

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF AMMONIA IN WATER SAMPLES

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Note: The effective date is considered to be the last approval date.

Revisions

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1.0 Scope and Application

This method covers the determination of ammonia in drinking, ground, surface and saline waters, domestic and industrial wastes. This SOP is based on EPA Methods 350.1 Rev 2.0 and AA100 Method E-1008-16 Rev. 3.

2.0 Summary

Samples are preserved at $\text{pH} < 2$ with sulfuric acid in the field. The sample is made alkaline and flows across a hydrophobic membrane in a gas dialysis module. Ammonia in the gaseous phase passes through the membrane into an acidic acceptor stream that is air segmented. The stream is made alkaline, and ammonia reacts with salicylate, nitroprusside, which is used as a catalyst, and dichloro-isocyanuric acid at 37°C to produce a green-blue indophenol complex measured at 660 nm. EDTA is added to the alkaline streams to prevent the precipitation of the hydroxides of calcium and magnesium.

3.0 Acronyms/ Definitions

DI – Deionized
RL – Reporting Limit
MDL – Method Detection Limit
QA – Quality Assurance
QC - Quality Control
ICV – Initial Calibration Verification
CCV – Continuing Calibration Verification
LFB – Laboratory Fortified Blank
LFM – Laboratory Fortified Matrix

4.0 Healthy and Safety

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.

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 Safety Data Sheets (SDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.0 Interferences

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

Metal ions in high concentrations, which precipitate as hydroxides, may cause poor reproducibility.

Low-molecular amines react similarly to ammonia and will consequently lead to erroneously high results.

Samples containing particles larger than 0.1 mm must be filtered.

Interferences may occur if the reaction mixture after addition of all reagent solutions does not reach a pH of at least 12.6. This mainly happens with strong acidic and buffered samples, which must be then approximately neutralized before analysis.

6.0 Personnel Qualifications

The analyst should have at least 4-year degree in physical science. The analyst must have a satisfactory IDC in place before analyzing samples. All personnel shall be responsible for complying with all QA/QC requirements that pertain to their organizational/technical function.

7.0 Equipment and Supplies

7.1 Segmented Flow Analyzer: Seal Analytical AA100

7.2 Glassware: Class A volumetric flasks and pipettes as required

7.3 Analytical Balance: Capable of accurately weighing to the nearest 0.01g

8.0 Procedure

8.1 Sample Handling and Preservation

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

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

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. On analysis, verify that the sample pH is less than 2 and document.

8.2 Reagent Preparation

- 8.2.1 **Reagent 1 - 0.036M H₂SO₄ diluent** for standards, to match preserved samples.
In a **1 L** volumetric flask add **500 mL DI water** and **2.0 mL of concentrated sulfuric acid**. Dilute to the mark with **DI water**. To be used for sample dilution. Store at room temperature. Stable for 6 months.
- 8.2.2 **Reagent 2 - Salicylate/Nitroprusside**
In a **200 mL** volumetric flask, dissolve **13 g sodium salicylate**, and **0.026 g sodium nitroferrocyanide dihydrate (nitroprusside)** in approximately **120 mL DI water**. Dilute to the mark with **DI water**, and invert to mix. Stable for one week in a brown glass bottle at 8°C.
- 8.2.3 **Reagent 3 - Dichloroisocyanurate (DCI)**
In a **200 mL** volumetric flask, dissolve **0.15 g sodium dichloroisocyanuric acid sodium salt dihydrate (0.14 g of the monohydrate)** into approximately **100 mL of DI water**. Dilute to the mark with **DI water** and invert to mix. Prepare fresh daily.
- 8.2.4 **Reagent 4 – Triton X-100, 30% Solution**
In a **100 mL** volumetric flask dissolve **30 mL of Triton X-100** into approximately **50 mL DI water**, dilute to the mark and invert to mix. Store at 4°C. Stable for 1 month. Discard if precipitate forms.
- 8.2.5 **Reagent 5 – System Wash**
Add **2 mL of Reagent 4 (Triton X-100, 30% solution)** to **1 L of DI water**. Replace weekly.
- 8.2.6 **Reagent 6 – Hydrochloric Acid, 1 M**
Cautiously add **85 mL of hydrochloric acid** to approximately **600 mL of DI water**, dilute to **1 L**, and add **1 mL of Reagent 4 (Triton X-100, 30% Solution)**. Stable for 6 months.
- 8.2.7 **Reagent 7 – EDTA/NAOH**
In a **1000 mL** volumetric flask, dissolve **56 g of EDTA**, and **30 g of sodium hydroxide** into approximately **800 mL of DI water**. Dilute to the mark with **DI water** and invert to mix. Add **5 mL of Reagent 4 (Triton X-100, 30% Solution)**. Prepare fresh weekly.
- 8.2.8 **Reagent 8 – Sulfuric Acid**
In a **1000 mL** volumetric flask, **20 mL of sulfuric acid** into approximately **800 mL of DI water**. Dilute to the mark with **DI water** and invert to mix. Add **1 mL of Reagent 4 (Triton X-100, 30% Solution)**. Prepare fresh weekly.

8.2.9 Reagent 9 – Working Buffer

In a 1000 mL volumetric flask, dissolve 16 g of di-sodium hydrogen phosphate-12-hydrate, 27 g of sodium hydroxide, and 24 g of potassium sodium tartrate tetrahydrate into approximately 600 mL of DI water. Dilute to the mark with DI water and invert to mix. Add 1 mL of Reagent 4 (Triton X-100, 30% Solution). Prepare fresh weekly.

8.3 Standard and QC Preparation

8.3.1 Preparation of Standards and QC samples

Three different vendors or lots of 1000 mg NH₃-N ammonia standard solution commercially prepared. Certificate of analysis is required.

8.3.2 Working standard 1 20 ppm standard solution as NH₃-N: To a 50 mL volumetric flask add 1.0 mL of stock standard and dilute to the mark with diluent.

Calibration curve

Calibration point/ level	1	2	3	4	5/CCV	6	7	8
Concentration as NH ₃ -N (µg N/L)	1200	800	600	400	200	100	40	0
Volume (mL) of Working Standard 1	1.5	1.0	0.75	0.50	0.25	0.125	0.10	-
Dilution volume with Reagent 1	25	25	25	25	25	25	50	-

8.3.3 Working standard 2 20 ppm standard solution as NH₃-N: To a 50 mL volumetric flask add 1.0 mL of stock standard 1000 ppm of ammonia (NH₃) as N and dilute to the mark with diluent.

ICV 0.20 ppm standard solution as NH₃-N: To a 25 mL volumetric flask, add 0.25 mL of working standard 2 and dilute to the mark with diluent.

8.3.4 Working Standard 3 20 ppm standard solution as NH₃-N: To a 50 mL volumetric flask add 1.0 mL of stock standard 1000 ppm of ammonia (NH₃) as N and dilute to the mark with diluent.

LFB 0.20 ppm standard solution as NH₃-N: To a 25 mL volumetric flask, add 0.25 mL of working standard 3 and dilute to the mark with diluent.

LFB L 0.040 ppm standard solution as NH₃-N: To a 50 mL volumetric flask, add 0.10 mL of working standard 3 and dilute to the mark with diluent.

Matrix Spike (MS) Prepared the same as the LFB and brought to 25 mL with sample.

8.3.5 Blanks Use diluent as blank.

8.4 Instrument Operation:

Start Up:

- Turn on the AA1 and the sampler.
- Fix the platen.
- Start pumping (click on charting)
 - 2 ml/L Triton, through the EDTA/NaOH and sulfuric acid lines
 - DI water, through the sample line and all other reagent lines
- Wait until the detector and heating coil have reached stable temperatures, there is a stable bubble pattern and there is a stable baseline.
- Place the straws into the corresponding reagents (salicylate/nitroprusside reagent last) and wait for a stable baseline.
- Right click on the chart and set the base
- Manually sample the 1200 ppb sample, wait until it shows up on the chart, right click, and set the gain to the high point

Shut Down:

- Pump for at least 15 minutes:
 - Remove straw for salicylate/nitroprusside reagent first to DI water
 - 2 ml/L Triton, through the EDTA/NaOH and sulfuric acid lines
 - DI water, through the sample line and all other reagent lines
- Wash dialysis system
 - DI water, 5 minutes through all lines to wash the Triton off the membrane.
- Empty dialysis system
 - Air, through the sample line, EDTA/NaOH and sulfuric acid lines.
 - DI water, through all other reagent lines
- Wait about 15 minutes until there is no liquid in the dialyzer
- Switch off all modules
- Release the platen.

Follow the manufacturer's instructions for proper operation.

9.0 Data and Record Management

9.1 Data package Documentation

All standards must be traceable back to the original vendor stock and that standard must be identified in detail (vendor, lot number, and certificate). Each data package must include a copy of the following:

- Sample preparation sheet
- Standards preparation sheet
- Raw instrument data and analytical sequence (printouts from the AA100 instrument)

Prep sheets are available in Y:\Wetchem\Forms

9.2 Archiving

Electronically archived data is in W:\Raw Data

9.3 Calculations

Prepare a calibration curve by plotting instrument response against standard concentration. Curves may need to be fit using the “slope only” option. This is done to correct for the fact that the eluent is DI water while the samples are all acidic.

Provided below is the conversion chart to convert NH_3 to $\text{NH}_3\text{-N}$
 $\text{NH}_3 = \text{NH}_3\text{-N} * 1.21589$

Assumed atomic weights:
H: 1.008, N: 14.007

9.4 Project Review

Upon completion of a project a project review form should be filled out and accompany the final report in the report folder. The first section (requested analysis and data folder completeness check) should be completed by the analyst. The last two sections (data evaluation and final report) should be completed by two different chemists that have knowledge of the method.

Project Review forms are available Y:\Wetchem\FORMS

10.0 Quality Control and Quality Assurance

10.1 Demonstration of capability.

The analyst must make an initial demonstration of capability (IDC) to generate acceptable accuracy and precision with this method. Continuing displays of proficiency (C-DOP) are repeated annually and each time a method modification is made.

10.2 Blank(s): There are three different types of blanks required by this method.

10.2.1 The Calibration Blank is 0.036M H_2SO_4 and run with the calibration curve.

10.2.2 Laboratory Reagent Blank is 0.036M H_2SO_4 . One laboratory reagent blank per batch of 20 samples or less.

10.2.3 Method Blanks should contain all the reagents and in the same volumes as used in processing the samples. Blanks shall be carried through the complete preparation, and analysis method process.

10.3 Laboratory Fortified Blanks (LFB)

One LFB with each batch of 20 samples or less. (See 8.3.4 for preparation)

10.4 Initial Calibration Verification Standards (ICVs)

The ICV is analyzed after calibration standards. Recoveries must be within 10% of the true value, otherwise re-calibration of the instrument is necessary. (See 8.3.3 for preparation.)

10.5 Continuing Calibration Verification Standards (CCV)

Analyze CCV after the ICV, after every ten injections, and at completion of analysis. Recoveries must be within 10% of the true value. If recoveries fall outside of this range the cause of the failure needs to be determined, corrected, and the instrument may need to be recalibrated.

10.6 Matrix Spike (LFM)

One for every 10 samples or less per project.

10.7 Sample Duplicates (DUP)

One for every 10 samples or less per project.

10.8 Method Detection Limit (MDL)

A low LFB is run with each analysis. At least once every thirteen months the MDL_s is calculated from low LFBs and the MDL_b is calculated from the blanks as described in 40 CFR 136 Appendix B. The verified MDL is the greater of the two values.

11.0 Waste Management and Pollution Prevention

NERL encourages all chemist and biologists to investigate analytical techniques, innovative technologies, and chemical substitution in laboratory processes to reduce waste and prevent pollution. As analytical SOPs are reviewed, on an annual basis, the responsible chemist or biologist will incorporate waste minimization practices where practicable and where these practices have been demonstrated to return data of equivalent quality.

Chemists and biologists must refer to the Waste Management Program SOP, most current revision, for proper disposal of laboratory waste. Personnel should contact the Environmental, Safety and Health Department if changes in the analytical SOP will generate new waste streams. Questions regarding the proper disposal of laboratory waste and purchase of new reagents should be directed to the Environmental, Safety and Health Department in advance of initiating a change in the analytical method.

12.0 Preventative Maintenance

Daily maintenance:

- Reposition the air valve tubing
- Check waste lines are clear and are not submerged
- Check baseline is stable and set base/gain
- Replace membrane as needed

Weekly maintenance:

- Replace peristaltic tubing
- Clean the sampler wash pot
- Record the reagent absorbance (AU)
- Record the sensitivity (AU)
- Perform cleaning by placing the EDTA, salicylate, and DIC lines in 1M hydrochloric acid (Reagent 6) for 15 minutes
- Check if any tubing joints need replacement

As needed maintenance:

- Clean the flow cell
- Replace waste lines
- Replace flow cell sleeving
- Replace sample line
- Check sampler probe
- Replace manifold transmission tubing
- Replace reagent inlet lines

13.0 References

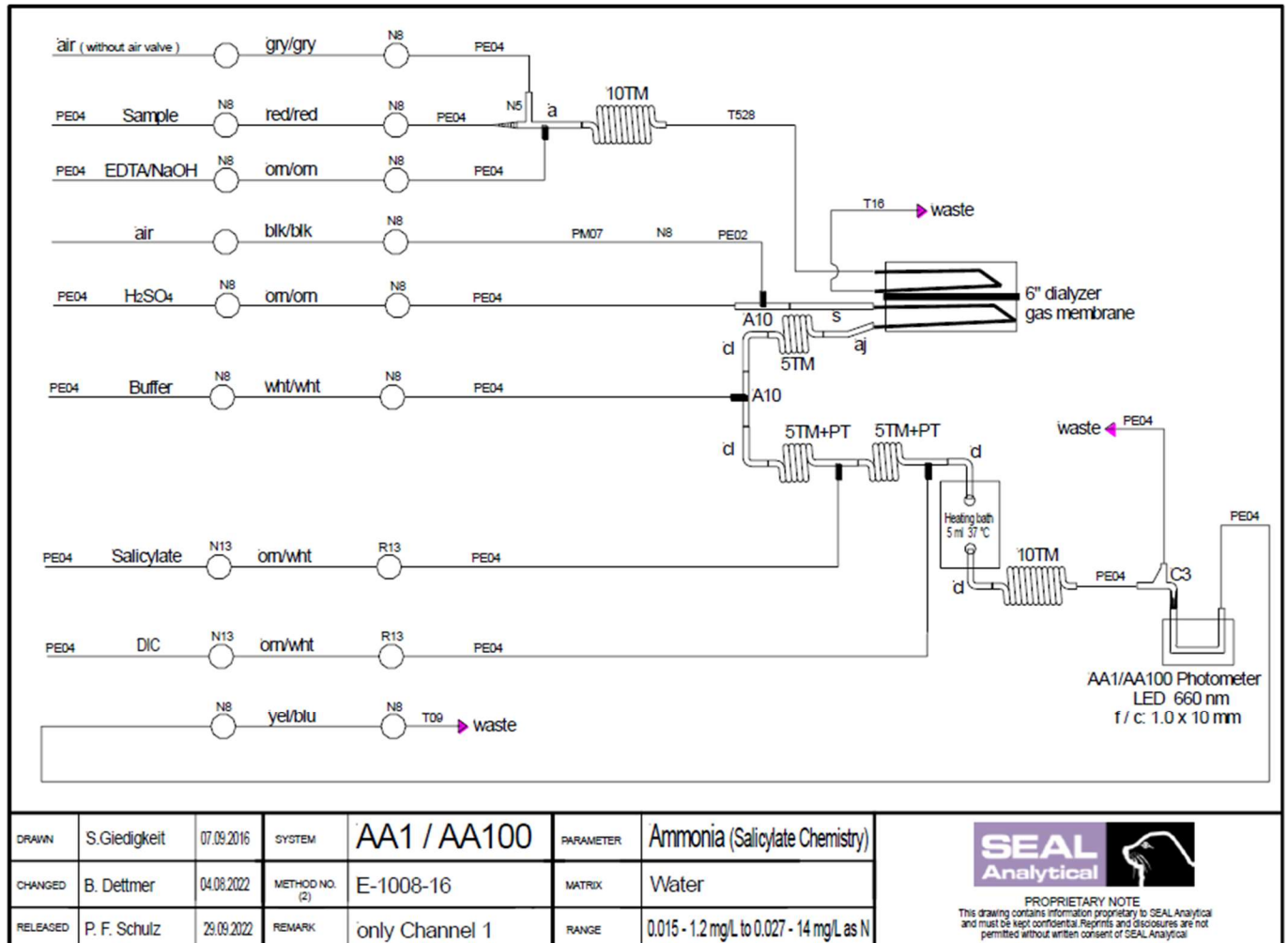
Method 350.1 Determination of ammonia nitrogen by semi-automated colorimetry.
Revision 2.0 August 1993.

AA100 Method E-1008-16 Rev.3 Ammonia in Water With Gas Diffusion (salicylate chemistry), April 2024.

Appendix 1: Acceptance Criteria

QA/QC Sample	Frequency	Acceptance Criteria	Corrective Action
Laboratory Blank	1 per 20 samples or less	Less than Reporting Limit	All samples associated with a contaminated blank should be re-analyzed or reported with qualifiers if not enough sample is available to analyze.
Sample Duplicate	1 for every 10 samples or less per project	$\leq 20\%$ RPD	Estimate results for that sample (J) and explain in comments section of the report
Holding Time		Samples must be analyzed within holding time of 28 days	If re-sampling is not available, results for samples are estimated (J) with explanation under comments in report.
IDC/LOQ	Run 4 replicates once a year of standard at concentration of 1 to 2 times the reporting limit, per analyst	90–110% recovery <20% RSD	Investigate problem and repeat
MDL/Low LFB	Analyze a low LFB with each run and calculate MDL at least once every thirteen months	N/A	N/A
Initial Cal. Verification standard	After calibration	$\pm 10\%$ of true value	Re-calibrate, prepare new ICV
LFB	1 per 20 samples or less	$\pm 10\%$ of true value	Re-analyze, qualify data
MS-Laboratory Fortified Matrix	1 for every 10 samples or less per project	$\pm 15\%$ of true value.	Qualify sample data with J1
Continuing Calibration Verification standard	Immediately after the ICV, after every 10 injections and at the end of the run	$\pm 10\%$ of true Value	Re-analyze all samples from last CCV that was within range.

Appendix 2: Flowchart for Ammonia Analysis by AA100



DRAWN	S.Giedigkeit	07.09.2016	SYSTEM	AA1 / AA100	PARAMETER	Ammonia (Salicylate Chemistry)
CHANGED	B. Dettmer	04.08.2022	METHOD NO. (2)	E-1008-16	MATRIX	Water
RELEASED	P. F. Schulz	29.09.2022	REMARK	only Channel 1	RANGE	0.015 - 1.2 mg/L to 0.027 - 14 mg/L as N



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