PREPARATION OF WATERS FOR TOTAL KJELDAHL NITROGEN ANALYSIS (COPPER CATALYST)

1. SCOPE AND APPLICATION

1.1. This method details the digestion of waters for Total Kjeldahl Nitrogen (TKN) analysis. Digestates produced by this method are suitable for analysis by the Seal AQ2 Discrete Analyzer (DEP SOP NU-092). For analytical details such as calibration range and the method detection limit (MDL) see DEP SOP NU-092.

1.2. The tests covered by this SOP are W-TKN and W-TKN-F.

1.3. Applicable matrices are surface and saline water, and domestic and industrial wastewater.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method number 351.2, Rev. 2.0 (1993) and AQ2 method No: EPA-111-A Rev.4

2.2. A digestion solution containing potassium sulfate, sulfuric acid and copper sulfate catalyst is added to the samples and then they are heated in a digestion block in order to convert organic nitrogen to its inorganic form (NH_4^+). The potassium sulfate raises the boiling temperature of the digestion and increases the rate of conversion.

2.3. Interferences: Nitrate in concentrations greater than 10 mg N/L can interfere with the digestion of TKN. The interference can be either negative or positive. For any samples with a known nitrate concentration of 10 mg N/L or higher the TKN result must be reported with a J qualifier or the sample diluted sufficiently to decrease the nitrate concentration to below 10 mg N/L PRIOR to digestion.

3. EQUIPMENT AND SUPPLIES

3.1. AI Scientific AIM 600 Digestion Block

3.2. 50 mL graduated digestion tubes, etched at 12.5 mL and 25 mL

3.3. Vortex mixer

3.4. Boiling beads

3.5. Metal rack/Cooling Rack

3.6. Parafilm

3.7. Graduated Cylinder (Class A)

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3.8. Beakers: 100-200 mL; 1-2 L

3.9. 200 µL, 10 mL, 20 mL Eppendorf pipettes

3.10. MadgeTech TC101A single-channel data logger with Type K thermocouple, or MadgeTech Quadtemp four-channel data logger with Type K thermocouple

Note: Each data logger and thermocouple combination is calibrated annually as a system, and the thermocouples and data loggers cannot be interchanged. Calibration certificates and system information are on file with the Nutrients supervisors. One USB interface cable allows all MadgeTech data logger devices to be connected to a PC running the MadgeTech4 software to start and stop recording, download temperature data, etc.

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

Note: All reagent and standard preparations must be documented in the LIMS 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and also documented in the prep worksheet in LIMS. If available, a Certificate of Analysis for each purchased reagent or standard is also maintained in the REAGENT_PREP folder on the Nutrients drive. A Certificate of Analysis for the standard reference material (4.3.3.) is kept in a binder in C307. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: Prior to using the chemicals in this analysis, read their MSDS sheet and the safety notes found in Section 8 of this SOP.

4.1. Chemicals:

4.1.1. Deionized (DI) water.

4.1.2. Potassium sulfate (K_2SO_4). Fisher, certified A.C.S. powder cat. # 7778-80-5.

4.1.3. Copper (II) sulfate pentahydrate. Fisher, certified A.C.S. crystals.

4.1.4. Sulfuric acid (H_2SO_4 conc.). Fisher, certified A.C.S.

4.2. Reagents:

4.2.1. Diluent - 0.2 % Sulfuric acid solution. Add 4 mL of concentrated sulfuric acid (4.1.4.) to 800 mL of DI water in 2 L volumetric flask. Dilute to mark and invert to mix. Holding time, 30 days.

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4.2.2. Copper sulfate digestion solution - Under a well ventilated fume hood in a graduated large beaker, slowly and carefully add 268 mL of concentrated sulfuric acid (4.1.4.) to 1.4 L of DI water while stirring with a magnetic stirrer. (**Caution:** This solution will get very hot due to the exothermic nature of this reaction. Add the acid in increments while allowing the mixture to cool between additions.) Next, add 268 g of K_2SO_4 (4.1.2.) in small increments and 22.8 g of copper (II) sulfate pentahydrate (4.1.3.) to the sulfuric acid solution with constant mixing using a magnetic stirrer. Let the solution stir under the hood until it reaches room temperature (approx. 60 min.). Pour into a 2 L volumetric flask and bring to volume with DI water. Cap and invert 5 times to mix. Pour into digestion solution bottle. Holding time, 30 days.

4.3. Standards and spiking solution (for detailed preparation instructions please refer to the DEP SOP NU-092):

4.3.1. Practical quantitation limit (PQL) solution (PQCK2) - 0.20 mg N/L TKN, 0.06 mg P/L TP (DEP SOP NU-092, 4.3.6.). This solution is also used for the analysis of TP and TKN in soils (DEP SOPS NU-084 and NU-081, respectively).

4.3.2. Spiking solution - 150 mg N/L TKN, 50 mg P/L TP (SOP NU-092, 4.3.5.). This solution is also used for the analysis of TP and TKN in soils (DEP SOPs NU-084 and NU-081, respectively).

4.3.3. ALTQC standard - the standard reference material solution (DEP SOP NU-092, 4.3.4.) is prepared from the stock standard according to the manufacturer's instructions. The supplier for the QC stock standard (e.g., Environmental Resource Associates) differs from the supplier of the calibration standards.

4.3.4. Working calibration standard (CAL STD) - 4 mg N/L (DEP SOP NU-092, 4.3.2.).

4.3.5. Continuing calibration verification (CCV) check standard - 1 mg N/L (DEP SOP NU-092, 4.3.3.).

5. SAMPLE PRESERVATION, PREPARATION AND HANDLING (SEE DEP SOP NU-033)

5.1. Aqueous samples are preserved at collection with sulfuric acid to a pH < 2 (0.2 % sulfuric acid). They are stored upon receipt in the Nutrient refrigerator located in the receiving room at ≤ 6 °C (not frozen) and have a 28 day holding time. Remove the samples from the refrigerator following the proper protocols to preserve the sample chain of custody. Allow samples to come to room temperature before digestion. Begin heating the digestion block to 180 °C using the appropriate program on the block controller (see section 5.4.10 below).

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5.2. While preparing the samples any extra sample waste not containing copper can be discarded down the sink. Any samples/solutions containing digestion solution that need to be discarded are to be collected in a hazardous waste container.

5.3. The following is required to setup a tray for digestion:

Note: All Samples having conductivities above 5000 umhos/cm (equivalent to 3.0 ppt salinity) are batched separately and diluted two-fold prior to digestion. If a sample conductivity exceeds 5000 umhos/cm it should be digested in a salt batch even if it is not labeled as a salt matrix. Samples with organic N concentrations above 10 mg N/L must be diluted prior to digestion in order to report results without a J qualifier. (Subtract the ammonia concentration, if known, from the TKN result to calculate the organic N concentration). Add a comment on the run report to document this observation.

5.3.1. One blank (MBLK) for each batch (10 samples) - the diluent (4.2.1.) is poured into the digestion tube.

5.3.2. One PQCK2 (4.3.1.) for each tray.

5.3.3. One ALTQC (4.3.3.) for each tray.

5.3.4. One laboratory fortified blank (LFB) per batch. The diluent is spiked at a concentration of 1.2 mg N/L TKN by adding 200 µL of 150 mg N/L TKN spiking solution (4.3.2.) into the 25 mL digestion tube.

5.3.5. Duplicate sample and/or duplicate matrix spikes (1.2 mg N/L) for each batch.

5.3.6. Samples, including samples to be redigested.

5.3.7. The appropriate prep worksheet (see section 5.4.3.) which contains the prep batch ID numbers and other batching information for the samples, and the temperature report for the digestion (see section 5.4.10.1. f.).

5.4. Preparation Procedure for Water Samples:

Note: For the preparation of all dilutions use the diluent, 0.2 % H_2SO_4 (4.2.1.).

5.4.1. Remove the following standard solutions from the refrigerator: spiking solution (4.3.2.), PQL solution (4.3.1.), ALTQC solution (4.3.3.), CAL STD (4.3.4.), and the CCV check solution (4.3.5.). Allow standards to come to room temperature before pouring them out for digestion.

5.4.2. For each tray one PQCK2, ALTQC, CAL STD and CCV check standard must accompany the batches. For every 10 samples digested, a blank (MBLK), an LFB spiked at a concentration of 1.2 mg N/L, and duplicate sample and/or duplicate matrix spikes are required.

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5.4.3. Create a new prep worksheet in the Prep Worksheet Manager module in LIMS:

5.4.3.1. Open the Prep Worksheet Manager module in LIMS and choose the first icon (which looks like a blank page) to create a new worksheet. Choose "351.2 (March 1983)" from the list of available preparation IDs, select the sample IDs to be added to the worksheet, assign the worksheet to the correct analyst, and click the checkmark to create the worksheet. The worksheet ID generated in LIMS will be the tray number for the digestion.

5.4.3.2. Open the new worksheet by clicking the spreadsheet icon in the Prep Worksheet Manager module and double-clicking the appropriate worksheet ID from the list. Complete the header information for the sheet: SOP (NU-091), prep analyst name, prep start date/time and prep completion date/time, equipment ID (e.g., digestion block 'Phoenix' or 'Winchester'), and the reagents and spike standard solution serial numbers (ensure that the corresponding identities and expiration dates are correct).

5.4.3.3. Update the sample table, beginning with the starter rows for QC samples digested on the tray, ensure that serial numbers, identities, and expiration dates for the standard solutions used are correct. A single prep batch ID will be generated automatically for every sample on the sheet; therefore, assign other prep batch IDs, as necessary, by 'Control+Clicking' the sample IDs to be batched together and generating a new prep batch ID for each batch. For the matrix spike sample in each batch, duplicate its row in the sample table **twice** by right-clicking on the row and selecting "Duplicate Row", then add the spike code '_O1_' and the letter A or B as a suffix to the sample ID. Save the updates by clicking the "Save" button at the bottom of the worksheet.

5.4.3.4. Once the information in the worksheet is complete and correct (see section 5.4.6. regarding dilution factors and sample volumes), ask a supervisor to review the worksheet.

5.4.4. Dry the outside of the clean tubes. If no clean tubes are available, clean the number of tubes needed before proceeding. The detailed procedure for cleaning tubes is given in DEP SOP NU-046.

5.4.5. Label the tubes using a permanent marker following the batching order on the digestion worksheet. The worksheet ID/tray number is written on the first tube on the tray.

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5.4.6. If the tubes were cleaned manually rinse them with DI water 10 times. Pour out any excess water in the clean tubes before pouring in the sample. Shake each sample well, and then rinse each tube with 1-2 mL of sample. Pipet 25 mL of each sample into the respective tubes. If a sample requires 1:2 dilution prior to digestion pipet 12.5 mL of the sample into the digestion tube and add 12.5 mL of 0.2 % sulfuric acid solution. For higher dilutions, use a volumetric flask to prepare the dilution by pipetting the appropriate amount of sample into the flask first and then bring the volume up to the mark with 0.2 % sulfuric acid solution. It is very important that the sample volume be accurate. NEVER dilute Equipment Blanks, Field Blanks or Trip Blanks samples unless told to do so by a supervisor. Record dilution factors and actual sample volume used on the prep worksheet. Pour extra blanks using the diluent for dilutions and continuing calibration blanks (CCB) on the analytical run.

5.4.7. One sample per batch is either digested in duplicate and/or spiked in duplicate (200 µL of 150 mg N/L spike solution (4.3.2.)) for precision information. The sample should be spiked at least once for matrix interference information.

5.4.8. Add 2 clean beads to each tube. Do not add more than 3 beads to water samples.

5.4.9. Add 5 mL of the copper sulfate digestion solution (4.2.2.) to each tube.

5.4.10. Place the tubes in the digestion block.

5.4.10.1. One spot on the block is chosen at random to place the thermocouple of the data logger system (3.10.). This system is used for temperature recording during the digestion process. The data logger program is started in advance using the MadgeTech4 software, as follows:

a. Plug data logger into the USB cable at the PC with the MadgeTech4 software installed.

b. Open the MadgeTech4 software. The data logger ID will appear in the "Connected Devices" panel of the software interface. Click on the ID once to highlight it.

c. In the Device tab menu bar, click Quick Start (this will use the most recent device settings, visible in the Connected Devices panel). When the data logger has been properly started, the status will read "Running".

d. Unplug the data logger from the USB interface cable, and attach to the appropriate thermocouple cable outside the hood where the digestion block sits.

e. After the digestion is complete (see section 5.4.13.), unplug the thermocouple cable and repeat steps a and b, above. Click "Stop" in the Device tab.

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f. Click “Download” to open the temperature report. Use the worksheet ID/tray number to name the temperature file in the dialog box that opens, and to save the report on the Chemistry drive \InorganicPrep\WRKSHTS\Templates\CURRENT\Nutrients\TKN-HighTP\Temperature logs. Print the report for the analyst to include with the run folder.

g. Close the MadgeTech4 software, and recharge the data logger battery if necessary.

5.4.10.2. If the block was preheated to 180 °C using program 2, continue with program 3. Program 3 holds the temperature at 180 °C for 2.2 hrs., after which it ramps up to 380 °C for 1.5 hrs. at a rate of 5 °C/min. Once at 380 °C, the block must stay at that temperature for a minimum of 30 minutes to meet the method requirements for complete digestion. Block temperatures above 395 °C should be avoided due to the possibility of side reactions. The block controller settings may be adjusted depending upon the actual temperature recorded by the data logger-thermocouple system; block temperatures should be verified whenever the controller settings are adjusted, and documented in the block maintenance manual located in B403.

5.4.10.3. If the block was not preheated at the time the tubes were placed in the block start the digestion by selecting program 3 (it will start by ramping up to 180 °C and then continue as described above). These initial heating stages are necessary to hydrolyze the organic nitrogen and drive off most of the water. Watch the samples to be sure they do not "bump" out of the tubes.

5.4.11. Return the ALTQC, spiking, CCV, PQL, and calibration standard solutions to the refrigerator.

5.4.12. Check samples back into the receiving room.

5.4.13. Once the digestion is complete place the tubes in a metal rack and allow the samples to cool to room temperature, approximately 15-20 minutes under the hood. Once the samples are cool, use a pipette or bottle-top dispenser to add 25 mL of DI-Water. WARNING: The samples at this point consist of a concentrated sulfuric acid/salt mixture. The addition of DI water may generate much heat and thus poses a significant hazard. Be sure to point the tube away from your face and swirl gently to mix.

5.4.14. Parafilm the sample tubes individually. Vortex until homogenous.

5.4.15. Check the bottom inside of the tubes for any precipitation. Salt samples are sometimes difficult to mix homogeneously because of the precipitation. If there

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is any difficulty getting the sample to mix well, remove the parafilm and slightly reheat the tube(s). Vortex and parafilm again. Digested solutions containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 Discrete Analyzer and then redigested with dilution to confirm the result.

5.4.16. The samples are now ready for analysis.

After the prep worksheet has been reviewed and any necessary notes, changes, or additions have been saved, complete the worksheet by clicking the "Complete" button in the lower left corner of the worksheet – this changes the status of the samples from "W" to "V" in LIMS. Active or completed worksheets can be printed or exported in excel format for convenience when setting up the AQ2 analysis.

Make sure the worksheet ID/tray number is marked clearly on the first tube in the tray, as well as on the data logger temperature report. The data logger temperature report is printed and submitted to the analyst for inclusion with the analysis folder. The digestion worksheet itself is reviewed in LIMS directly and archived as part of the LIMS program.

6. SAMPLE ANALYSIS/QUANTITATION/DATA ARCHIVAL

Refer to the DEP SOP NU-092 for analysis procedure and quantitation information.

7. QUALITY CONTROL (SEE DEP SOPS NU-038 AND NU-043)

Samples with similar background matrices are grouped as a digestion batch and prepared together. A digestion batch is defined as a set of 10 samples or less from the same matrix group. The following are possible matrices that can be grouped together as a matrix group; each is prepared with one set of quality control samples. If a sample or group of samples is visibly different from the rest, they should be batched separately.

Matrix Group	Matrices grouped together as one batch
A	W-GROUND, W-SURF-FRH, and W-DRINKING
B	Conductivity > 5000 umhos/cm; 1:2 predilution
C	W-EFFLUENT, W-INFLUENT

Samples with the matrix WATER should be batched with Group A unless the conductivity is greater than 5000 umhos/cm in which case they should be batched with Group B.

7.1. Digested PQCK2 - one per tray.

7.2. Digested standard reference material (ALTQC) - one per tray.

7.3. Digested LFB - one per batch.

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7.4. Method Blank - one per batch.

7.5. Select samples for quality control - Randomly choose a sample to be used as the quality control sample for each batch. This sample may be changed if upon observation it seems not to be a representative of the rest of the samples e.g., presence of a great deal of particulate or much different color. In this case you may treat this sample as a separate batch and continue with the sample preparation.

7.6. Sample matrix spike/s - Using the sample selected as QC sample (7.5.), prepare and digest a matrix spike or a set of duplicate spikes for each batch. If only one matrix spike is prepared the QC sample must be digested in duplicate.

7.7. For method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to the following DEP SOP'S: NU-092 and NU-043.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

8.1. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

8.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

8.3. Read the MSDS information for safe reagent and sample handling.

8.4. The solution used to digest samples contains copper, which is a hazardous waste and toxic. All sample waste that contains digestion solution (i.e., prior to or after digestion) should also be collected in bottles labeled as 'Hazardous Waste', and disposed as acidic metals waste. No more than three full bottles of digestion solution waste should be stored in the prep lab. A full waste bottle should not be stored more than three days in the lab.

9. REFERENCES

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use. Method validation data is on file with the supervisor.

9.1. EPA Method 351.2, Rev. 2.0 (1993).

9.2. Seal AQ2 Method EPA-111-A, Rev. 4, Total Kjeldahl Nitrogen-N (Copper Catalyst) in Drinking, Ground and Surface Waters, Domestic and Industrial Wastes.

9.3. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

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9.4. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

9.5. DEP SOP NU-041, Reagent Preparation Procedure.

9.6. DEP SOP NU-042, Standard Preparation Procedure.

9.7. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

9.8. DEP SOP NU-046, Procedure for TKN/TP/LLTP Glassware Cleaning.

9.9. DEP SOP NU-081, Determination of Total Kjeldahl Nitrogen in Soils using the Westco SmartChem Discrete Analyzer.

9.10. DEP SOP NU-084, Determination of Total Phosphorus in Soils using the AQ2 Discrete Analyzer.

9.11. DEP SOP NU-092, Determination of Total Kjeldahl Nitrogen in Water using the Seal AQ2 Discrete Analyzer.

9.12. Quality Manual, Bureau of Laboratories, FDEP.

9.13. Laboratory Health and Safety Plan.

9.14. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

Appendix of Significant Changes

08/20/2010	Clarify instructions on procedure and requirements for the digestion of high conductivity samples (Sections 4, 5, and 7).
08/12/2014	Instructions are added for the use of the temperature logger systems and the preparation worksheet manager module within the LIMS program. (Section 3.10 and 5.4)
05/06/2015	Use of pipettes to transfer sample aliquots into the digestion tubes. (Section 5.4.6. and 5.4.13)

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