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) (CASRN 6381-92-6) and approximately six pellets of NaOH in 1 L of reagent water.

7.10 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside (CASRN 14402-89-2) in 1 L of reagent water.

7.11 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH₄Cl (CASRN 12125-02-9), dried at 105°C, in reagent water, and dilute to 1 L. 1.0 mL = 1.0 mg NH₃-N.

7.12 Standard Solution A: Dilute 10.0 mL of stock solution (Section 7.11) to 1 L with reagent water. 1.0 mL = 0.01 mg NH₃-N.

7.13 Standard Solution B: Dilute 10.0 mL of standard solution A (Section 7.12) to 100.0 mL with reagent water. 1.0 mL = 0.001 mg NH₃-N.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H₂SO₄ to a pH <2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9.0 QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2 Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by ± 10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ±10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

\[
\text{MDL} = (t) \times (S)
\]

where, \(t\) = Student’s t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [\(t = 3.14\) for seven replicates]

\(S\) = standard deviation of the replicate analyses

MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

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9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (\(x\)) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

\[
\text{UPPER CONTROL LIMIT} = x + 3S  \\
\text{LOWER CONTROL LIMIT} = x - 3S
\]

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to 10 new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to established an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every 10th sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift, the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.
9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculated using the following equation:

\[ R = \frac{C_s - C}{s} \times 100 \]

where, \( R \) = percent recovery

\( C_s \) = fortified sample concentration

\( C \) = sample background concentration

\( s \) = concentration equivalent of analyte added to sample

9.4.3 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solutions (Sections 7.12 and 7.13) to 100 mL with reagent water.

10.2 Process standards and blanks as described in Section 11.0, Procedure.

10.3 Set up manifold as shown in Figure 1.

10.4 Prepare flow system as described in Section 11.0, Procedure.
10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

10.6 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based regression curve fitting techniques. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.7 After the calibration has been established, it must be verified by the analysis of a suitable QCS. If measurements exceed ±10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

11.1 Preparation of equipment: Add 500 mL of reagent water to an 800 mL Kjeldahl flask. The addition of boiling chips that have been previously treated with dilute NaOH will prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia.

11.2 Sample preparation: Remove the residual chorine in the sample by adding dechlorinating agent (Section 7.5) equivalent to the chlorine residual. To 400 mL of sample add 1 N NaOH (Section 7.4), until the pH is 9.5, check the pH during addition with a pH meter or by use of a short range pH paper.

11.3 Distillation: Transfer the sample, the pH of which has been adjusted to 9.5, to an 800 mL Kjeldahl flask and add 25 mL of the borate buffer (Section 7.3). Distill 300 mL at the rate of 6-10 mL/min. into 50 mL of 2% boric acid (Section 7.2) contained in a 500 mL Erlenmeyer flask.

Note: The condenser tip or an extension of the condenser tip must extend below the level of the boric acid solution.

11.4 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples.

11.5 Allow analysis system to warm up as required. Feed wash water through sample line.

11.6 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

11.7 Switch sample line from reagent water to sampler and begin analysis.
12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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 results in mg NH₃-N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH₃-N/L, the standard deviation was ±0.005.

13.2 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 0.16 and 1.44 mg NH₃-N/L, recoveries were 107% and 99%, respectively.

13.3 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg NH₃-N/L.

14.0 POLLUTION PREVENTION

14.1 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 to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 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.

14.3 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 U.S. 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 14.3.
REFERENCES


4. A Wetting Agent Recommended and Supplied by the Technicon Corporation for Use in AutoAnalyzers.


9. Code of Federal Regulations 40, Ch. 1, Pt. 136, Appendix B.
### TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

<table>
<thead>
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<th>Number of Values Reported</th>
<th>True Value (T)</th>
<th>Mean (X)</th>
<th>Residual for X</th>
<th>Standard Deviation (S)</th>
<th>Residual for S</th>
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REGRESSIONS: $X = 1.003T - 0.003$, $S = 0.052T + 0.019$
**Figure 1**

Ammonia Manifold

**Table**

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<th>mL/min</th>
<th>Substance</th>
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<td>1.00</td>
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**Notes**

- TO RESAMPLE
- TO F/C PUMP TUBE
- HEATING BATH 37°C
- COLORIMETER 660 nm 50mm F/C
- PUMP 30 PER HOUR
- SAMPLE 96 SEC.
- WASH 24 SEC.