DETERMINATION OF TOTAL PHOSPHORUS IN SOILS USING THE AQ2 DISCRETE ANALYZER

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1. SCOPE AND APPLICATION

1.1. This method details the determination of phosphorus as Total Phosphorus (TP) in solid matrices (soils, sediments etc.). The applicable range for this method is from the method detection limit (MDL) of 2.5 mg P/kg (0.020 mg P/L) to 125 mg P/kg (1.0 mg P/L). The range is dependent on the percent solids of the sample analyzed and may be extended by dilution. The practical quantitation limit (PQL) is 7.5 mg P/kg (0.060 mg P/L).

1.2. The test covered by this SOP is S-TP.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on SEAL Analytical AQ2 Method: EPA-120-A Rev. 4 and EPA Method 365.4.

2.2. Prior to analysis the samples are acid digested according to DEP SOP NU-048. This process converts inorganic and organic forms of phosphorus to orthophosphate.

2.3. Orthophosphate reacts with molybdenum and antimony in an acidic medium to form a phosphoantimony/molybdenum complex, which is reduced with ascorbic acid. The AQ2 Discrete Analyzer is used to measure the absorbance of the complex at 880 nm.

2.4. Interferences - Ferric iron, copper, and silica. Also, samples with background absorbance at the analytical wavelength may interfere, see EPA Method 365.4.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation: AQ2 Discrete Analyzer

3.2. Glassware (pipettes and volumetric flasks Class A):

- Various pipettes for stock/working standard preparation
- 1 x 100 mL volumetric flask
- 3 x 250 mL volumetric flasks
- 3 x 500 mL volumetric flasks
- 2 x 2 L volumetric flasks
- 4 x 500 mL brown Nalgene bottles
- 1 x 500 mL Nalgene bottle

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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-Hg \. 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. Acetone, C_3H_6O

4.1.2. Ammonium Molybdate, $(NH_3)_6Mo_7O_{24}*4H_2O$

4.1.3. Antimony Potassium Tartrate, $K(SbO)C_4H_4O_6*1/2\ H_2O$

4.1.4. Ascorbic Acid, $C_6H_8O_6$

4.1.5. DI Water

4.1.6. Potassium Dihydrogen Phosphate, KH_2PO_4

4.1.7. Sodium Chloride, NaCl

4.1.8. Glycine, $C_2H_5NO_2$

4.1.9. β-Glycerophosphoric acid, $C_3H_7Na_2O_6P*5H_2O$

4.1.10. Bleach, (e.g. Clorox)

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4.1.11. Sulfuric Acid, concentrated H_2SO_4

4.1.12. Soil ALTQC, Standard Reference Material (SRM) purchased from ERA (see also DEP SOP NU-048)

4.2. Reagents:

4.2.1. **5 N Sulfuric Acid** - In a 500 mL volumetric flask, slowly add 70 mL of concentrated sulfuric acid (4.1.11.) to approximately 400 mL of DI water. Dilute to the mark with DI water. Invert to mix. Holding time, 180 days.

4.2.2. **Working Acid/Salt Reagent** - To a 200 mL volumetric flask containing about 50 mL DI water, add 4.0 g NaCl (4.1.7.) and 90 mL of 5 N sulfuric acid (4.2.1.). Dilute to the mark with DI water. Store the solution in a Nalgene bottle. Holding time, 30 days.

4.2.3. **Stock Ammonium Molybdate Reagent** - Using a 250 mL beaker, add 4.0 g of ammonium molybdate tetrahydrate (4.1.2.) to 100 mL of DI water. Final volume is 100 mL. Stir for at least 2 hours. Store this solution in a brown Nalgene bottle at 4 ^{o}C. Discard this reagent if it becomes blue or turbid. Holding time, 60 days.

4.2.4. **Stock Antimony Potassium Tartrate Reagent** - Using a 500 mL beaker dissolve 1.5 g of antimony potassium tartrate (4.1.3.) in 250 mL of DI water. Dilute to 500 mL. Store at 4 ^{o}C in a brown Nalgene bottle. Holding time, 30 days.

4.2.5. **Working Color Reagent** - To a 200 mL volumetric flask, add 40 mL of the stock ammonium molybdate reagent (4.2.3.) and 13.3 mL of stock antimony potassium tartrate reagent (4.2.4.). Dilute to the mark and invert several times to mix. Store at 4 ^{o}C in a brown Nalgene bottle. Holding time, 14 days.

4.2.6. **Ascorbic Acid Reagent** - Using a 500 mL beaker and a magnetic stir plate dissolve 15.0 g of ascorbic acid (4.1.4.) in approx. 150 mL of DI water. Transfer to a 250 mL volumetric flask and bring to volume with DI water. Transfer to a brown Nalgene bottle. Add 0.5 mL of acetone (4.1.1.) and invert several times. Store in the refrigerator. Holding time, 7 days.

4.2.7. **1 % Bleach Solution** - For instrument cleaning. When using bleach containing 5.25 % NaOCl add 40 mL of bleach (4.1.10.) and 160 mL DI water to a 250 mL beaker. Adjust volumes as needed, as commercial bleach concentration varies. Prepare fresh when needed and discard leftover solution immediately after use.

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4.3. Standards and spiking solutions:

4.3.1. **1000 mg P/L Stock Standard** - To a 1 L volumetric flask containing 400 mL of DI water, add 4.394 g anhydrous potassium dihydrogen phosphate, (4.1.6.) which has been dried at 105 ^{o}C overnight or to a constant weight. Dilute to the mark and invert 12 times. Refrigerate in an amber bottle. This standard solution is also used for TP and orthophosphate water analyses (see DEP SOPS NU-090 and NU-070, respectively). Holding time, 180 days.

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 (DEP SOP NU-048, 4.2.3.) and 0.5 mL conc. H_2SO_4 (4.1.11.). Add 1.0 mL of NH_3 1000 mg N/L stock standard (DEP SOP NU-095, 5.3.1.) and 250 µL of TP 1000 mg P/L stock standard (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 TKN in soils (DEP SOP NU-101) and TKN in water matrices (DEP SOP NU-092). 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:

Calibration Standard	Conc. (TP, mg P/L)	Source
C1	0.05	Auto-dilution of C5
C2	0.10	Auto-dilution of C5
C3	0.30	Auto-dilution of C5
C4	0.60	Auto-dilution of C5
C5	1.0	Working Cal. Standard (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 (4.1.11.). Add 250 µL of NH_3 1000 mg N/L stock standard (DEP SOP NU-095, 5.3.1.) and 100 µL of TP 1000 mg P/L stock standard (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 TKN in soils (DEP SOP NU-101). Holding time, 30 days.

4.3.4. **TP/TKN Organic Spiking Stock Solution, (TP: 50 mg P/L, TKN: 150 mg N/L) -** Dry approx. 1.1 g glycine (4.1.8.) overnight at 60 ^{o}C. Add

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0.8040 g dried glycine and 0.4941 g beta-glycerophosphoric acid disodium salt (4.1.9.) to a 1 L volumetric flask containing about 500 mL of DI water. Bring to volume and invert 12 times. Do not acidify. Store in refrigerator. This solution is also used for the analysis of TKN in soils (DEP SOP NU-101). Holding time, 180 days.

4.3.5. **TP/TKN Practical Quantitation Limit (PQL) standard (PQHLTP) (TP: 0.060 mg P/L, TKN: 0.20 mg N/L)** - To a 1 L volumetric flask containing 500 mL of DI water, add 60 µL of 1000 mg P/L TP stock standard (4.3.1.) and 200 µL of 1000 mg N/L NH_3 stock standard (DEP SOP NU-081, 4.3.1.) and 2 mL conc. H_2SO_4 (4.1.11.). Bring to volume with DI water and invert 12 times. Refrigerate. This solution is also used for the analysis of TKN in soils (DEP SOP NU-101). Holding time 30 days.

4.3.6. Soil **ALTQC standard** - The SRM quality control standard (ERAQCSOIL#, 4.1.12.) is prepared according to the manufacturer's instructions and digested according to DEP SOP NU-048. The supplier (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.

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

5. SAMPLE PREPARATION PROCEDURE (SEE DEP SOP NU-033)

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

6. SAMPLE ANALYSIS AND QUANTITATION

Note: • Digested solutions containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 Discrete Analyzer. Samples with undigested organic matter must be redigested with dilution to confirm the result.
• Observe routine maintenance as recommended by the instrument manufacturer and enter any maintenance performed in the maintenance logbook.
• Samples with TP concentrations above 10 mg P/L must be prediluted and redigested.

6.1. Initial Preparation:

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6.1.1. Remove stock standard, any working standards, and reagents from the refrigerator. Check the expiration dates on all standards and reagents. Discard expired standards and/or reagents and reprepare as necessary.

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

6.2. Calibration:

6.2.1. The instrument is calibrated using the 1.0 mg P/L working calibration standard (4.3.2.).

6.2.2. The diluent for TP is the digested blank (4.3.7.).

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled AQ2 reagent vessels with the following: working acid/salt reagent (4.2.2.), working color reagent (4.2.5.) and ascorbic acid reagent (4.2.6.).

6.3.2. Reagent Location - The reagent tray is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. Place working acid/salt reagent in position 1, working color reagent in position 2, and the ascorbic acid reagent in position 3.

6.3.3. Controls - Fill one reagent vessel with check standard (CCV, 4.3.3.) and place it in position 10. Fill another vessel with digested blank and place it in position 14.

6.4. Analysis Setup/Sequence setup:

6.4.1. Turn on the AQ2 Analyzer (large button on the right side behind the Instrument) and the computer. To start the software double click on the AQ2 Icon on the screen. The username is '**Instrument 1'** and the password is 'password'.

6.4.2. Check the DI water reservoir and the waste containers. Replace all used reaction segments.

6.4.3. In the software, go to 'Settings' menu and select maintenance. Zero the reaction segment counter.

6.4.4. AQ2 System Flush - Remove AQ2 water inlet sensor and place in a beaker with 1 % bleach solution (4.2.7.). From the maintenance menu perform the following:

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a. Prime the syringe - 10 primes (2 times).

b. Prime the syringe - 2 primes (at least 15 times). Verify flow through the probe wash assembly.

c. Wash the cuvette - Auto Wash (at least 10 times).

d. Flush the beaker and the water inlet sensor with DI water.

e. Using fresh DI water repeat steps a (once) and c (7 times).

6.4.5. Go back to the 'settings' menu and select the daily start-up option.

6.4.6. Setup of the analytical run - Click on the scheduling icon on the left side of the screen. Select a free tray and name the run (in the format HLTPyyyymmdd001K, e.g. HLTP20190324001K, with the suffix K referring to instrument 1). Now the sequence screen appears. Leave lines one and two empty. Type the sample sequence in the 'Sample ID' column. Now, highlight the typed lines and select the HLTP test. The test should appear in the requested test column to the right of the sample ID's. Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

An example run sequence includes:

- **Calibration - the instrument auto-dilutes all calibrants from the high standard (4.3.2.)**
- **CCV (4.3.3.) and CCB (4.3.7.) – these are scheduled to bracket every 10 cups on the run**
- **MBLKs (4.3.7.) – for each of the batches**
- **PQHLTP (4.3.5.)**
- **LFBs (4.3.4.) – for each of the batches**
- **ERAQCSOIL# (4.3.6.)**
- **Batched samples with duplicate spikes – check prep worksheet for batching information**
- **CCV and CCB – final bracket**

6.4.7. Pouring the analytical run - Cup positions one and two are reserved for the calibration standards. For HLTP the calibration standard (4.3.2.) is placed in position #1. Pour the samples according to the cup position on the bench sheet.

6.4.8. Double-click on the 'Run' icon on the left side of the screen. A dialog box appears. Select the tray that contains the sequence to be analyzed. Click 'OK'. Another dialog box appears: check the auto-standardize box and click 'OK' (if the previous calibration is to be reused do not check the auto-standardize option). Select 'OK' on the following dialog box.

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6.4.9. The reagent levels are checked by the instrument and are displayed on the upper right corner of the screen. If the levels are sufficient for the entire run the analysis will start. If not, the run will stop and display the reagent which has insufficient volume. Fill up the reagent and re-start the run according to 6.4.8. It is advisable to start the run with full reagent containers if a lot of dilutions are expected as the calculated volumes do not account for dilutions.

6.5. Quantitation/Calculations:

6.5.1. The instrument will report TP concentrations in mg P/L.

6.5.2. The QC-Manager converts the results to mg P/kg. It also calculates the recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and 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. After the run has finished, print the AQ2 Test Parameters Report from the 'Settings' menu on the top of the screen.

6.6.2. Click on the 'Acceptance' menu located on the left side of the screen.

6.6.3. Click on the 'Accept All' Icon, this adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the convention described in 6.4.6. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in: \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\HLTP\

6.6.4. Convert the excel file into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 1'.

6.6.5. Click on the 'Reporting' icon on the left side of the screen. Check the reporting format 'TKN/TP' and print the run report. It contains the calibration and all the data. Select the chronological order report function to print your report.

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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-TP), filename/s (HLTPYYYYMMDD001K), 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. AQ2 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\AQ2\HLTP\

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 contingency plans for handling out-of-control or unacceptable data refer to the following DEP SOPs NU-038 and NU-043.

8.2. The 0.40 mg P/L working check standard (CCV, 4.3.3.) and the MBLK/MDL (digested blank) will be run every 10 samples and at the end of each run. Acceptance limits for the MBLK and MDL are < 0.020 and > - 0.020 mg P/L. The check standard recovery acceptance limits are 85 - 115 %.

8.3. Each tray of digested samples contains a digested PQL-level standard (PQHLTP, 4.3.5.) and SRM quality control standard (soil ALTQC, 4.3.6.). Acceptance limits for the recovery of the PQL-level standard are 70 – 130 %. Acceptance criteria for the SRM standard are specified in the Certificate of Analysis provided by the supplier.

8.4. A method blank (MBLK), 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 are 80 -120 %. The RPD of sample/matrix spike duplicates must be less than 20 % for results above the PQL.

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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 all calibrants above the PQL and 30 % at the PQL.

8.6 Every day samples are analyzed a new calibration is 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.

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. AQ2 Operator Handbook and AQ Software guide.

10.2. EPA Method 365.4

10.3. SEAL Analytical AQ2 METHOD NO: EPA-120-A Rev. 4

10.4. DEP SOP NU-033, Summary of Analysis Procedure 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.

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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-101, Determination of Total Kjeldahl Nitrogen in Soils Using the Westco SmartChem Discrete Analyzer.

10.11. DEP SOP NU-070, Analysis of Orthophosphate Using the Bran+Luebbe Segmented Flow Analyzer.

10.12. DEP SOP NU-090, Determination of Total Phosphorus in Water Using the AQ2 Discrete Analyzer.

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

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

10.15. Quality Manual, Bureau of Laboratories, FDEP.

10.16. Laboratory Health and Safety Plan.

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

11. TECHNICAL SUPPORT CONTACTS

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Appendix of Significant Changes

06/30/2008 Modifications to reagent preparation procedures and method setup to conform to SEAL Analytical AQ2 Method: EPA-120-A Rev. 4.

07/08/2010 Edited to remove reference to the W-TP and W-TP-F tests which are no longer performed.

07/05/2011 Removed the standard preparation sections for salt blank and check standard solutions that were used for W-TP and W-TP-F tests. Quality control section was modified and corrected. Included use of a sediment ALTQC sample.

06/29/2013 Holding time for the 5 N sulfuric acid was set to 180 days.

06/08/2015 Data archival information was clarified in Section 7.

12/13/2018 Reference to S-TKN analysis updated to DEP SOP NU-101.

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