

Ammonia (Ammonium) Spectroquant® Test Kits Analytical Method

**Spectroquant® Test Kits of Merck KGaA, Darmstadt, Germany:
1.14739, 1.14752, 1.14558, 1.14544, 1.00683, 1.14559**

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Scope and Application

1. This method determines the level of ammonium-nitrogen ($\text{NH}_4\text{-N}$), ammonium (NH_4^+), ammonia-nitrogen ($\text{NH}_3\text{-N}$), and ammonia (NH_3) in waters and wastewater matrices. In a strong alkaline solution $\text{NH}_4\text{-N}$ is present entirely as ammonia. This reacts with hypochlorite ions to form monochloramine, which in turn reacts with 2-chlorophenol or Thymol to form indophenol blue. This is then determined photometrically at or near 690-712 nm. The result is expressed as mg $\text{NH}_4\text{-N}$ or $\text{NH}_3\text{-N}$ mg/L, but can be converted to mg NH_4^+ /L or NH_3 /L.
2. This method is for use in the United States Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
3. This method is intended for the analysis of $\text{NH}_4\text{-N}$ or $\text{NH}_3\text{-N}$ on drinking and surface waters, ground water, industrial wastes, and other wastewater matrices.
4. The method detection limit has been determined by a single laboratory validation study summarized in the Method Performance section. [1]
5. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedures in Quality Control section.

Summary Method

1. This method consists of convenient, ready to use cell test kits for determination of ammonium-nitrogen ($\text{NH}_4\text{-N}$), ammonium (NH_4^+), ammonia-nitrogen ($\text{NH}_3\text{-N}$), and ammonia (NH_3).
2. This method is based on "Standard Methods for the Examination of Water and Wastewater", 22nd edition, SM 4500 $\text{NH}_3\text{-F}$ (1997/2011) [2], and International Standard Organization ISO Standard No.7150-1 [3].
3. The test kit is suitable for both on-site testing and typical laboratory testing.
4. The solid reagent for monochloramine formation and color reaction is used.
5. The blue compound which is formed is determined photometrically at a wavelength at or between 690- 712 nm.
6. The photometric determination can be conducted on either a Spectroquant[®] system photometer or any other spectrophotometric device.
7. Quality is assured through the use of quality control samples (QCS), calibration of the instrumentation by using calibration test solutions and operation of a formal quality assurance program.

Definitions

1. Ammonia Stock Standard Solution: A concentrated solution containing method analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.
2. Calibration Blank: A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ammonia analyzer
3. Calibration Standard: A solution prepared from the dilution of stock standard solutions. These solutions are used to calibrate the instrument response with respect to analyte concentration.
4. Detection Limit (DL), also called 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.
5. Dynamic Range (DR): The concentration range over which the instrument response to an analyte is first order linear or second order quadratic.
6. Instrument Performance Check (IPC) Solution: A solution of method analyte, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.
7. Laboratory Fortified Blank (LFB) - An aliquot of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added. The LFB is processed and 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.
8. Laboratory Fortified Sample Matrix/Duplicate (LFM/LFMD) also called Matrix Spike/Matrix Spike Duplicate (MS/MSD): An aliquot of an environmental sample to which known quantities of ammonia is 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.
9. Laboratory Reagent Blank (LRB) - A volume of reagent water or other blank matrix that is processed exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, surrogates and internal standards that are used in the extraction and analysis batches. The LRB is used to determine if the method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
10. Minimum Reporting Level (MRL) - The minimum concentration that can be reported by a laboratory as a quantitated value for a method analyte in a sample following analysis. This

concentration must not be any lower than the concentration of the lowest calibration standard for that instrument.

11. Water Sample: For the purpose of this method, a sample taken from one of the following sources: drinking water, surface water, groundwater, and storm runoff, industrial or domestic wastewater.

Interferences

1. Suspended matter and turbidity may interfere with the photometric measurement. To counter this potential positive interference, filter the sample through a membrane filter with 0.45 micrometer pore diameter or distill as per SM 4500 NH₃ – B (1997/2011) [2]
2. Primary amines interfere heavily and should be absent. Secondary amines interfere in concentration > 250 mg/L, amino phenols in concentration > 10 mg/l and aniline in concentration > 50 mg/L. These compounds react similar than ammonium does with reagents to produce a blue color similar to that formed.
3. Sulfide will interfere if more than 50 mg/L is present in the sample due to the reaction with hypochlorite ion.
4. Iron (III)-ion in concentrations >25 mg/L will interfere

Safety

1. This method does not address all safety issues associated with its use. The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical and environmental sample should be regarded as a potential health hazard and exposure should be minimized. The laboratory is responsible for maintaining a safe work environment and 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 (SDSs) should be available to all personnel involved in these analyses.
2. The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. It is suggested that the laboratory perform personal hygiene monitoring of each analyst using this method and that the results of this monitoring shall be made available to the analyst.
3. Samples of unknown origin may possess potentially hazardous compounds. Samples should be handled with care (e.g., under a hood), so as to minimize exposure.
4. As samples of unknown origin may contain compounds which could react violently with the reagents, pipette the sample into the test tube under a hood, and direct the opening of the test tube away from anyone in the area.
5. This method employs the use of Spectroquant® Ammonium Tests containing pre-measured reagents, which limits the handling of hazardous chemicals.

Equipment and Supplies

1. Membrane filters, 0.45 μm pre-wash by soaking in deionized water.
2. Class A volumetric flasks-various sizes, acid washed.
3. Class A volumetric pipettes-various sizes, acid washed.
4. Rectangular cuvettes 1-5 cm, or round cuvettes 13-16 mm or equivalent cuvettes, as needed
5. Pipetter- Fixed or adjustable volume.
6. Pipetter tips, Ammonia free.
7. Visible spectrophotometer capable of measuring absorbance within a wavelength range of 690-712 nm, and with cell compartment for rectangular cuvettes 1-5 cm, or round cuvettes 13-16 mm or equivalent.
8. Laboratory timer.
9. Rack for cells.
10. Dry cloth or wipe for cleaning cells.

Reagents and Standards

1. Ammonia free deionized (DI) water
2. Spectroquant[®] Ammonium Test kit.
3. Ammonium Chloride (NH_4Cl) 99.99% [CAS - 12125-02-9]. Sigma-Aldrich P/N 326372 or Equivalent.
4. Purchased $\text{NH}_4\text{-N}$ / $\text{NH}_3\text{-N}$ Standard Stock Solution (1,000 mg/L) or prepared $\text{NH}_4\text{-N}$ stock solution (1000 mg/L).
 - 4.1. Dissolve 3.819 g of anhydrous ammonium chloride, dried at 105°C, in 800 mL DI water in a Class a 1000 mL volumetric flask.
 - 4.2. Add DI water to a total volume of 1000 mL.
 - 4.3. Mix well and store in a plastic bottle. Shelf life of 360 days or as per manufacturer's directions.

Analytical Standards

1. Standard Stock Solution 1 (1000 mg/L).
2. Standard Stock Solution 2 (100 mg/L $\text{NH}_3\text{-N}$, 121.6 mg/L NH_3 , 100.00 mg/L $\text{NH}_4\text{-N}$, 128.8 mg/L NH_4^+):
 - 2.1. 100 mL of Standard Stock Solution 1 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.

- 2.2. Ammonia free DI water was added to the mark (1000 mL).
- 2.3. Stock Solution 2 was mixed completely by flask inversion.
3. Standard Stock Solution 3 (10.00 mg/L NH₃-N, 12.16 mg/L NH₃, 10.00 mg/L NH₄-N, 12.88 mg/L NH₄⁺):
 - 3.1. 100 mL of Standard Stock Solution 2 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
 - 3.2. Ammonia free DI water was added to the mark (1000 mL).
 - 3.3. Stock Solution 3 was mixed completely by flask inversion.
4. All standards and spike solutions were prepared as per Table 1 below.
 - 4.1. Volume in mL of Standard Stock Solution was transferred with a Class A pipet to a Class A 100 mL volumetric flask.
 - 4.2. Ammonia free DI water was added to the mark (100 mL).
 - 4.3. Standard Solution was mixed completely by flask inversion.

Table 1: Standard Preparation	
Standard (mg/L NH₃-N or mg/L NH₄-N)	Standard Stock Solution 1 (mL) (Diluted to 100 mL)
150	15
120	12
Standard (mg/L NH₃-N or mg/L NH₄-N)	Standard Stock Solution 2 (mL) (Diluted to 100 mL)
100	Use as is.
80	80
60	60
40	40
20	20
16	16
15	15
Standard (mg/L NH₃-N or mg/L NH₄-N)	Standard Stock Solution 3 (mL) (Diluted to 100 mL)
10	Use as is
8	80
4	40
3	30
2	20
1	10
0.5	5
0.2	2
0.1	1
0.05	<i>Note: 1 ml diluted to 200 mL</i>

Distillation or Other Sample Preparation

1. The samples used in this method are:
 - 1.1. Standards prepared in ammonia free water.
 - 1.2. Water samples that can be:
 - 1.2.1. Used as is if there is no turbidity.
 - 1.2.2. Filtered through a 0.45 µm Whatman PES syringe filter to remove turbidity.
 - 1.2.3. Distilled as per 4500-NH₃ B (1)-1997
2. The Standard Methods for the Examination of Water and Wastewater [2] methods approved by EPA [4] allows the analyses of these ammonia samples without distillation. Filtration is identified in the Spectroquant® product flyers and is acceptable under the EPA approved method 4500-NH₃ F (1)-1997.
 - 2.1. 4500-NH₃ A (1)-1997 “*Methods D, E, F, G, and H may be used either with or without sample distillation.*”
 - 2.2. 4500-NH₃ F (1)-1997 “*Remove interfering turbidity by distillation or filtration*”.

Sample Collection, Preservation, and Storage

1. Collect approximately 1-L, or a minimum of 100 ml, of a representative sample in a new plastic or glass bottle, following conventional sampling techniques.
 - 1.1. For dissolved NH₄-N/ NH₃-N filter samples through a 0.45 µm Whatman PES syringe filter for dissolved NH₄-N/ NH₃-N immediately upon sampling.
2. Analyze the samples for NH₄-N/ NH₃-N as soon as possible.
3. Preserve samples with conc. sulfuric acid to pH 1.
4. Refrigerate samples at ≤ 6°C from the time of collection. Maximum holding time is 28 days. 40 CFR 136, Table II [4].
5. Collect an additional two aliquots of a sample for each batch (of at least 20 samples) for the matrix spike and matrix spike duplicate.
6. Analyze the samples for NH₄-N/ NH₃-N as soon as possible.

Quality Control

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 Capability, a LRB, LFB, LFM, LFMD, and CCV for each Sample Batch and the use of control charts to determine Ongoing Demonstration of Capability. The laboratory is required to maintain performance records that define the quality of the data that are generated.

2. A LRB, LFB, LFM, LFMD, and CCV will be run with each sample batch. Failure of any QC sample in sample batch will require a corrective action and may require the sample batch to be reanalyzed.

Initial Demonstration of Capability (IDC)

1. Before new analysts run any samples, verify their capability with the method. Run a LFB at least four times.
2. Calculate the standard deviation of the four samples.
3. All 4 LFB values must fall within the LFB recovery limits for the analyst to pass the IDC
4. The LFB's recovery limits are:

$$\text{LFB's initial recovery limits} = \text{Mean} \pm (5.84 \times \text{Standard Deviation})$$

5. Where:

5.84 = the two-sided Student's t factor for three degrees of freedom.

Method Detection Limit (MDL)

1. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using Type 1 Ammonia Free Deionized (DI) Reagent Water fortified at a concentration of one to five times the estimated instrument detection limit.
2. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method.
3. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = t \times S$$

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

4. 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.

MDLs must be determined as per the current promulgated 40 CFR part 136 Appendix B.[5]

Laboratory Reagent Blank (LRB)

1. DI water that is analyzed as a sample. Analyzed at a frequency of no less than once per sample batch. The blank result will be below the MDL.
2. If LRB fails, immediately cease analyzing samples and initiate corrective action.

Laboratory Fortified Blank (LFB)

1. A sample of known concentration of ammonia in DI water. Analyzed at a frequency of no less than once per sample batch.
2. The concentration of the LFB should be between 10% and 50% of the calibration range set by the calibration standards.
3. The minimum acceptable recovery will be 90% to 110%.
4. If LFB fails, immediately cease analyzing samples and initiate corrective action.
5. The LFB QC (ODC) charts will set the acceptable recovery limits
6. Percent Recovery LFB calculation:

$$\left(\frac{\text{Experimental Value}}{\text{Expected Value}} \right) * 100 = \text{Percent Recovery LFB}$$

Experimental Value = LFB Concentration determined experimentally

Expected Value = Known LFB concentration

Laboratory Fortified Matrix Spikes (LFM and LFMD) or Matrix Spikes (MS/MSD)

1. A duplicate set of water samples spiked with a known amount of ammonia so that the overall concentration of the spiked sample is within the dynamic calibration range of the instrument.
2. Analyzed at a frequency of no less than once per sample batch.
3. A Relative Percent Difference will be calculated for each LFM/LFMD or MS/MSD set.
4. The LFM/LFMD or MS/MSD recovery and RPD should be between a minimum acceptable level of
 - 4.1. . LFM/LFMD or MS/MSD recovery of 90% to 110%
 - 4.2. RPD \leq 20%
5. If LFM/LFMD fails, initiate corrective action.
6. Percent Recovery LFM Calculation

$$\left(\frac{\text{Spiked Value} - (s \times \text{Unspiked Value})}{\text{Concentration of Spike}} \right) * 100 = \text{Percent Recovery LFM}$$

Spiked Value = LFM concentration determined experimentally

Unspiked Value = Concentration of sample before spiking

s = Dilution Correction

7. Relative Percent Difference (RPD) Calculation

$$\left(\frac{\left(\frac{LFM - LFMD}{2} \right)}{\left(\frac{LFM + LFMD}{2} \right)} \right) * 100 = RPD$$

LFM = Concentration determined for LFM

LFMD = Concentration determined for LFM duplicate

Continuing Calibration Verification (CCV)

1. Check standards will be prepared so as to have a concentration between the ML and upper calibration standard. They will be run at least once per batch.
2. For the calibration verification to be valid, check standard results must not exceed $\pm 10\%$ of its true value,
3. If calibration verification fails, immediately cease analyzing samples and initiate corrective action.

Dynamic Range (DR)

1. The DR must be determined initially and with each day of use. This is performed by calibrating the instrument for either a linear or quadratic range as per the manufacturer's directions.

Instrument Performance Check (IPC) Solution

1. Solutions consisting of the LRB, LFB, LFM/LFMD and CCV ran per each batch of samples. These results will be charted on control charts to determine ODC for the laboratory.

Sample Batch

1. A group of samples which behave similarly with respect to the sampling or the testing procedures being employed and which are processed as a unit.
2. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch.
3. A batch cannot span between laboratory work days (24 hrs.).
4. New batches must be started each laboratory work day.
5. Sample Batch: Typical sample analysis sequence .
 - 5.1. Calibration zero
 - 5.2. Calibration Standards, Linear or Quadratic
 - 5.3. LRB
 - 5.4. LFB

- 5.5. Sample used for LFM/LFMD
- 5.6. LFM
- 5.7. LFMD
- 5.8. Samples (First half of batch)
- 5.9. CCV
- 5.10. Samples (Second half of batch)
- 5.11. Repeat CCV (Optional)

Control Charts (ODC)

1. Control charts will be kept for LFB and LFM percent recovery per sample batch, LFM and LFMD RPD per sample batch, LRB concentration and CCV concentration per sample batch.
2. Trends will be graphed and warning limits calculated to show whether the values determined go outside the set by the laboratory. (*Refer to minimum acceptable values above for each QC parameter*)
3. If these trends exceeds the maximum limits of this method, then corrective action (Root Cause Analysis) must be performed.

Corrective Action (Root Cause Analysis)

1. The laboratory analyst(s) and laboratory management will perform a root cause analysis for any QC failures. The analysis will have at a minimum the following areas described in detail:
 - 1.1. Identify the problem: Identify the QC failure. Include instrument, reagent, sampling, personnel and any other problems.
 - 1.2. Investigate to identify the root cause: Determine how each problem identified interacted with each other to create the QC problem.
 - 1.3. Come up with the solution: Develop an encompassing solution to address all problems that created the QC failure.
 - 1.4. Implement the solution: Develop an implementation plan that includes all components of the developed solution and have laboratory management implement it.
 - 1.5. Document the solution: Document all corrective action steps taken under laboratory management implementation of the corrective action.
 - 1.6. Communicate the solution: Develop training and management programs to communicate and evaluate all personnel included in the corrective action solution.
 - 1.7. Evaluate the effectiveness of the solution: Document QC results in trend charts and laboratory staff performance to validate corrective action solution.

Calibration and Standardization

1. Visible Spectrophotometer

1.1. The Spectroquant® system photometer

1.1.1. Has factory calibrations included. These factory calibrations can be used for *non-regulatory* ammonia analyses. The calibration curve can be verified, and the data from this verification can be stored, modified or re-entered at anytime. However, the factory program settings cannot be changed by the user. When appropriate, the manufacturer supplies a new microchip (transponder) containing new calibration data.

1.1.2. Can be calibrated by the user to meet the requirements of this method.

1.2. For visible spectrophotometers, other than the Spectroquant® system photometer, plot a calibration curve with a minimum of five (5) data points, from standards prepared from a NH₄-N standards solution appropriate to the range to be tested. The calibration curve should also include a blank DI water.

2. Prepare standard curves from the NH₄-N/ NH₃-N solution. The curve should include the lowest and highest concentrations for the range tested as set by the Spectroquant® test kit product insert.
3. Apply linear or polynomial curve-fitting statistics, as appropriate, to analyze the concentration–instrument response relationship. The appropriate linear or nonlinear correlation coefficient for standard concentration-to-instrument response should be ≥ 0.995 .
4. Back calculate the concentration of each calibration point. The back-calculated and true concentrations should agree within ± 10 . At the lower limit of the operational range, acceptance criteria are usually wider.
5. Repeat initial calibration daily or when starting a new batch of samples, unless the method permits calibration verification between batches.
6. Run CCVs as per the QC requirements.

Procedure

1. Choose a Spectroquant® Ammonium Test concentration range appropriate for the sample matrix to be tested, using prior knowledge of the particular waste stream.
2. Table 2 contains the Dynamic Range for each test kit. Standard 1 cm rectangular or round cells round cuvettes 13-16 mm were used unless noted.

Table 2: Spectroquant® Test Kit Dynamic Range	
Test Kit	Dynamic Range NH₄-N/ NH₃-N (mg/L)
1.00683	2.0-75 5-150
1.14739	0.010-2.000
1.14559	4.0-80.0
1.14544	0.5-16.0
1.14558	0.20-8.00
1.14752 * 2 cm Cuvette ** 5 cm Cuvette	0.05-3.00 0.03-1.50* 0.010-0.500**

Note: Refer to the specific Spectroquant® product insert for additional detailed information.

3. Pipette sufficient sample depending on the concentration range of pre-treated sample (pH 4-13, if necessary add sodium hydroxide solution or diluted sulfuric acid to adjust to this pH-range) into a Spectroquant® test kit tube or other reagent tube.
4. Mix well by inversion.
5. Proceed with the additional reagent addition and reaction time as per the specific Spectroquant® Test Kit.
6. Warm up the instrument as per manufacturer's suggestion for operation.
 - 6.1. Set the instrument to a wavelength between 690-712 nm.
 - 6.2. Zero the instrument with a reagent water / blank which has been prepared in the same manner as the standards and samples.
 - 6.3. Place the cell into the cell compartment/cell holder.
 - 6.4. Record the absorbance reading from the instrument.
 - 6.5. Plot the absorbance reading on the calibration curve, to obtain the concentration NH₄-N/ NH₃-N in mg/L.

Data Analysis and Calculations

1. If no pre-dilution was performed upon the sample, no calculation is necessary.
2. If pre-dilution was required, calculate the NH₄-N (mg/L) as follows:

3. Report results to two significant digits for concentrations found above the ML (Section 1.6) in all samples. Report results below the ML as 2.0 mg NH₄-N/L.

Method Performance

1. The method performance was measured by a single analyst/laboratory and is detailed in Tables 3-13.[1]

Calibration and Linear Correlation Coefficient

1. Utilizing both the absorbance maximum determined experimentally for the Spectroquant® test kit and 690 nm, the Thermo Scientific Genesys 10S was calibrated with a minimum of six (6) standards and a linear correlation coefficient was determined. The standards bracketed the Spectroquant® test kit concentrations listed in the product flyer.
2. The correlation coefficient was >0.999 in all cases indicating that the Spectroquant® test kits can produce a linear calibration. The calibration standards using mg/L (NH₃-N or NH₄-N) for each test kit along with the linear Correlation Coefficients are summarized in Table 3.
3. The calibration values for the traditional phenate Berthelot reaction Standard Methods 4500-NH₃ (F) [2] produced a linear calibration line and a correlation coefficient > 0.999 as summarized in Table 4. Therefore, the substituted phenols used in the Spectroquant® test kit performed in an equivalent manner as the classical phenate method.

Accuracy (Spectroquant® QC Sample-LFB)

1. Quality control check samples were obtained from Spectroquant® (Table 5) and used in the equivalency evaluation of the Spectroquant® test kits ability to recover free ammonia (ammonium).
2. The control check concentration (accuracy) is summarized as the percent recoveries for each Spectroquant® test kit and Standard Methods 4500-NH₃ (F) [2] for the quality control check samples used and are summarized in Table 8.

Precision (Spectroquant® QC Sample-LFB)

1. Precision is defined in Standard Methods for the Examination of Water and Wastewater, Part 1000 and in EPA guidance documents as either the confidence interval about the mean or the Percent Relative Standard Deviation (%RSD).[6-8]

$$\text{Percent Relative Standard Deviation} = \left(\frac{s}{\bar{X}} \right) \times 100$$

2. The %RSD was calculated for the Spectroquant® and Standard Methods 4500-NH₃ (F) [2] check samples that were used for the accuracy evaluation. The number of replicate concentration determinations at the maximum wavelength determined for each Spectroquant® test kit and at 690 nm was five (5) replicates. The mean and standard deviation was then calculated and these values were used to determine %RSD. The %RSD

for reach quality controls sample is summarized in Table 9.

3. Some of the experimental data had a standard deviation of zero (0). In these cases, the %RSD was set to 0.00%. In other cases the standard deviation was of such a low order of magnitude, that the calculated %RSD was $< 0.0049\%$ and the value was rounded to 0.00%.
4. The accuracy or recovery of the Spectroquant® check samples by either the Spectroquant® test kits or the classical phenate method was no lower than 96.77% and no higher than 110.61%. The %RSD of the Spectroquant® check samples by either the Spectroquant® test kits or the classical phenate method was no greater than 0.12%.
5. These efficient recoveries of 3rd party check samples and the low %RSD shows that the Spectroquant® will produce equivalent accurate and precise results when compared to Standard Methods 4500-NH₃ (F) [2]. This is emphasized in a direct comparison between a Spectroquant® test kit and the classical phenate method with a calibration range that brackets two Spectroquant® Check sample, Tables 6-7.

Laboratory Reagent Blank (LRB)

1. A volume of reagent water or other blank matrix was processed exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, that are used in the analysis. The LRB was run five times for each Spectroquant® test and the average is reported in Table 10 below. In all cases, ammonia was not seen at a level above 0.00 mg/L NH₃-N or NH₄-N

Matrix Spikes

1. A wastewater matrix was utilized in determining the matrix effects on the spike percent recovery, precision and the relative percent difference between the matrix spike (MS) and the duplicate spike (MSD).
2. A sample of wastewater effluent was obtained from a wastewater plant final clarifier prior to disinfection. This sample was then filtered through a 0.45 µm syringe filter prior to matrix analyses.
3. The wastewater plant laboratory staff indicated that the ammonia analyses performed in their laboratory consistently had results of ammonia concentrations of < 0.5 mg/L NH₃-N.
4. The Spectroquant® Test Kit 14739 was selected for the test as it has the lowest reporting limit. Matrix spike concentration was selected to be at the midpoint of the Spectroquant® test kit range (1 mg/L NH₃-N). Each spectroscopic analysis was measured five (5) times and the average is reports and the standard deviation was used in the % RSD calculation.
5. The percent recovery, RPD and %RSD for the samples are summarized in Table 11-12.

Method Detection Limits

1. The Method Detection Limit (MDL) was calculated for each Spectroquant® test kit

following the requirements in Appendix B, 40 CFR part 136 [9].

2. Each Spectroquant® test kit product flyer and test kit contained an ammonia (ammonium) range for which the test kit reagents were at a concentration to produce complete reaction of the ammonia to form in indophenol.
3. The concentration of sample utilized for each Spectroquant® test kit MDL was determined to be 2-5 times the concentration of the lowest concentration listed on the Spectroquant® test kit or product flyer.
4. The results for the Spectroquant® test kit MDL are at or below the MRL listed on the Spectroquant® product flyer (Table 13).
5. These MRL vs. MDL results for the Spectroquant® test kits concludes that the Spectroquant® test kits with the substituted phenols can provide an indophenol chromophore that responds like the classical phenate method and that the dynamic range listed on each Spectroquant® product flyer is accurate.

Pollution Prevention

1. The reagents used in this method pose little threat to the environment, when managed properly.
2. Reagents should be ordered consistent with laboratory use, to minimize the amount of expired materials to be disposed.

Waste Management

1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restriction. 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 sewer discharge permits and regulations.
2. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste Reduction," both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

References

1. Askew, E.F., *Equivalency of Ammonia (Ammonium) Spectroquant® Test Kits: Ammonia (Ammonium) by Indophenol Reaction and Photometry*. 2016.
2. Eaton, E., Baird, R., Rice, E., , ed. *Standard Methods for the Examination of Water and Wastewater, 22nd Edition*. 22 ed. 2012, APHA, AWWA, WEF.
3. ISO, *Water Quality - Determination of ammonium -Part 1 : Manual spectrometric method*. 1984.
4. EPA, *Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures*. 2012. p. 29758-29846.
5. EPA, *Part 136-Guidelines Establishing Test Procedures for the Analysis of Pollutants Appendix B*. 1973, EPA.
6. EPA, *Chapter 5: Calculation Of Precision, Rias, And Method Detection Limit For Chemical And Physical Measurements*. 1984.
7. EPA, *Protocol for the Evaluation of Alternate Test Procedures for Organic and Inorganic Analytes in Drinking Water*. 2015.
8. EPA, *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*. 2016.
9. EPA, *Subchapter D 40 CFR part 136 Guidelines Establishing Test Preoceedures for the Analysis of Pollutants*. 2001.

Tables, Diagrams, Flowcharts, and Validation Data

Table 3: Spectroquant® Test Kit Calibration Standards and Correlation Coefficient

Test Kit	Wavelength	Standard 1*	Standard 2*	Standard 3*	Standard 4*	Standard 5*	Standard 6*	Standard 7*	Standard 8*	Standard 9*	Correlation Coefficient
1.00683 High	690 nm	0.000	8.000	10.000	20.000	40.000	80.000	100.000	120.000	150.000	0.99972
	710 nm	0.000	8.000	10.000	20.000	40.000	80.000	100.000	120.000	150.000	0.99973
1.00683 Low	690 nm	0.000	2.000	4.000	8.000	10.000	20.000	40.000	60.000		0.99993
	703 nm	0.000	2.000	4.000	8.000	10.000	20.000	40.000	60.000		0.99994
1.14739	690 nm	0.000	0.050	0.100	0.500	0.700	1.000	2.000			0.99995
	708 nm	0.000	0.050	0.100	0.500	0.700	1.000	2.000			0.99996
1.14559	690 nm	0.000	4.000	8.000	10.000	20.000	40.000	60.000	80.000		0.99989
	710 nm	0.000	4.000	8.000	10.000	20.000	40.000	60.000	80.000		0.99991
1.14544	690 nm	0.000	0.500	1.000	2.000	4.000	8.000	10.000	15.000		0.99973
	711 nm	0.000	0.500	1.000	2.000	4.000	8.000	10.000	15.000		0.99973
1.14558	690 nm	0.000	0.500	1.000	2.000	4.000	8.000		0.99982		
	710 nm	0.000	0.500	1.000	2.000	4.000	8.000		0.99975		
1.14752	690 nm	0.000	0.100	0.500	1.000	2.000	3.000				0.99992

Table 4: Standard Methods 4500-NH₃ (F) Phenate Calibration Standards and Correlation Coefficient

Test Kit	Wavelength	Standard 1*	Standard 2*	Standard 3*	Standard 4*	Standard 5*	Standard 6*	Standard 7*	Standard 8*	Correlation Coefficient
Phenate	633 nm	0.000	0.050	0.100	0.500	0.700	1.000	2.000	120.000	0.99986

Table 5: Spectroquant® Quality Control Check Samples (LFB-IPC)

Spectroquant® Check Sample	Label Concentration (NH₄-N mg/L)	Check Sample Actual Concentration (NH₄-N mg/L)	Check Sample Precision (± mg/L)
1.25022	0.400	0.396	0.012
1.25023	1.00	0.98	0.04
1.25024	2.00	2.03	0.07
1.25025	6.00	5.98	0.13
1.25026	12.0	12.0	0.4
1.25027	50.0	51.0	1.2

Table 6: LFB Recovery Comparison Classical Phenate - Spectroquant®				
Spectroquant® Check Sample	Label Concentration (NH₄-N mg/L)	Classical Phenate	1.14739	
		633 nm	690 nm	708 nm
1.25022	0.4	100.51%	107.32%	107.07%
1.25023	1	107.55%	110.61%	102.45%

Table 7: Classical Phenate %RSD Comparison Classical Phenate - Spectroquant®				
Spectroquant® Check Sample	Label Concentration (mg/L)	Classical Phenate	1.14739	
		633 nm	690 nm	708 nm
1.25022	0.4	0.00%	107.32%	107.07%
1.25023	1	0.00%	110.61%	102.45%

Table 8: Spectroquant® and Classical Phenate Quality Control Accuracy (LFB-IPC NH₃-N or NH₄-N % Recovery)

Label Concentration (mg/L)	1.00683 High Range Check Samples		1.00683 Low Range Check Samples		1.14739 Check Samples		1.14559 Check Samples		1.14544 Check Samples		1.14558 Check Samples		1.14752 Check Samples	Classical Phenate
	690 nm	710 nm	690 nm	703 nm	690 nm	708 nm	690 nm	710 nm	690 nm	711 nm	690 nm	710 nm	690 nm	633 nm
0.4					107.32%	107.07%								100.51%
1					110.61%	102.45%					105.20%	104.69%	105.71%	107.55%
2									101.13%	101.92%	100.94%	100.49%	100.00%	
6	100.89%	101.61%	97.06%	101.61%			98.93%	99.88%	97.53%	98.11%	99.33%	99.18%		
12	102.86%	103.49%	99.12%	99.86%			100.89%	101.13%	97.37%	97.28%				
50	97.80%	98.01%	99.63%	99.54%			96.96%	96.77%						

Table 9: Spectroquant® and Classical Phenate Quality Control Precision (LFB % RSD)

Label Concentration (mg/L)	1.00683 High Range Check Samples		1.00683 Low Range Check Samples		1.14739 Check Samples		1.14559 Check Samples		1.14544 Check Samples		1.14558 Check Samples		1.14752 Check Samples	Classical Phenate
	690 nm	710 nm	690 nm	703 nm	690 nm	708 nm	690 nm	710 nm	690 nm	711 nm	690 nm	710 nm	690 nm	633 nm
0.4					0.00%	0.00%								0.00%
1					0.00%	0.00%					0.05%	0.05%	0.00%	0.00%
2									0.05%	0.05%	0.12%	0.02%	0.00%	
6	0.07%	0.07%	0.03%	0.03%			0.05%	0.05%	0.12%	0.02%	0.01%	0.02%		
12	0.02%	0.04%	0.02%	0.03%			0.02%	0.02%	0.01%	0.02%				
50	0.00%	0.01%	0.02%	0.03%			0.01%	0.01%						

Table 10: Spectroquant® Laboratory Reagent Blank (mg/L NH₃-N or NH₄-N)

1.00683 High		1.00683 Low		1.14739		1.14559		1.14544		1.14558 s		1.14752
690 nm	710 nm	690 nm	703 nm	690 nm	708 nm	690 nm	710 nm	690 nm	711 nm	690 nm	710 nm	690 nm
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 11: Matrix Spike Percent Recovery and RPD		
	690 nm	708 nm
Final Effluent (NH₃-N or NH₄-N mg/L)	0.165	0.174
Matrix Spike (NH₃-N or NH₄-N mg/L)	1.150	1.147
Matrix Spike Percent Recovery	98.50%	97.30%
Matrix Spike Duplicate (NH₃-N or NH₄-N mg/L)	1.158	1.153
Matrix Spike Duplicate Percent Recovery	99.30%	97.90%
Relative Percent Difference	0.69%	0.52%

Table 12: Matrix Spike and Spike Duplicate %RSD		
	690 nm	708 nm
Final Effluent	0.00%	0.00%
Matrix Spike	0.00%	0.00%
Matrix Spike Duplicate	0.00%	0.00%

Table 13: Method reporting Limit - Method Detection Limits

Spectroquant® Test Kit	Wavelength	<i>MRL</i> <i>Lowest Concentration Listed for</i> <i>Spectroquant® Test Kit</i> <i>(NH₃-N or NH₄-N mg/L)</i>	MDL (NH₃-N or NH₄-N mg/L)
1.00683 High Range	690 nm	5.00	0.438
	710 nm	5.00	0.331
1.00683 Low Range	690 nm	2.00	0.257
	703 nm	2.00	0.321
1.14739	690 nm	0.010	0.010
	708 nm	0.010	0.008
1.14559	690 nm	4.00	0.285
	710 nm	4.00	0.295
1.14544	690 nm	0.50	0.066
	711 nm	0.50	0.057
1.14558	690 nm	0.20	0.061
	710 nm	0.20	0.061
1.14752	690 nm	0.05	0.031