Laboratory Manager Approval: Kristy E. Hrehor / 08/19/2021

QA Manager Approval: Jeffney Moore / 08/19/2021

EPA Method 351.2 – Total Kjeldahl Nitrogen

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1 Scope and Application

1.1 EPA Method 351.2 covers the determination of Total Kjeldahl Nitrogen (TKN) in drinking, ground, and surface waters, as well as domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. This method is modified by the instrument manufacturer Lachat Instruments using QuikChem Method 10-107-06-2-H.

1.2 Restricted Procedure:

This procedure is restricted to use by an analyst experienced in the operation of a Lachat QuikChem Analyzer and Block Digester. Additionally, the analyst must complete the requirements of the GAEPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GAEPD Chemical Hygiene Plan for additional information regarding chemicals required by this method.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Plan (see SOP reference 13.3) for Quality Control Definitions.

2.2 Primary Source (PS) – A standard that is used to make up the calibration points of a curve.

2.3 Second Source (SS) – A standard made from a manufacturer other than that of the primary source.

2.4 Initial Calibration Verification (ICV) – An ICV is a second source standard that is used to verify the correctness of the primary source calibration curve. The ICV is run at a level equal to that of a Laboratory Control Sample (LCS) or the midpoint on the calibration curve.

2.5 Continuing Calibration Check (CCC) or Continuing Calibration Verification (CCV) – A standard used to verify that the response of the instrument has not changed since initial calibration. The CCC is run at a level equal to that of a Laboratory Control Sample (LCS) or the midpoint on the calibration curve.

2.6 Calibration Blank (CB), Initial Calibration Verification Blank (ICB), Method Blank (MBLK), MDLB or Continuing Calibration Blank (CCB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

2.7 MDLS (Method Detection Limit Spike) – MDLB spiked with analytes at the lowest calibration level to be used for determination of MDL.

3 Interferences

3.1 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

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

4 Safety

4.1 Refer to the EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision (See Section 13.8)

5 Apparatus and Equipment

5.1 Analytical balance, capable of accurately weighing to the nearest 0.0001g.

5.2 Glassware -- Class A volumetric flasks, graduated cylinders, and pipettes.

5.3 Lachat Quikchem Flow Injection Analysis (FIA) System designed to deliver and react sample and reagents in the required order and ratios.

5.3.1 Lachat autosampler

5.3.2 Reagent pump

5.3.3 Reaction unit or manifold

5.3.4 Colorimetric detector with 660 nm interference filter

5.4 Computer with Microsoft Windows operating system and Lachat Omnion software

5.5 8.0 ml plastic culture tubes, 13mm OD x 100 mm, Fisher Scientific Part # 14-956-8E or equivalent or glass culture tubes 12 mm x 75 mm VWR part # 60825-502 or equivalent for loading samples on auto-sampler.

5.6 Seal BD50 Block Digester and AIM 600 Block Digester

5.7 50 place digestion tube racks

5.8 75mL Glass digestion tubes

5.9 Glass Cold Finger stoppers

5.10 PTFE boiling stones

5.11 NIST traceable digital thermometers. Recertification or replacement required quarterly.

5.12 Type K Heavy Duty 12” Long temperature probe. Recertification required yearly.

5.13 Sonicator
5.13.1 Vacuum source for degassing
5.13.2 Rubber tubing, serological pipette, and rubber stopper for degassing wand
5.14 10mL (fixed) and 0-50mL bottle top dispensers
5.14.1 Each day of use, the volume dispensed by each bottle top dispenser must be verified for the specific volume for which it is being used.
5.14.2 Bottle top dispensers may be verified by measuring the volume dispensed with a class A graduated cylinder. The volume dispensed must be within ± 2.5 percent of the nominal volume.
5.15 Air displacement pipettes of various volumes, auto-pipettors, pipette tips in various sizes. Air displacement pipettes and auto-pipettors may also be described as mechanical pipettes.
5.15.1 Each day of use, the volume dispensed by each mechanical pipette must be verified for the specific volume for which the pipette is being used.
5.15.1.1 Mechanical pipette volumes are verified by measuring the weight of a volume of water dispensed by the unit. At room temperature, 1 ml of water is equal to 1g. Mechanical pipettes must be verified to be within ± 2.5 percent of the nominal volume.
5.15.1.2 Mechanical pipettes must be professionally calibrated every 6 months.
5.15.2 Auto-pipettors may be verified by measuring the volume dispensed with a class A graduated cylinder. The volume dispensed must be within ± 2.5 percent of the nominal volume.
5.16 Autosampler racks, 90-position
5.17 Vortex mixer
5.18 Cooling rack
5.19 Heatproof tiles
5.20 HDPE bottles, containing 2.5 ml of 10% sulfuric acid (preservative), for sampling.
5.21 HDPE bottles, various sizes, for storage of standards.
5.22 Glass bottles, dark amber in color, for storage of reagents and standards.
5.23 Magnetic stir plate
5.24 Magnetic stir bars of various sizes
5.25 Disposal pipette tips, 101-1000 µl - Fisher PN# 02-707-507 or equivalent.
5.26 Disposable transfer pipettes:
5.26.1 Plastic - VWR® Disposable Transfer Pipets PN# 16001-190 or Fisherbrand™ Standard Disposable Transfer Pipettes PN# 13-711-7M or equivalent

6 Reagents and Standards

6.1 Reagent water:
Purified water which does not contain any measurable quantities of target analytes or interfering compounds for each compound of interest (Deionized, HPLC, Milli-Q water, or equivalent. Milli-Q water has a resistivity of 18.2[MΩ.cm] @ 25oC and a TOC of 50 ug/L or less).

6.2 Digestion Solution:
In a 2 L beaker, add 268 g of Potassium sulfate (K_2SO_4) and 14.6 g Copper sulfate ($CuSO_4$) to 800 ml Reagent water. Slowly add 268 ml concentrated Sulfuric acid (H_2SO_4) while stirring with a magnetic stir bar on a magnetic stir

plate. ***Caution:*** The solution will get hot. Allow the solution to cool to ambient temperature while stirring. When cool, transfer to a 2L volumetric flask and dilute to volume. Prepare fresh every 3 months.

6.3 Hypochlorite Solution:
In a 250 ml volumetric flask, dilute 15.0 ml Clorox Bleach (5.25% Sodium hypochlorite, The Clorox Company, Oakland, CA) to volume with Reagent water. Invert to mix. For Clorox Bleach (6.0% Sodium Hypochlorite) dilute 13.1 ml of bleach to 250 ml with Reagent water. For Clorox Bleach (6.15% Sodium Hypochlorite), dilute 12.8 ml of bleach to 250 ml with Reagent water. For Clorox Bleach (8.25% Sodium hypochlorite), dilute 9.54 ml of bleach to 250 ml with reagent water. Prepare fresh daily. Degas before use.

6.4 Salicylate-Nitroprusside:
In a 1 L volumetric flask dissolve 150.0 g Sodium salicylate [Salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$] and 1.00 g Sodium nitroferricyanide dihydrate [$Na_2Fe(CN)5NO \cdot 2H_2O$] in approximately 800 ml of DI water. Dilute to volume and invert to mix. Store in an amber glass or brown plastic bottle and prepare fresh monthly. Degas before use.

6.5 Buffer:
To a 1 L volumetric flask add 900 ml of Reagent water. Completely dissolve 35.0 g Sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). Add 20.0 g disodium EDTA (ethylenediaminetetraacetic acid disodium salt). Add 50.0 g Sodium hydroxide (NaOH), dilute to volume and invert to mix. De-gas before use. Solution is stable for 1 month.

6.6 Sodium Hydroxide (0.8M):
In a 1 L volumetric flask dissolve 32.0 g Sodium hydroxide (NaOH) in approximately 800 ml of Reagent water. Dilute to the volume and stir to mix. Solution is stable for 3 months.

6.7 Digestion Diluent (for carrier and simulated standards):
In a 1 L volumetric flask dissolve 400 ml digestion solution (reagent 6.2) in approximately 400 ml of Reagent water. Dilute to the volume and stir to mix. De-gas before use. Prepare fresh weekly.

6.8 Primary Source(PS) Ammonium Chloride Stock Standard(1000 mg/L NH_3-N):
Weigh out 3.819 g of Ammonium chloride that has been dried for 2 hours at 110° C, transfer to a 1 L volumetric flask containing 100 ml of Reagent water, dissolve and bring to volume with Reagent water. Commercially prepared stock standard may also be purchased and used. Solution is stable for 6 months.

6.9 Calibration Standards:
Using the Primary Source (PS) Ammonium Chloride Stock Standard (1000 mg/L NH_3-N), prepare calibration standards at six concentrations in Reagent water. The calibration standards range from 0.00 mg/L NH_3-N to 5.00 mg/L NH_3-N. After the standards are brought to volume with Reagent water, 10 ml of 10% Sulfuric acid is added. Prepare every three months. The standards have been modified from the Lachat method.

Table 6.9.1 - Calibration Standards

Ammonium Chloride Stock Solution (ml)	**Final Volume(ml)** (filled to volume with Reagent water)	**Concentration (mg/L NH_3-N)**
0.00*	1000	0.00
0.20	1000	0.20
0.60	1000	0.60
1.0	1000	1.00
3.0	1000	3.00
5.0	1000	5.00

* 0.00 mg/L standard is Reagent water preserved with 10ml of 10% Sulfuric acid added.

6.10 10% Sulfuric acid:
Purchased from VWR, Part # BDH 3358-4 or equivalent.

6.11 100 mg/L Spiking Solution:
Pipette 10 mL of Ammonium chloride Stock
standard into a 100 ml volumetric flask and bring to volume.

6.12 0.00 mg/L Standard, ICB, CCB, MBLK, MDLB and Dilution water):
To prepare an ICB/CCB, Pipette 10ml of 10% H2SO4 into a 1L flask that already contains 1L of Reagent water. This solution is stable for 28 days. The volume of the reagent may be altered as long as the final concentration remains the same.

6.12.1 Prepare fresh every 28 days.

6.13 Volumes and amounts of reagents, chemicals and standards may be altered as long as final concentrations remain the same. Sample volumes and injection amounts may be altered as long as required detection limits can be met and sample/reagent ratios remain the same.

6.14 Ammonium Chloride ICV Stock Solution or Second Source (SS)

6.14.1 The ICV stock standard is used as a second source standard.

6.14.3 This stock standard must be from a different source than the stock standard used to make the calibration standards.

6.14.4 The prepared standard is stable until expiration date on bottle or within 6 months of opening date, whichever is sooner.

6.15 Ammonium Chloride (TKN) ICV Second Source Solution (SS)

6.15.1 Prepare the ICV to a concentration as close to 3.0 mg/L NH_3-N as possible.

6.15.2 The ICV solution must be prepared fresh every 3 months.

6.15.3 Prepare the ICV using reagent water. Once ICV is diluted with reagent water, make sure to preserve ICV using appropriate amount of 10% Sulfuric Acid (See 6.10). Use 10 ml of 10% Sulfuric acid per 1 L of ICV solution.

7 Sample Collection

7.1 Samples are collected in 250 ml plastic bottles and pre-preserved with 10% H_2SO_4 to a pH of < 2.

7.2 Sample preservation is checked in the receiving lab at the time of receipt.
7.2.1 The pH is checked with disposable transfer pipets. A clean disposable transfer pipet is used to draw up a few drops of the sample. The drops are then placed on an appropriate narrow range pH paper. Never dip the pH paper into the sample.
7.2.2 If the sample pH is not < 2 the collector is notified. The sample is then preserved in the receiving lab and a comment is placed on the affected analysis that the sample was preserved in the laboratory. The result is "J" flagged as estimated due to sample not preserved at time of collection.
7.3 Holding time is 28 days.
7.4 Samples are stored at 0-6°C (not frozen).

8 Calibration

8.1 Calibration Standards

The calibration curve consists of the calibration standards at the following concentrations: 0.00 mg/L NH_3-N, 0.20 mg/L NH_3-N, 0.60 mg/L NH_3-N, 1.00 mg/L NH_3-N, 3.00 mg/L NH_3-N, and 5.00 mg/L NH_3-N.

8.2 Calibration Curve

The Lachat QuikChem is calibrated daily. Six standards are used to construct the calibration curve. Minimum acceptable correlation coefficient is 0.995 using a linear regression.

8.3 Calibration Verification

An initial calibration verification standard (ICV), continuing calibration check (CCC) and a continuing calibration blank (CCB) must be analyzed immediately after the calibration standards. The initial calibration verification standard must be prepared with a stock from a different source than the standards used in the calibration of the instrument. The ICV value must be within 10% of its true value and the CCB value must be less than the method RL or the run will have to be repeated. A continuing calibration check (CCC) and a continuing calibration blank (CCB) must be analyzed every 10 samples and at the end of the sample run and must meet the same criteria as the ICV and ICB. The continuing calibration check may come from the same source as the calibration standards. If the CCC or CCB does not meet acceptance criteria, then all samples affected by the out of control CCC or CCB are to be rerun. The CCC can be prepared using the same source as the calibration standards.

8.4 A MDLS (low level mdl spike) at the concentration of 0.20 mg/L NH_3-N must be analyzed with each batch to perform an ongoing MDL study. All batch QC must be valid to report this result.
8.5 A MDLB (MDL blank) must be analyzed once per analytical batch to perform an ongoing MDL study. All batch QC must be valid to report this result.

9 Quality Control

9.1 Refer to Table 14.1 for Reporting Limits (RL's), Refer to Table 9.4.1 for Default QC limits, Appendix A for Quality Assurance Criteria, and Table 14.3 for Quality Control Procedures associated with this method.
9.2 See SOP reference 13.4 for training and certification procedures.

9.3 See SOP reference 13.5 for control charting procedures.

9.3.1 For Initial Demonstrations of Capability (IDC), Method 351.2 requires a recovery range of 90% - 110% (see calculation 11.8.).

9.3.2 The EPD Laboratory uses the most current accuracy and precision control ranges in use for samples for Continuing Demonstrations of Capability (CDC). If 4 replicates are performed (as opposed to two LCS/LCSD pairs) a 15% RSD is required (see calculation 11.4.).

9.4 Control Limits:

9.4.1 Method 351.2 requires control limits to be adjusted through the use of control charts. For DW samples, control charting performed every six months will be used to adjust the acceptance limits within the default limits. For all other samples, control charting will be performed every six months for trend monitoring purposes only.

9.4.2 Default control limits for recovery for LCS/LCSD pairs are based on Section 9.3.2 of EPA Method 351.2 (reference 13.1) as noted in Table 9.4.1 below. The default limits are 90% - 110% recovery.

9.4.3 The EPD laboratory sets LCS/LCSD precision control limits to 0-20% RPD.

9.4.4 Default control limits for recovery for MS/MSD pairs are based on Section 9.4.2 of EPA Method 351.2. The default limits are 90% -110% recovery.

9.4.4.1 10% of all routine samples must be spiked.

9.4.5 MS/MSD precision limits are set by the EPD lab as 0-20% RPD.

9.4.6 In-house limits based on control charts may never exceed the default limits.

9.4.7 See Administrative SOP for Control Charting and Control Limits, SOP reference 13.5 for further details.

9.5 MDL (method detection limit) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.

9.5.1 The actual MDL varies depending on instrument and matrix.

9.5.2 The MDL must be determined annually for each instrument prior to results being reported for that instrument. The MDL determined for each compound must be less than the reporting limit for that compound.

9.5.3 The Method Detection Limit Study for all analytes must be performed initially on a new instrument and performed after major instrument repairs or changes to procedures. There are two ways to perform the MDL. The first is with 7 samples and 7 blanks over 3 separate days. The second preferred way the MDL is run as a continuous format.

9.5.4 The 7 MDL samples study is performed by preparing 7 spiked vials, MDLSpike, spiked at the lowest calibration point of the curve, and preparing 7 clean blank vials filled with DI water, MDLBlank. These 7 sets of spiked and blank vial "pairs" are analyzed over 3 separate days, there may or may not be a non-analysis day between each of the 3 days. A total of 14 vials are prepared, 7 spiked and 7 blank.

9.5.5 A continuous format MDL study is performed where one vial is spiked as an MDLSpike, at the lowest point of the calibration curve and analyzed with every batch of samples along with the method blank vial as an MDLBlank.

9.5.6 The results of the MDLBlank will be entered into Labworks using the Method Blank test code, B_TKN. The MDLSpike result will be entered using the MLTKN. The MDL Spiked Amount will be entered into the test code

MATKN. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-TKN.

9.5.7 MDL study must be performed every six months and before the MDL for the instrument expires.

9.5.8 Data for the MDL study is pulled from a two year period.

Table 9.4.1 - Default QC Limits for Method EPA 351.2

QC Type	Analyte	Accuracy (%R) LCL	UCL	Precision (%RPD
LCS/LCSD	TKN	90 -	110[1]	20[2]
MS/MSD	TKN	90 -	110[3]	20[4]

[1]EPA 351.2 specifies initially for the LCS a 90-110% recovery range. The EPD Lab applies LCS recovery limits to the LCSD. Recovery limits are determined through the use of control charts.
[2]By default, the EPD laboratory sets LCS/LCSD precision control limits to 0-20% RPD. Precision limits are determined through the use of control charts.
[3]EPA 351.2 sets MS recovery limits to 90-110%. The EPD Lab applies the MS limits to the MSD. MS/MSD recovery limits are static.
[4]MS/MSD precision control limits are set to 0-20% RPD. MS/MSD precision limits are static.

10 Procedure

10.1 Sample Pretreatment – Digestion Procedure

10.1.1 Remove sample bottles, standards, and reagents from cold storage and allow them to equilibrate to room temperature prior to sample preparation and/or analysis.

10.1.2 Obtain the standards, nutrient bottles containing the samples to be analyzed, and the sample tubes to be used in digestion.

10.1.3 Using 25 ml volumetric glassware, measure 25 ml of each standard and place in appropriate sample tube. Measure the ICV and CCC's in the same way.

10.1.4 The CCB/MBLK/MDLB must be poured into a 250ml sample collection bottle before 25 ml is placed into the appropriate tubes. Record lot # of bottle used.

10.1.5 The MDLS/0.20 mg/L NH_3-N standard must be poured into a 250ml sample collection bottle before 25 ml is placed into the appropriate tube. Record lot # of bottle used.

10.1.6 The CCC's and LCS's are analyzed at the 3.00 mg/L NH_3-N concentration. Matrix Spikes and Matrix Spike duplicates are prepared at 3.00 mg/L by pipetting 0.75 ml of 100 mg/L spiking solution into a 25 ml volumetric flask and filling to volume with the selected QC sample. Prepare the LCS and LCSD by pipetting 0.75 ml of 100 mg/L spiking standard into a 25 ml volumetric flask and filling to volume with acidified blank that was stored in a 250ml sample collection sample bottle. Make sure that auto-pipettor volume has been verified and recorded prior to use. Record lot # of bottle used.

10.1.7 Next, shake the samples completely to ensure homogeneity. Using volumetric glassware, transfer 25 ml of each sample to be analyzed into its appropriate tube. When the tubes are placed in the block digester, the positions of the QC and thermometer should be shifted with each batch so that over time, the QC and the thermometer are checked in each position.

10.1.8 After all samples, standards, and blanks have been transferred to their tubes, add 10 ml of Digestion solution to each tube using the bottle top dispenser. Make sure that the bottle top dispenser volume has been verified and recorded prior to adding the digestion solution. Note: The amount is verified and recorded using the Inorganic Lab Bottle Top Dispenser Verification Sheet in the Inorganic Lab Bottle Top Dispenser Log.

10.1.9 Next, add 4 to 5 Teflon boiling chips to each tube and vortex well for complete mixing of the sample and digestion reagent.

10.1.10 Turn on the block digester and press the Run key, and then the OK (checkmark) key to start heating the block. The digester will beep when it reaches the set temperature of 200ºC. At that time, place the loaded sample rack onto the block and press the Continue button to continue the program. Each digester will hold the temperature at 200ºC for two hours.

10.1.11 At this point, most of the water will have evaporated from the samples. The block will then heat to 380ºC. During this heating step, place a glass cold finger on top of each digestion tube to prevent loss of sulfuric acid by evaporation. The digester will hold the temperature at 380ºC for 1 hour. The digestion will take approximately 4 hours.

10.1.12 After the digestion has taken place, the digester will beep when finished; remove the rack and place it on a heatproof tile or metal cooling rack. Allow the tubes and glass cold fingers to cool until the upper half of the tubes can be handled comfortably - approximately 15 minutes. Remove the glass cold fingers. Do not allow the tubes to cool completely or the samples will solidify.

10.1.13 Using the bottle top dispenser that is set to 22 ml, add 22 ml of fresh Reagent water to each tube, then vortex for complete mixing. Make sure that the bottle top dispenser volume has been verified and recorded prior to adding the DI water. Note: The amount is verified and recorded using the Inorganic Lab Bottle Top Dispenser Verification Sheet in the Inorganic Lab Bottle Top Dispenser Log.

10.1.14 Samples should be at room temperature before analysis. If digestates need to be stored before analysis, cover tubes with aluminum foil and place in the refrigerator.

10.2 Analysis

10.2.1 Prior to analysis remove samples and standards from cold storage and allow them to equilibrate to room temperature.

10.2.2 Turn on the computer and instrument, and log in to the computer.

10.2.3 Fill Reagent water container with fresh Reagent water. Prepare hypochlorite solution and carrier if necessary.

10.2.4 Click on the Omnion icon on the desktop to open the Omnion program.

10.2.5 Under the Run dropdown menu in the upper left corner of the screen, click Open and then select the file “TKNTemplate.omn.”

10.2.6 Type in all samples, standard numbers, and reagent numbers according to the template format. Print and save the file as “TKN (date)” with the date in MM-DD-YY format.

10.2.7 Vortex all digestion tubes and then pour standards and samples into auto-sampler vials and place in position as noted on tray worksheet printed above (10.2.6).

10.2.8 When prompted to change the set point of the relevant heater to 60°C, click OK.

10.2.9 Tighten down the platens and place all lines in DI H_2O first to check for leaks. Turn on pump by pressing the Manual Run button on pump. The speed should be set at 35.

10.2.10 After 5 minutes of pumping DI H_2O put the appropriate lines in the 0.80M NaOH solution and buffer and allow pump to run for 5 minutes. Make sure to degas reagents before use.

10.2.11 Next place all remaining lines except for the salicylate-nitroprusside line into appropriate reagents. Make sure that reagents have been degassed as required. Allow system to pump 5 minutes.

10.2.12 Place the salicylate-nitroprusside reagent line into the appropriate reagent container and allow pump to run for 5 minutes. Check to make sure there are no clogs or backups in the system flow.

10.2.13 When all samples are loaded, Click on Run icon at top center of screen.

10.2.14 After the run is finished, select "Export Worksheet Data" under the Run dropdown menu. Print two copies of the run report.

10.2.15 Click the Tools dropdown menu and select "Custom Report". Click the yellow Format icon at the top of the screen, then click the Layout tab on the pop-up menu. Under the Header field, enter analyst initials after "Author:", and click Apply. Then click the Charts tab, select "Show N Peaks per Chart" and set N equal to 20, and click Apply. Click Close to close the Format menu.

10.2.16 Print the Custom Report by clicking the Run dropdown menu, and then Print.

10.2.17 Re-digest all samples (at a dilution) for which the responses are greater than 5.0 mg/L NH_3-N.

10.2.18 To prepare dilutions, use the ICB/CCB/Dilution water solution (see section 6.12) to dilute the samples.

10.2.19 If TKN result of a sample is less than the Ammonia result for that sample, re-analyze the sample for both TKN and Ammonia to confirm the results. TKN results should always be greater than or equal to the Ammonia results for a given sample.

10.2.20 Volumes and amounts of reagents, chemicals and standards may be altered as long as final concentrations remain the same. Sample volumes and injection amounts may be altered as long as required detection limits can be met and sample/reagent ratios remain the same.

10.2.21 If a sample must be filtered, do so after digestion. If any sample in a batch requires filtration, the LCS, LCSD and Method Blank must also be filtered.

10.2.22 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by placing transmission lines in 1:11 HCl (1M) and pump for several minutes. Then place all transmission lines in water and pump for several minutes. Resume pumping reagents.

10.3 Shutdown Procedure

10.3.1 When the run is finished, exit all windows, and exit the Omnion program.

10.3.2 Shut down the computer and turn off the monitor.
10.3.3 Place the salicylate-nitroprusside line in Reagent water and allow to pump for 5 minutes. Next place all remaining lines into Reagent water and allow to pump for 10 minutes.
10.3.4 Finally run air through lines until tubing is dry.
10.3.5 Turn off autosampler, reagent pump, and Lachat QuikChem 8500. Release the platens on the reagent pump and replace cap on waste container.

11 Calculations

11.1 The weighted external standard calibration is calculated by the instrument software and is documented in the instrument software.

11.2 Mean ($\overline{X}$):

$$\overline{X} = \frac{X_1 + X_2 + \cdots X_n}{n}$$

11.2.1 Where:
$X_1 + X_2 + \cdots X_n$ = The sum of a set of values Xi, i = 1 to n
n = The number of values in the set

11.3 Standard Deviation (n – 1) (σn - 1):

$$\sigma_{n-1} = \sqrt{\sum_{i=1}^{n} \frac{(X_i - \overline{X})^2}{n-1}}$$

11.3.1 Where:

$\overline{X}$ = Mean of the values
X_i = Individual values 1 through i
n = Number of values

11.4 Percent Relative Standard Deviation (%RSD):

$$\%RSD = \frac{\sigma_{n-1}}{\overline{X}} * 100$$

11.4.1 Where:

$\sigma n - 1$ = Sample Standard Deviation
$\overline{X}$ = Mean of the values

11.5 Relative Percent Difference (%RPD or RPD):

$$\%RPD = \frac{|X_1 - X_2|}{\frac{(X_1 + X_2)}{2}} * 100$$

11.5.1 Where:

$|X_1 - X_2|$ = Absolute difference between two values

$\frac{(X_1 + X_2)}{2}$ = Average of two values

11.6 Percent Drift, %Drift:

$$\%\text{Drift} = \frac{(\text{Concentration}_{\text{Calculated}} - \text{Concentration}_{\text{Expected}})}{\text{Concentration}_{\text{Expected}}} * 100$$

11.6.1 Where:

$\text{Concentration}_{\text{Calculated}}$ = Concentration calculated from result
$\text{Concentration}_{\text{Expected}}$ = Theoretical concentration of the standard

11.7 Extract Concentration:

11.7.1 The extract concentration is calculated relative to the calibration curve by the instrument software.

11.8 Percent Recovery:

11.8.1 LCS/LCSD:

$$\%\text{Recovery} = \frac{\text{Conc}_{\text{spiked}}}{\text{Conc}_{\text{expected}}} * 100$$

11.8.1.1 Where:

$\text{Conc}_{\text{spiked}}$ = Concentration found in the spiked sample
$\text{Conc}_{\text{expected}}$ = Expected concentration

11.8.2 MS/MSD:

$$\%\text{Recovery} = \frac{\text{Conc}_{\text{spiked}} - \text{Conc}_{\text{unspiked}}}{\text{Conc}_{\text{expected}}} * 100$$

11.8.2.1 Where:

$\text{Conc}_{\text{spiked}}$ = Concentration found in the spiked sample
$\text{Conc}_{\text{unspiked}}$ = Concentration found in unspiked sample
$\text{Conc}_{\text{expected}}$ = Expected concentration

11.9 Calculation of Dilution Factors

$$C \times D = F$$

11.9.1 Where:

C = concentration from instrument in mg/L
D = dilution factor, if any
F = final concentration in mg/L

12 Waste Management

12.1 See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating Procedures, SOP reference 13.6.

13 References

13.1 Environmental Monitoring Systems Laboratory, Office of Research and Development. U.S. Environmental Protection Agency, Cincinnati, Ohio. Revision 2.0, August 1993, Method 351.2.

13.2 Determination of Total Kjeldahl Nitrogen by Flow Injection Analysis Colorimetry (Copper Catalyst/Block Digester Method), Lachat Instruments QuikChem Method 10-107-06-2-H. Revision 13 May 2008.

13.3 EPD Laboratory Quality Assurance Plan, online revision.

13.4 GA EPD Laboratory SOP's – Initial Demonstration of Capability SOP 6-001, online revision or Continuing Demonstration of Capability SOP 6-002, online revision.

13.5 GA EPD Laboratory SOP-EPD Laboratory Procedures for Control Charts and Control Limits SOP, SOP 6-025, online revision.

13.6 GA EPD Laboratory SOP-EPD Laboratory Waste Management SOP, SOP 6-015, online revision.

13.7 GA EPD Laboratory SOP – Determination of Method Detection Limit, Method Detection Limit SOP 6-007, online revision.

13.8 GA EPD Laboratory Safety Plan – EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

14 Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

Table 14.1 RL's for EPA 351.2

Parameter/Method	Analyte	Matrix (aqueous)	
		RL	Unit
EPA 351.2	TKN	0.20	mg/L

Table 14.2 Summary of Calibration and QC Procedures for Method EPA 351.2

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
EPA 351.2	TKN	Initial Calibration for all analytes	Initial calibration prior to sample analysis	Correlation coefficient ≥ 0.995 linear regression	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once per initial calibration or quarterly, whichever is sooner.	TKN concentration within 10% of expected value	Correct problem then repeat initial calibration	
		Initial Calibration Verification Blank (ICB)	Once per calibration	TKN value must be < RL	Correct problem then repeat initial calibration	
		Initial Demonstration: Demonstrate ability to generate acceptable accuracy and precision using four analysis of a QC check sample, a method blank, and a blind sample. In addition, the analyst must prepare one standard.	Once per analyst	QC Acceptance Criteria Table, SOP 3-011 Appendix A and Initial Demonstration SOP	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Continuing Demonstration: Demonstrate ability to generate acceptable accuracy and precision using a variety of analysis options of a QC sample(s)	Every 6 months	QC Acceptance Criteria Table, SOP 3-011 Appendix A and Continuing Demonstration SOP	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Method Blank (MBLK)	One per analytical batch	TKN value must be < RL	Correct problem then analyze method blank and all samples processed with the contaminated blank	If unable to reanalyze, flag with a "B"
		Laboratory Control Sample (LCS/ LCSD)	One LCS/LCSD per analytical batch	QC Acceptance Criteria Table, SOP 3-011 Appendix A	Correct problem then reanalyze the LCS/LCSD and all samples in the affected batch	If unable to reanalyze, flag with a "J"
		Matrix Spike (MS/MSD)	10% of samples	QC Acceptance Criteria Table, SOP 3-011 Appendix A	Evaluate out of control event, reanalyze or flag data	
		Continuing Calibration Check (CCC)	Prior to analysis, after every 10 samples and at the end of sample run	TKN concentration within 10% of expected value	Correct problem then reanalyze CCC and all samples associated with failed CCC.	

Table 14.2 Summary of Calibration and QC Procedures for Method EPA 351.2

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
EPA 351.2	TKN	Continuing Calibration Blank (CCB)	After every 10 samples and at the end of sample run	TKN concentration must be < 0.20 mg/L	Correct problem then reanalyze CCB and all samples associated with out of control CCB.	
		MDL Low Level Spike (MDLS) 0.20 mg/L NH_3-N	Once per analytical batch	All batch QC must be valid	Correct problem then reanalyze the affected batch	
		MDL Blank (MDLB)	Once per analytical batch	All batch QC must be valid	Correct problem then reanalyze the affected batch	
		MDL Study	Every six months or after major maintenance of the instrument	All Spiked MDLs must have a value greater than 0. Minimum Detection Limits established shall be < the RLs in Table 14.1	Re-do MDL Study	None
		MDL analysis	Once per batch or as needed to acquire data points per SOP 6-007, online revision	All Spiked MDLs must have a value greater than 0. All other QC in the MDL blank and MDL sample (i.e. Surrogate Spike or Internal Standard, etc. if included) must meet established criteria	Correct problem and re-run the MDL sample or MDL blank once and initiate a corrective action. If the re-run fails a second time, do not use MDL data. Update corrective action, and use associated sample data	None

Appendix A – Quality Assurance Criteria for Method EPA 351.2

Table A.1 Quality Assurance Criteria for Method EPA 351.2

QC Type	Analyte	Accuracy(%R) LCL		UCL	Precision (%RPD)
LCS/LCSD	TKN	90	-	110	20
MS/MSD	TKN	90*	-	110*	20*

*MS/MSD Control limits are static by EPA Method/EPD Lab default.

Control charts are generated twice annually for trend monitoring purposes only

Control Chart data generated from 01/01/2019 - 01/01/2021

Updates to Previous Version:
Section 9
Section 14
Table A.1
Updated for online version.