DETERMINATION OF TOTAL KJELDAHL NITROGEN IN SOILS USING THE WESTCO SMARTCHEM DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. The Westco SmartChem Discrete Analyzer is used for the determination of total Kjeldahl nitrogen (TKN) in soils. The calibration range is from the method detection limit (MDL) 10 mg N/kg (0.08 mg N/L) to 500 mg N/kg (4 mg N/L) TKN. The range is dependent on the percent solids of the sample and may be extended by reducing the amount of sample prepared for analysis. The practical quantitation limit (PQL) is 25 mg N/kg (0.20 mg N/L).

1.2. This SOP is based on EPA method 351.2, Rev. 2.0 (1993). The test covered by this SOP is S-TKN.

2. SUMMARY OF THE METHOD

2.1. Prior to analysis, mercuric sulfate catalyzed digestion converts free ammonia and organic nitrogen compounds to ammonium sulfate (see DEP SOP NU-048).

2.2. Ammonium reacts with salicylate and hypochlorite in a buffered alkaline solution in the presence of sodium nitroferricyanide (pH=12.8-13) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm.

2.3. Interferences: The working buffer eliminates precipitation of calcium and magnesium hydroxides. Samples that are turbid must be filtered prior to determination. High nitrate concentrations (>10 mg N/L) interfere with the TKN preparation procedure. If interference is suspected samples are diluted prior to digestion.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation - Westco SmartChem Discrete Analyzer.

3.2. Glassware and pipettes: Class A type volumetric (vol.) glassware, graduated cylinders and pipettes.

Stock standard:	1 L vol. flask
Working standards:	1-100 mL vol. flask 4-250 mL vol. flasks
Reagents:	3-500 mL Nalgene bottles with caps 2-500 mL flasks 1-100 mL flask 50 mL graduated cylinder 10 mL graduated cylinder 2-1 L flasks 1-1 L beaker 2-1 L Nalgene bottles 1-500 mL brown Nalgene bottle

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Misc.: magnetic stirrer and stir bar
spatulas for weighing out reagents
variable Eppendorf type pipettes
vacuum filtration apparatus

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

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water.

Note: Before preparing reagents or standards, or running samples, read the safety notes in the safety/hazardous waste section of this SOP and the MSDS's for the chemicals used in this analysis.

Note: All reagent preparations should be documented in the Reagent Preparation Log sheet located on the network at: \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\TKN-TP_Soil_Digestion\. This sheet needs to be updated whenever a reagent is newly prepared. All reagent and standard preparations must be documented in the Laboratory Information Management System (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 or in the reagent sheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased reagent is also maintained in the REAGENT_PREP folder. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

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.

4.1. Chemicals:

4.1.1. Ammonium Chloride, NH_4Cl

4.1.2. Glycine, $C_2H_5NO_2$

4.1.3. β-Glycerophosphoric Acid Disodium Salt, Pentahydrate, $C_3O_{11}H_{17}PNa_2*5H_2O$

4.1.4. Westco Probe Rinse Solution

4.1.5. Westco Cleaning Solution

4.1.6. DI Water, ammonia-free

4.1.7. Sodium Salicylate, $NaC_7H_5O_3$

4.1.8. Sulfuric Acid, concentrated H_2SO_4

4.1.9. Sodium Hydroxide, NaOH

4.1.10. Sodium Hypochlorite, NaOCl (from Ricca, approx. 3 % NaOCl)

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4.1.11. Sodium Nitroferricyanide, $Na_2Fe(CN)_5NO*2H_2O$

4.1.12. Sodium Chloride, NaCl

4.1.13. Sodium Potassium Tartrate, $KOCO(CHOH)_2COONa*4H_2O$

4.1.14. Sodium Phosphate, Dibasic Heptahydrate, $Na_2HPO_4*7H_2O$

4.1.15. Standard Reference Material (SRM) purchased from ERA (soil ALTQC)

4.2. Reagents:

4.2.1. **Stock sodium hydroxide, 20 % solution** - In a 1 L vol. flask, add 200 g NaOH (4.1.9.) to approx. 800 mL DI water while stirring slowly. Bring to volume with DI water. Do not filter. Holding time, 180 days.

Note: Solution will become hot as sodium hydroxide is added to water. Use a plastic tray containing water as a water bath to cool the NaOH solution.

4.2.2. **Stock buffer** - Using a 1 L beaker and magnetic stirrer, add 134.0 g sodium phosphate dibasic heptahydrate (4.1.14.) and 20 g NaOH (4.1.9.) to approx. 700 mL DI water. Stir until dissolved, approx. 10 min. Transfer to a 1 L vol. flask and bring to volume with DI water. Store at room temperature. Holding time, 60 days.

4.2.3. **Sodium potassium tartrate, 20 % solution** - Dissolve 100 g of sodium potassium tartrate (4.1.13.) in approx. 300 mL of DI water. Add one pellet of NaOH (4.1.9.) and a few boiling chips, boil gently for 45 minutes. Cover, and after the solution has cooled down to room temperature, bring it to volume in a 500 mL vol. flask. Adjust the pH to that of DI water (approx. pH 5) with conc. H_2SO_4 (4.1.8.). Holding time, 180 days.

4.2.4. **Working stock salicylate/nitroferricyanide solution**:

Note: Use caution when weighing out sodium salicylate; it may have a small particle size and be dusty.

4.2.4.1. Using a 1 L beaker and magnetic stirrer apparatus, dissolve 150 g sodium salicylate (4.1.7.), 0.3 g sodium nitroferricyanide (4.1.11.) in approx. 350 mL DI water. Mix for a few minutes, transfer to a 500 mL vol. flask and bring to volume.

4.2.4.2. Filter using the vacuum filtration apparatus and a filter with a 0.45 micron pore size. To prevent clogging of the filter, filter 100 mL per filter. Store at room temperature. Holding time, 30 days.

4.2.5. **Working buffer** – Always combine the reagents in the stated order. Use a clean and empty 100 mL vol. flask. Add 25 mL of stock sodium potassium tartrate (4.2.3.) to 20 mL of stock buffer solution (4.2.2.) and mix. Add 12 mL of 20 % NaOH solution (4.2.1.) to the mixture. Dilute to the mark with DI water. **Prepare daily**.

4.2.6. **Working hypochlorite** - To a 100 mL vol. flask containing approx. 50

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mL DI water, add 12 mL hypochlorite (3 % NaOCl, 4.1.10.). Bring to volume. **Prepare daily**.

4.3. Standard and spiking solutions:

4.3.1. **NH_3 stock solution (1000 mg N/L) for working standards** - To a 1 L vol. flask containing about 500 mL DI water, add 3.819 g ammonium chloride (NH_4Cl) (4.1.1.), which has been dried for 12-24 hours at 105 ^oC. Preserve with 2 mL H_2SO_4 (4.1.8.). Dilute to the mark and invert 12 times. Refrigerate. Holding time for the stock standard is 180 days. This solution is also used for ammonia analysis (DEP SOP NU-095, section 5.3.1.).

4.3.2. **TKN/TP working calibration standard (TKN: 4 mg N/L, TP: 1 mg P/L)** - To a 250 mL vol. flask containing approximately 150 mL DI water add 50 mL of digestion solution (see DEP SOP NU-048, section 4.2.3.) and 0.5 mL conc. H_2SO_4 (4.1.8.). Add 1.0 mL of NH_3 stock standard (1000 mg N/L, 4.3.1.) and 250 µL of TP stock standard (1000 mg P/L, see DEP SOP NU-084, 4.3.1.). Fill up to the mark with DI water. Invert 12 times to mix. Store this standard at room temperature. This solution is also used for the analysis of TP in soils (DEP SOP NU-084). Holding time, 30 days.

The other standards used in the linear calibration are auto-diluted by the instrument from the TKN (4 mg N/L)/TP (1 mg P/L) working calibration standard. Their concentrations are as follows:

Standard	Conc. (TKN, mg N/L)	Source
C1	0.0	Digested Blank (4.3.7.)
C2	0.2	Instrument auto-dilution of C6
C3	0.5	Instrument auto-dilution of C6
C4	1	Instrument auto-dilution of C6
C5	2	Instrument auto-dilution of C6
C6	4	Working Cal. Std (4.3.2.)

4.3.3. **CCV (TKN: 1 mg N/L, TP: 0.4 mg P/L)** - To a 250 mL vol. flask containing approximately 150 mL DI water add 50 mL of digestion solution (DEP SOP NU-048, 4.2.3.) and 0.5 mL conc. H_2SO_4 (4.1.8.). Add 250 µL of NH_3 stock standard (1000 mg N/L, 4.3.1.) and 100 µL of TP stock standard (1000 mg P/L, see DEP SOP NU-084, 4.3.1.). Fill up to the mark with DI water. Invert 12 times to mix. Store this standard at room temperature. This solution is also used for the analysis of TP in soils (DEP SOP NU-084). Holding time, 30 days.

4.3.4. **ALTQC standard** - The quality control standard (ERAQCSOIL#, 4.1.15.) is prepared according to the manufacturer's instructions and

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digested according to DEP SOP NU-048. The supplier (e.g., Environmental Resource Associates) differs from the supplier of the calibration standards. The stock ALTQC standard may be diluted appropriately (by weighing out a smaller amount of soil standard) so that the concentrations falls within the calibration range. For the concentration and expiration date see the manufacturer's certificate.

4.3.5. **TKN/TP Spiking Stock Solution (TKN: 150 mg N/L, TP: 50 mg P/L)** - Dry approx. 1.1 g glycine (4.1.2.) overnight at 60 ^{o}C. Add 0.8040 g of dried glycine and 0.4941 g β-glycerophosphoric acid disodium salt (4.1.3.) to a 1 L vol. flask containing approx. 400 mL DI water. Bring to volume with DI water. Invert 12 times. Store in refrigerator. This solution is also used for the analysis of TKN in waters (DEP SOP NU-092) and TP in soils (DEP SOP NU-084). Holding time, 180 days.

Note: **Glycine** $C_2H_5NO_2$: FW = 75.07 g/mol. Molecular weight factor = 14.0067/75.07 = 0.1866.
β-Glycerophosphoric acid disodium salt pentahydrate, $C_3O_{11}H_{17}PNa_2$: FW = 306.11 g/mol. Molecular weight factor = 30.9738/306.11 = 0.1012.

4.3.6. **TKN/TP Practical Quantitation Limit (PQL) standard (PQCK2) (TKN: 0.20 mg N/L, TP: 0.06 mg P/L)** - To a 1 L vol. flask, containing about 500 mL of DI water, add 200 µL of NH_3 stock standard (1000 mg N/L, 4.3.1), 60 µL of TP stock standard (1000 mg P/L, DEP SOP NU-084, section 4.3.1.) and 2 mL of conc. H_2SO_4 (4.1.8.). Bring to volume with DI water. Digest this standard according to DEP SOP NU-048. This solution is also used for the analysis of TKN in waters (DEP SOP NU-092) and TP in soils (DEP SOP NU-084). Holding time: 30 days.

4.3.7. **Blank** (Diluent) - 0.2 % sulfuric acid (digested according to DEP SOP NU-048).

5. SAMPLE HANDLING AND PREPARATION

For sample collection, preservation, shipment, storage, and preparation instructions details see DEP SOP NU-048.

6. SAMPLE ANALYSIS AND QUANTITATION (SEE DEP SOP NU-033)

6.1. Read the Westco SmartChem operations manual before proceeding.

6.2. Initial Preparation:

6.2.1. Select digested trays to be analyzed and vortex samples thoroughly.

Note: Samples containing particulate matter should be filtered through a 0.45 µm filter prior to analysis using the Westco SmartChem Discrete Analyzer. If there is particulate matter in the digestate the sample should be diluted and re-digested.

6.2.2. Check solution levels of the three large Westco reservoirs (located on the

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right side of the instrument):

- Probe Rinse - Prepare this by adding 1 mL of stock Probe Rinse solution (4.1.4.) to 2 L of DI water.
- DI Water.
- Cleaning Solution - Prepare this by adding 100 mL of stock cleaning solution (4.1.5.) to the reservoir and bringing the final volume to 2 L with DI water.

Note: These reservoirs should be emptied, cleaned with 1:2 HCL in DI water, and refilled every two to three weeks.

6.2.3. Empty old sample cups into the hazardous waste bottle and dispose of all the old sample cups. Perform any other maintenance, e.g., replacing the drying pad, pump tubing, cuvettes, or lamp as needed.

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled SmartChem reagent vessels up to the mark (approx. 33 mL) with the following:

- Working Buffer (4.2.5.)
- Working stock salicylate/nitroferricyanide (4.2.4.)
- Working hypochlorite (4.2.6.)

6.3.2. Add 500 µL of stock probe rinse solution (surfactant, 4.1.4.) to the salicylate reagent (4.2.4.). Invert several times carefully to mix. Make sure that the solution is not covered with foam.

6.3.3. Load the reagents in the correct order (6.3.1.) on the reagent rack of the SmartChem instrument. Fill one reagent vessel with diluent (digested blank, 4.3.7.) and place in the diluent position #1.

6.3.4. Place the following controls in the controls tray in order:

1. 4 mg N/L working calibration standard (4.3.2.)
2. 1 mg N/L CCV standard (4.3.3.)
3. MDL (digested blank, 4.3.7.)

6.4. Analysis Setup/Sequence Setup:

6.4.1. Double click the SmartChem icon and another login window will appear, log in using the name *westco* and password *joe*. Click on start and allow 15- 20 minutes for the instrument to warm up.

6.4.2. Perform a *WBL* (water baseline) which takes 20 min. to perform. This checks the absorbance of each cuvette at all operating wavelengths. After completion view the results. Any cuvettes with red results will not be used by the instrument and MAY need to be changed. Many red numbers could indicate a problem with the detector or lamp. Consult a supervisor if this occurs.

6.4.3. Click on *Sample Entry*. There is a list of methods on the bottom of the

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screen. Select ***TKNX-EPA351.2-Instrument 1*** by double clicking on it.

6.4.4. A file name will automatically be assigned to the sequence run and it is named by year/month/day001.

6.4.5. At the top left hand side locate the *Accept Samples* box. Type in the total number of cups required for the analysis.

6.4.6. A sequence table will be generated and in the column labeled *ID Sample* type in the sample numbers to be analyzed.

An example run sequence includes:

- **Reagent Baseline (RBL) – 3 results are collected and averaged automatically**
- **Calibration - the instrument auto-dilutes all calibrants from the high standard (4.3.2.)**
- **MBLKs (4.3.7.)**
- **PQCK2 (4.3.6.)**
- **LFBs (4.3.5.)**
- **ERAQCSOIL# (4.3.4.)**
- **Batched samples with duplicate spikes – check prep worksheet for batching information**
- **CCV (4.3.3.) and CCB (4.3.7.) – these are scheduled to bracket every 10 cups on the run, including a final bracket**

6.4.7. After this is completed click on *Save* (the floppy disk symbol). If you forget to save before changing directories, the sequence run will be deleted and you will have to repeat the sample entry. Save the file as "TKN-Instrument 1" and print this as a bench sheet.

6.4.8. Choose the *SystemMonitor* directory and under *Plan List* select your run sequence.

6.4.9. When pouring samples, rinse once with sample first. Do not fill sample cups all the way to the top.

6.4.10. Once the trays are loaded start the run by clicking on the green arrow button, and the analysis will begin. To use the previous calibration (only for runs performed on the same day) uncheck the boxes for calibration and RBL.

6.4.11. After the run is completed go to the *Options* menu on the left hand side and click on *Data Retrieval*. Open the just finished run plan and highlight the method in the box with the white background underneath. You can print the results, calibration, and method by selecting each of the three buttons to the right of the method field.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for TKN in mg N/L.

6.5.2. The LIMS QC-Manager calculates results for the soils in mg N/kg and

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recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and/or spikes. For general calculations including RPDs and spike recoveries see Section 5.9 of the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to section 4.3.2. The other standards used in the linear calibration are auto-diluted by the instrument from the high standard. The correlation coefficient for the calibration is calculated by the instrument software.

6.6. Post-Run Procedure:

6.6.1. Choose the *Options* menu on the left hand side. Click on *Export* and find the date and run desired. Select the plan and click on *Excel.* Navigate to the directory at

\\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\SmartChem 2\TKN\ and save.

6.6.2. Open the file in excel and save as text, tab delimited (*.txt).

6.6.3. Upload into LIMS and save file as TKNyyyymmdd001.

7. DATA ARCHIVAL

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test (S-TKN), filename/s (TKNyyyymmdd001), and initials of the analyst.

7.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, reagent logsheet, digestion temperature data logger report, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. The sample preparation worksheet is archived electronically in the Prep Worksheet Manager module in the LIMS system.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. Westco instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\SmartChem2\TKN\.

8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE NU-038 AND NU-043)

8.1. 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 DEP SOPs NU-038 and NU-043.

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8.2. The 1 mg N/L working check standard (CCV, 4.3.3.) and the MBLK (method blank)/MDL (digested blank, 4.3.7.) are run every 10 samples and at the beginning and end of each run. Acceptance limits for the MBLK and MDL are < 0.08 and > - 0.08 mg N/L. The check standard recovery acceptance limits are 85 - 115 %.

8.3. Each tray of digested samples contains a digested PQL level standard (PQCK2, 4.3.6.) and a digested ALTQC standard (ERAQCSOIL#, 4.3.4.). Acceptance limits for the recovery of the PQCK2 are 70 - 130 %. Refer to the manufacturer's Certificate of Analysis for the acceptance limits of the soil ALTQC.

8.4. A method blank (MBLK), a laboratory fortified blank (LFB), a duplicate sample and/or duplicate matrix spikes are digested and analyzed for every batch of 10 samples (see DEP SOP NU-048). Acceptance limits for the LFB and matrix spike recoveries for soils and sediment samples are 80 - 120 %. The RPD of sample/matrix spike duplicates must be less than 20 % for results above the PQL.

8.5. Linear Calibration Range (LCR) - See FDEP Quality Manual Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'. The correlation coefficient of the linear calibration must be equal to or larger than 0.995. The % error calculated for each calibrant should not exceed 10 % for calibrants above the PQL and 30 % at the PQL level.

8.6. Every day samples are analyzed a new calibration has to be performed at the beginning of the analysis and the LCR must be verified (8.5.). Consecutive runs on one day may use the same calibration. To verify the calibration range the recoveries for calibration standards above the PQL must be 90 -110 %.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

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

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

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

9.4. The solution used to digest samples contains mercury, which is hazardous and toxic. All waste from the instrument, digested samples, and standards made with digestion solution should be collected in bottles clearly labeled as hazardous waste.

10. 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.

10.1. EPA method 351.2, Rev. 2.0 (1993).

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10.2. Quality Manual, Bureau of Laboratories, FDEP.

10.3. Laboratory Health and Safety Plan.

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

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

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

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

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

10.9. DEP SOP NU-048, Preparation of Sediments for Total Kjeldahl Nitrogen and Total Phosphorus Analyses.

10.10. DEP SOP NU-095, Determination of Ammonia in Water by Gas Diffusion Segmented Flow Analysis

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

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

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

Appendix of Significant Changes

08/24/2010 SOP revised for the analysis of TKN in soils only. Some procedural clarifications in section 4 and the addition of subsection 6.5

08/19/2011 Preparations of the working calibration standard, CCV and PQL solutions were modified and now include phosphorous (solutions also used for the analysis of TKN in waters and TP in soils).

07/08/2015 Data archival information was clarified in Section 7.

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