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

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 using the OI Analytical Flow Solution® 3100 Automated Chemistry Analyzer for Segmented Flow Analysis. For analytical details such as calibration range and the method detection limit (MDL) see DEP SOP NU-098.

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

1.3. Applicable matrices are surface, ground, effluents, influents and saline water.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method 351.2, Rev. 2.0 (1993) and OI Analytical Method 327152 utilizing gas diffusion. The volume of sample digested for this method is 10 mL.

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 glass digestion tubes.

3.3. Vortex mixer

3.4. Teflon boiling beads

3.5. Metal rack/Cooling rack

3.6. Parafilm

3.7. Graduated Cylinder (Class A)

3.8. 2 L Beaker

3.9. Variable Eppendorf type pipettes (10 µL-100 µL, 100 µL-1000 µL, 1 mL-10 mL)

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3.10. 100 mL, 500 mL, 1 L, and 2 L volumetric flasks for working standards and reagent preparation

3.11. MadgeTech TC101A single-channel data logger with Type K thermocouple, or MadgeTech Quadtemp four-channel data logger with Type K thermocouple connected to channel 4 of the Quadtemp. Each data logger and thermocouple combination is calibrated annually as a system, and cannot be interchanged. 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 entered in the LIMS 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the prep worksheet in LIMS. The Certificate of Analysis for the standard reference material is kept in a binder in C307. If available, a Certificate of Analysis for each purchased reagent is maintained on the nutrients drive at: \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\TKN-Digestion\. All containers of 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.

4.1.3. Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$). 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. Store at room temperature.

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

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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. Store at room temperature.

4.3. Standards and spiking solution (for detailed preparation instructions see DEP SOP NU-098):

4.3.1. Working calibration standards (CAL STD), practical quantitation limit (PQL) solution (PQCK2), and continuing calibration verification (CCV) check standard (DEP SOP NU-098, 5.3.3.): Prepare these standards each day that samples are digested. Holding time, 7 days.

4.3.2. Spiking solution: 150 mg N/L TKN, 50 mg P/L TP (DEP SOP NU-098, 5.3.5.). This solution is also used for the analysis of TKN and TP in soils (DEP SOP NU-081 and NU-084, respectively). Holding time, 90 days.

4.3.3. ALTQC working standard: The standard reference material solution (DEP SOP NU-098, 5.3.4.). Holding time, 30 days.

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

5.1. Samples are preserved at collection with sulfuric acid to a final concentration of 0.2 % (v/v), shipped on ice, and stored at $\leq 6\ ^{o}C$ (not frozen). 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 using program 2 on the block controller.

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 for digestion:

Note: 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 TKN concentrations above 10 mg N/L must be prediluted to a concentration of less than 10 mg N/L before digestion in order to report the result without a J qualifier.

5.3.1. One method blank (MBLK) for each batch (max.10 samples): Pipet 10 mL of the diluent (4.2.1.) 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 set of working calibration standards, and at least one CCV, one CCB, and an additional blank (CAR) for each day that samples are digested (4.3.1).

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5.3.5. One laboratory fortified blank (LFB) per batch: Add 10 mL of diluent into a digestion tube. Spike with 80 µL of 150 mg N/L TKN spiking solution (4.3.2.). (Spike final concentration: 1.2 mg N/L.)

5.3.6. Duplicate sample and/or duplicate matrix spikes (1.2 mg N/L) for each batch. Same preparation as for the LFB, but pipet 10 mL of the sample instead of the diluent.

5.3.7. Samples, including samples to be redigested.

5.3.8. Dilution factors recorded on the digestion sheet.

5.3.9. The appropriate digestion sheet for each tray digested.

5.4. Preparation Procedure:

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.), ALTQC working solution (4.3.3.), and the TKN stock standard (DEP SOP NU-098, 5.3.1.). Allow standards to come to room temperature.

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

5.4.2.1. 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.

5.4.2.2. 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-097), prep analyst name, prep start date/time and prep completion date/time, equipment ID (e.g. digestion block 'Phoenix' or 'Zagros'), and the spike standard solution serial number (ensure that the corresponding identity and expiration date is correct).

5.4.2.3. 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.

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5.4.2.4. Once the information in the worksheet is complete and correct (see section 5.4.5. regarding dilution factors and sample volumes), ask a supervisor to review the worksheet. When the prep is complete and after the worksheet has been reviewed and any necessary 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..

5.4.3. 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. If the tubes were cleaned manually and left to soak in 1:2 HNO_3, rinse them with DI water 10 times.

5.4.4. Label the tubes using a permanent marker following the order on the digestion sheet.

5.4.5. 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 10 mL of each sample into the respective tubes. If a sample requires dilution prior to digestion use the diluent (4.2.1.) to make up the volume. Use calibrated pipettes or volumetric flasks to prepare the dilutions. It is very important that the sample volume be accurate. NEVER dilute Equipment Blanks, Field Blanks or Trip Blanks unless told to do so by a supervisor. Pour extra blanks using the diluent for dilutions, continuing calibration blanks (CCB), and other blanks (CAR) on the analytical run.

5.4.6. One sample per batch is either digested in duplicate and/or spiked in duplicate (80 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.

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

5.4.8. Add 2 mL of the copper sulfate digestion solution (4.2.2.) to each tube.

5.4.9. Place the tubes in the digestion block.

5.4.9.1. A well on the block is chosen at random for the thermocouple of the data logger system (3.11.). 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).

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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 5.4.12.), unplug the thermocouple cable and repeat steps a and b, above. Click 'Stop' in the Device tab.

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 at \InorganicPrep\WRKSHTS\Templates\CURRENT\Nutrients\TKN-HighTP\Temperature logs. Print the report for the analyst to include with the run folder. Check and initial the report.

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

5.4.9.2. 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 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. Record block controller adjustments in the maintenance manual.

5.4.9.3. 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.

5.4.10. Return the ALTQC, spiking solution, and TKN stock standard to the refrigerator. Discard the working standard solutions.

5.4.11. Check samples back into the receiving room.

5.4.12. 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, pipet 10 mL of DI water into each tube to make up the volume.

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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.

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

5.4.14. Check the bottom inside of the tubes for any precipitation. Salt samples are sometimes difficult to mix homogeneously because of the precipitation. If there 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 and then redigested with dilution to confirm the result.

5.4.15. The samples are now ready for 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 temperature report (5.4.9.1.f) is printed and submitted to the analyst for inclusion with the analysis folder. The prep worksheet itself is reviewed in LIMS and archived as part of the LIMS program.

6. SAMPLE ANALYSIS/QUANTITATION/DATA ARCHIVAL

Refer to the DEP SOP NU-098 for analysis procedure, quantitation information, and data archival.

7. QUALITY CONTROL/METHOD PERFORMANCE/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (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:

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

If a sample or group of samples is visibly different from the rest, they should be batched separately. 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. One set of working standards and checks are prepared and digested each day samples are digested.

7.2. Digested PQL: one per tray.

7.3. Digested standard reference material (ALTQC): one per tray.

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7.4. Digested LFB: one per batch.

7.5. Method Blank: one per batch.

7.6. 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, much different color, etc. In this case you may treat this sample as a separate batch and continue with the sample preparation.

7.7. Sample matrix spike/s: Using the sample selected as QC sample (7.6.), 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.

7.8. 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-098, NU-038, 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.

9. REFERENCES/DEFINITIONS/VALIDATION DATA

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. OI Analytical method # 327152: In-line Gas Diffusion Total Kjeldahl Nitrogen (TKN) and Ammonia, by Gas Diffusion Segmented Flow Analysis (SFA)

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

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.

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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-098, Determination of Total Kjeldahl Nitrogen in Water using by Gas Diffusion Segmented Flow Analysis

9.12. Quality Manual, Bureau of Laboratories, FDEP.

9.13. Laboratory Health and Safety Plan.

Appendix of Significant Changes

10/05/2015 Reagent and Standard documentation was updated in Section 4.

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