**Update items in red font at a minimum, make sure to update any steps to reflect your procedures.**

**Revision Date: October 10, 2021.**

*The SOP must be read completely and followed by laboratory staff doing analysis. An IDC maybe completed by analysis of passing standard and blank results, quarterly blinds, or PT samples, or simply reading and following the SOP.*

1. **Analyte:** Ammonia (NH3-N)
2. **Scope and Application**: This method is applicable to domestic and industrial wastes matrices. Studies using this vendor method have shown, with data, that distillation of domestic wastewater effluents using TNTplus TM 830 is not required. Sample results are submitted on the electronic Discharge Monitoring Report (eDMR), are used for to determine compliance of discharge limits.
3. **Summary of the Test Method:** Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol. The amount of color formed is directly proportional to the ammonia nitrogen present in the sample. Test results are measured at 690 ± 5 nm.
4. **Method Sensitivity:** The Method Detection Limit (MDL/LOD) must be determined initially and then recalculated each year according to the approved MDL / LOD procedure.

For the ongoing LOD, all method blanks analyzed with samples that are reported are entered into the WDNR spreadsheet (or equivalent) along with two different LOD spikes (in different batches) each quarter. Calculate the ongoing LOD every 13 mo. The ongoing LOD spikes are the same concentration as the initial LOD in order to compare if the LOD should be updated. The LOD must be at or below the permit limit.

The LOQ is typically 10/3 the LOD. Alternatively, the low calibration standard concentration may be used for the LOQ (which must be above the LOD).

1. **Potential Interferences:** the ions listed in the Hach reference method up to the concentration ranges of those analytes do not cause interferences. A 10,000-fold excess of urea does not interfere. All reducing agents interfere and cause low-bias results. An analyte which is in excess of the stated range will affect color formation resulting in a false reading. Measurements can be verified using sample dilutions. Samples with a high level of interference require distillation.
2. **Sample Handling:** Collect samples in clean plastic (or glass) bottles. The hold time for a preserved sample is 28 days. Store refrigerated (above freezing to 6oC) – separate from standards or contaminating sources.

**Preserve** with H2SO4 at collection unless the samples will be analyzed immediately (~15minutes). This can be done by adding ~ 0.5ml of 5 NH2SO4 per 200 mL of sample.

Periodically (at least quarterly) verify the pH is <2 using pH paper and document the pH.

1. **Equipment and Supplies:**
2. Spectrophotometer, Model \_\_\_\_\_\_\_ for use at 690 nm
3. Class A pipets (cleaned) or verified mechanical pipettors and disposable tips
4. Glassware such as beakers, volumetric flasks and graduated cylinders – if needed these may be acid-rinsed.
5. Test tube rack
6. pH paper, narrow range (0 – 6.0); used to confirm pH of <2 for preservation
7. pH paper, narrow range (5.5 – 8.0); used to confirm preserved samples are neutralized to 6.0 to 8.0
8. Distilled water, ammonia-free (referred to as distilled or reagent water)
9. Hach Company TNTplusTM 830 Reagent Set, Cat. No. TNT830
10. NH3-N stock standard (purchased), 100 mg/L NH3-N. Store in a ≤ 6ºC refrigerator after opening when required. The expiration date is provided by the vendor.
11. NH3-N working standard solution (prepared), 10 mg/L NH3-N: Fill a 100mL volumetric flask about ½ full of reagent/distilled water and pipetting in 10 mL of 100 mg/L standard. Fill to the line with reagent water and mix by inverting approximately 7 times. Store this working standard the same way as the stock standard. Replace as needed.
12. CCV/LCS (1 mg/L) solution from the working standard (prepared) solution: Prepare by pipetting 9 ml of distilled water in a clean beaker and 1ml of the 10 mg/L stock standard, swirl well to mix. Remake this with each batch. If additional volume is desired (make sure to note preparation details in the prepared chemical log): fill a 100mL volumetric flask about ½ full of reagent/distilled water and pipetting in 10 mL of 100 mg/L standard. Fill to the line with reagent water and mix by inverting approximately 7 times. Store this working standard the same way as the stock standard. Replace as needed.
13. Prepare the calibration standards from the 10 mg/L ammonia working standard. Pipet both the standard and then the distilled water into the Hach TNT vial:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration of the working ammonia standard: | | **10** | mg/L | mg/L = ppm |
|  |  |  |  |  |
|  | **Concentration** of Standard to prepare (mg/L) | Amount of distilled **water** volume (mL) | Amount of stock **standard** to pipet (mL) | Final volume (mL) |
|  |
| Standard 1 | **0.20** mg/L | 4.9 | 0.1 mL | 5 |  |
| Standard 2 | **0.40** | 4.8 | 0.2 | 5 |  |
| Standard 3 | **1.00** | 4.5 | 0.5 | 5 |  |
| Standard 4 | **2.00** | 4.0 | 1.0 | 5 |  |

*\*Alternatively, pipet the volumes of the standard at a specific concentration into distilled water into volumetric flasks. Record the initial and final volumes used in the prepared chemical log.*

1. ICV (initial calibration verification standard), second source ammonia stock standard, 100 mg/L NH3-N. Must be a lot # different from the calibration standard source above. Use This the manufacturer’s storage & expire date.
2. ICV ammonia working standard solution, 1.0 mg/L NH3-N.

Option 1: Step a: Make a 10 mg/L intermediate standard by pipetting 0.5 ml (of a 100 mg/L standard) into 4.5 mL of distilled water in a clean beaker/container followed by step b:

Step b: pipet 0.5 ml of the 10.0mg/L intermediate standard into the Hach vial and add 4.5 mL of distilled water. This is a 1.0 mg/L concentration.

Option 2: Add 1.0 mL of the stock ICV standard to a 100 mL volumetric flask that is ~1/2 full of distilled water. Bring up to volume with distilled water. Store according to the directions for the stock standard. If any standard is refrigerated, it must be separate from samples and also from contaminating sources. Replace as needed.

1. Sulfuric acid solution, 5 N (for preserving samples)
2. Sodium hydroxide solution, 5 N (for neutralizing preserved samples)
3. Proficiency Testing samples from an approved provider
4. **Calibration**
   1. Prepare the calibration standards and the calibration blank in the Hach vials. A linear calibration requires at least 3 non zero standards. The correlation coefficient (r) must be at least 0.995. If this does not pass, fix the problem and recalibrate the instrument.
   2. The calibration must be initially verified with a 2nd source standard, the recovery must meet 90-110% recovery. Then on an ongoing basis the calibration is verified using a CCV must be analyzed and pass 90-110%.
   3. When compliance samples are analyzed, the onboard/preprogrammed calibration must NOT be used.
   4. In order to enter the information onto (or into) the benchsheet, make sure to open it or print it. Saving the benchsheets on the computer will require renaming it on each analysis day.
   5. Zero the spectrophotometer with a method blank sample that has NO reagent from under the DosiCap™ added (this is the instrument blank). Insert the vial, close the cover, and press “ZERO” to zero the meter.
   6. The calibration the same as the samples (see the procedure below). After the timer goes off, insert the 0 standard (calibration blank) and enter the concentration (0.00), press enter, press read to get the absorbance value, and record absorbance on the bench sheet. Record the actual absorbance for the calibration blank this is rarely exactly 0.
   7. Insert the 0.2 /lowest standard and enter the concentration (0.2), press enter, press read, and record the absorbance. Do this with each of the prepared standards. After the 2.0 mg/L standard concentration was entered, press exit, display will show “Force Zero on/off,” and select force Zero **off**. Press down, display will show Calibration Formula, press enter, and select formula for r2=, press enter. Press down arrow to Store Program, and press enter.
   8. Entering the calibration standards on the applicable NH3-N benchsheet will allow the analyst to view the calibration and will also provide warnings if the QC is failing or the samples are over range.
   9. Refer to the QC table for frequency, criteria and corrective actions.

Example sequence on calibration days:

Calibration blank, followed by each calibration standard

ICV

CCV/LCS

MB/CCB

Samples 1-10

CCV (same as the CCV/LCS above)

CCB (same as the MB/CCB above)

Example sequence on non- calibration days:

CCV/LCS

MB/CCB

Samples 1-10

CCV (same as the CCV/LCS above)

CCB (same as the MB/CCB above)

If there are more than 20 samples, make sure to make another MB and LCS (labeling these MB-2 and LCS-2). Analyze MB-2 and LCS-2 prior to the 21st sample.

1. **Quality Control:**

Each Analyst must have an IDC on file.

The MDL/LOD study must be current (the Ongoing LOD needs to be recalculated within 13 months).

Table

|  |  |  |  |
| --- | --- | --- | --- |
| Name of QC sample | Analysis Frequency | Criteria | Comments and corrective actions |
| **ICV**  Initial calibration verification | After each calibration curve | 90-110% recovery | If the ICV fails to meet 90-110%, stop and correct the problem (remake the standard and reanalyze). If it still fails recalibrate. |
| **ICB**  Initial calibration blnk | After the ICV | < LOD | If it is above the LOD then stop and fix the problem (recheck the blank). |
| **CCV/LCS** (combined continuing calibration verification and laboratory control sample) | As the CCV/LCS: Beginning of each day before analyzing samples (*also meets frequency of the LCS for 20 samples or less*).  As the CCV this is also analyzed after every 10 samples and at the end if 350.1 is the reference method. | 90-110% recovery | If the CCV/LCS fails to meet 90-110%, stop and correct the problem (remake the standard and reanalyze). If it still fails recalibrate. |
| **MB/CCB** Method blank and continuing calibration blank (combined) | As the MB and CCB: Run after the CCV, prior to samples, and  as the MB analyzed with each batch maximum of 20 samples  As the CCB analyze it every 10 samples and at the end. | < LOD or  < 5% of permit limit or  <10% of sample | If the MB fails, recheck the MB to confirm (optional), qualify if criteria is not met |
| **PT** from an approved provider.  Prepare according to directions and analyze it with the same procedure used for samples. | Annually (Jan-August)  Report the result with the correct method code. | Acceptable result by PT vendor | If it fails, review possible causes. A new PT will be needed. If the laboratory is unsure what caused the failure, ordering a blind QC to analyze before analyzing the 2nd PT is optional.  If it fails a 2nd time, reach out to your laboratory contact at WDNR for assistance. |

10.0 Procedure for sample analysis

*Make sure to also follow the manufacturer’s instructions.*

1. If the samples were preserved with acid, neutralize to a pH of 6-8 with 5 N sodium hydroxide solution. Use either a pH meter or pH paper. Record the neutralization pH.

The amount of 5 N sodium hydroxide will be about the same as the amount of 5 N sulfuric acid used to preserve the sample. Using less than a 2% dilution when adding the acid or base would not require adjusting the sample for a dilution.

1. Warm all samples and reagents to room temperature.
2. Turn on the spectrophotometer and press the program # (make sure the wavelength is set to 690 ± 5 nm).
3. Label all vials that will be needed including vials for QC samples.
4. Carefully remove the protective foil lid from the DosiCap™ *Zip*. Unscrew the cap from the vial.
5. Pipet 5 mL of samples and QC samples to the appropriate labeled vials.
6. Zero the spectrophotometer with a method blank sample with no reagent from under the DosiCap™ added (this is the instrument blank). Insert the vial, close the cover, and press “ZERO” to zero the meter.
7. Flip the DosiCapTM *Zip* over so that the reagent side faces the vial. Screw the cap tightly onto the vial.
8. Shake the capped vial 2 to 3 times to dissolve the reagent in the cap.
9. Verify that the reagent has dissolved by looking down through the open end of the DosiCapTM *Zip*.
10. Set the timer for 15 minutes. Try to be consistent for all samples.
11. After the timer goes off, invert the sample an additional 2 to 3 times to mix. The color remains stable for an additional 15 minutes after the timer expires.
12. Thoroughly clean the outside of the prepared vial, insert it into the cell holder, push “READ,” and record the absorbance reading on the ammonia bench sheet.
13. To see the absorbance reading, press “shift ABS,” then “READ.”
14. If samples have color or turbidity, add the sample to a vial but do not remove the foil lid. Put the cap on the vial and zero the spectrophotometer (similar to zeroing with the instrument blank but with a portion of unreacted sample).
15. If any sample has an absorbance reading higher than the highest standard in the calibration curve, the sample must be diluted and reanalyzed. Assess this as a part of the data assessment.

To dilute the sample, estimate a concentration that will be within the calibration range (nearest the middle of the curve is recommended). Dilute the sample in a new vial using distilled water. Record the initial and final volumes.

1. Enter the dilution factor into the benchsheet if needed. Make sure to adjust the result, the LOD and LOQ by the dilution factors.
2. If the result is less than the LOD, report < that value.
3. Qualifiers (after Data Assessment):

If the MB did not meet the criteria, make sure to qualify the affected data.

If the LCS did not meet the criteria, and it is also the CCV, fix the problem prior to analyzing samples.

If the LCS is not the same as the CCV then qualify the affected sample data.

Report results with any comments/qualifiers in the eDMR.

1. Enter any failures into the Corrective Action Log, filling out as much detail as needed. The goal is to make the necessary adjustments to prevent the failure from happening again.
2. Reach out for technical assistance if the problem is not resolved.

Waste Management

All laboratory waste, excess reagents, and samples must be disposed of in a manner that is consistent

with applicable rules and regulations.

The Hach TNT 830, 831, and 832 vials use a reagent called nitroprusside (Na2[Fe(CN)5NO]2H2O). Hach has determined that there are trace levels (6 – 14 µg/L) of free cyanide in the reacted samples. It is the waste generator’s responsibility to determine if it is a hazardous waste. The waste generator in this case is the laboratory. Under s. NR 661.23 (1)(e), cyanide bearing waste could be a hazardous waste when it is a cyanide or sulfide bearing waste which, when exposed to pH conditions between 2 and 12.5, can generate toxic gases, vapors or fumes in a quantity sufficient to present a danger to human health or the environment.

References

* 1. EPA 600/R-93-100, August 1993, Method 350.1, Determination of Nitrogen Ammonia by Semi-Automated Colorimetry – Revision 2.0.
  2. Ammonia – Spectrophotometric Measurement of Ammonia Nitrogen and Total Kjeldahl Nitrogen in Water and Wastewater, Method 10205, Hach Company TNTPlus™, revision 2.0, August 2008. Hach Company, Loveland Colorado.
  3. Evaluation of the need for preliminary distillation prior to analysis of ammonia using Hach method 10205 (TNTplus™ 830, 831, 832), Hach Applications Laboratory Report, the Hach Company, Loveland, Colorado. 2011.

Disclaimer

*The mentioning of company or product names does not constitute endorsement by the Wisconsin Department of Natural Resources or the authors.*