DETERMINATION OF TOTAL PHOSPHORUS IN WATER USING THE AQ2 DISCRETE ANALYZER

1. SCOPE AND APPLICATION

1.1. This method details the determination of phosphorus as total phosphorus (TP) in drinking, surface, saline waters and wastewaters. The applicable range for this method is from the practical quantitation limit (PQL) of 0.050 to 1.0 mg P/L. The method detection limit (MDL) is 0.005 mg P/L. The range may be extended by dilution. All samples with concentrations below the PQL on the AQ2 are analyzed using the segmented flow analyzer (see DEP SOP NU-082).

1.2. The tests covered by this SOP are W-S-A-TP and W-S-A-TP-F.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA Method 365.1, Rev. 2.0 (1993) and SEAL Analytical AQ2 Method EPA-119-A Rev. 5.

2.2. Prior to analysis the samples are acid-persulfate digested according to the DEP SOP NU-049. This process converts inorganic and organic forms of phosphorus to ortho-phosphate.

2.3. Ortho-phosphate 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 365.1. High conductivity can cause interferences in matrix spike recoveries. To minimize this interference samples having conductivities above 5000 µmhos/cm are batched together and diluted two-fold prior to digestion, see DEP SOP NU049 section 5.3.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – Seal AQ2 Discrete Analyzer

3.2. Glassware (Class A)

- Various pipettes for reagent solution preparation
- 1- 1 L volumetric flask
- 1- 2 L volumetric flask
- 6- 500 mL volumetric flasks
- 2- 500 mL brown Nalgene bottles
- 2- 500 mL Nalgene bottles

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

Note: Refer to DEP SOP NU-049 for the preparation of all standard solutions.

Note: Wash all glassware with 1:2 HCl and rinse at least three times with deionized (DI) water. Before preparing reagents, standards, or running samples, please read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

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 documented in the Reagent and Standard Logsheet (NU-RGTSTD) located in the Reagent Prep folder on the Nutrients drive at, \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\. It needs to be updated whenever a reagent or standard is newly prepared. The Logsheet must be submitted with each analysis folder. If available, a Certificate of Analysis for each purchased reagent or chemical is maintained in the REAGENT_PREP folder on the Nutrients drive at \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\. A Certificate of Analysis for the standard reference material is kept in a binder in C307. 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 Molybdate, $(NH_3)_6Mo_7O_{24}*4H_2O$

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

4.1.3. Ascorbic Acid, $C_6H_8O_6$

4.1.4. DI Water

4.1.5. Potassium Dihydrogen Phosphate, KH_2PO_4

4.1.6. Sodium Hydroxide, NaOH

4.1.7. Sodium EDTA dihydrate, $Na_2EDTA*2H_2O$

4.1.8. Sulfuric Acid, concentrated, H_2SO_4

4.1.9. Sodium hypochlorite, NaOCl (Clorox Bleach, 8.25%)

4.2. Reagents:

4.2.1. 5 N Sulfuric Acid - In a 500 mL volumetric flask, slowly add 70 mL of concentrated sulfuric acid (4.1.8.) to approximately 400 mL of DI water. Invert to mix and make up to volume. Holding time, 180 days.

4.2.2. Stock Ammonium Molybdate, 4 % (w/v) - Using a 500 mL beaker, add 20.0 g of ammonium molybdate tetrahydrate (4.1.1.) to 400 mL of DI water. Dilute to 500 mL with DI water. Stir for at least 2 hours. Store

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this solution in a plastic bottle and refrigerate. Holding time, 60 days. Discard this reagent if it becomes visibly blue or turbid.

4.2.3. Stock Antimony Potassium Tartrate Reagent, 3 g/L - Using a 500 mL beaker dissolve 1.5 g of antimony potassium tartrate (4.1.2.) in 250 mL of DI water. Dilute to 500 mL with DI water. Store refrigerated in a dark bottle. Holding time, 30 days.

4.2.4. Color Reagent - To a 500 mL volumetric flask, add 200 mL of 5N sulfuric acid (4.2.1.), followed by 30 mL of stock antimony potassium tartrate (4.2.3.) reagent. Swirl the mixture and then add 100 mL of the stock ammonium molybdate reagent (4.2.2.) and dilute to the mark. Invert several times to mix. Store refrigerated in a plastic bottle. Holding time, 14 days.

4.2.5. Ascorbic Acid Reagent (40 g/L) - Using a 500 mL beaker and a magnetic stir plate dissolve 20.0 g of ascorbic acid (4.1.3.) in approx. 120 mL of DI water. Transfer to a 500 mL volumetric flask and bring to volume with DI water. Transfer to a dark bottle and store in the refrigerator. Holding time, 7 days.

4.2.6. Alkaline EDTA Rinse (cuvette wash) - Add approximately 250 mL DI water to a 500 mL volumetric flask. Add 5 g sodium EDTA dihydrate (4.1.7.) and 10 g NaOH (4.1.6.) to the flask and swirl until the NaOH dissolves. Dilute to volume with DI water and mix well. Holding time, 60 days.

4.2.7. Sodium hypochlorite wash solution - Prepare a 1 % v/v bleach solution by adding 1 mL Clorox Bleach (4.1.9.) to 100 mL DI water. The bleach solution is made fresh when needed.

4.2.8. Calibration and Quality Control Standards - Refer to DEP SOP NU-049 for the preparation of all standard solutions.

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

Refer to DEP SOP NU-049 for preparation instructions and sample handling details. The carrier solution and all samples, working standards and QC standard solutions, and blanks are digested prior to analysis.

6. SAMPLE ANALYSIS AND QUANTITATION\CALIBRATION

Note: • Samples containing particulate matter should be filtered through a 0.45 µm filter prior to analysis on the AQ2 Discrete Analyzer. Redigestion will also be necessary - consult supervisor to determine if predilution will be required.

- If the digested sample has color, it must be pre-diluted and redigested.
- Samples with TP concentrations above 5 mg P/L must be prediluted and redigested.

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• Observe routine maintenance as recommended by the instrument manufacturer and enter any maintenance performed in the maintenance logbook.

6.1. Initial Preparation:

6.1.1. Remove reagents from the refrigerator and check their expiration dates. Discard expired reagents and re-prepare if necessary.

6.1.2. Select digestion 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 (AQ2 CAL STD; see DEP SOP NU-049, section 4.3.3.).

6.2.2. The diluent for TP is the digested blank, and is also used for the MDL and continuing calibration blank (CCB) (see DEP SOP NU-049, section 4.2.3.).

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled AQ2 reagent vessels with the following, diluent (see DEP SOP NU-049, section 4.2.3.), color reagent (4.2.4.) and ascorbic acid reagent (4.2.5.).

6.3.2. Reagent Location - The reagent tray, with plastic wedge shaped containers is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. The color reagent is in position 7, the ascorbic acid reagent in position 8, and the stock standard (1.0 mg/L) is in position 9.

6.3.3. Controls - Fill one reagent vessel with check standard (CCV, 0.40 mg/L, see section 4.3.6. in DEP SOP NU-049) and place it in position 11. Fill another vessel with digested blank (CCB, 6.2.2.) and place it in position 10.

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 % v/v sodium hypochlorite (4.2.7). From the maintenance menu perform the following,

a. Prime the syringe - 10 primes (3 times)

b. Verify flow through the probe wash assembly.

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c. Wash the cuvette - Auto Wash (at least 5 times).

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

e. Using fresh DI water repeat steps a - c and double the number of primes (i.e., 6 times for a.) and washes (i.e., 10 times for c.).

f. Add alkaline EDTA rinse solution (4.2.6.) to the reagent wedge at position 1 and perform Extra Wash 3 times.

g. Perform Auto Wash with DI water 5 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. Choose **Reagent Set 3**. Select a free tray and name the run. The file name follows the TPYYYYMMDD001Q data format, where TP stands for Total Phosphorus, YYYY is the year, MM is the month and DD is the day of the month. Postscripts 001,002 etc. are used to denote the first or second run of the day, and Q indicates the AQ2-2 instrument (K indicates the AQ2-1 instrument). For example, the first run on instrument AQ2-2 on Sept 1, 2015 would be named TP20150901001Q. Now the sequence screen appears.

Then using the prep worksheet (see DEP SOP NU-049, section 5.18.), begin typing up the run in order of the batches listed on the worksheet. Starting at line 1, type the sample sequence in the 'Sample ID' column. Include the tray number with the PQL sample line, and the prep batch ID number with each MBLK sample line. Include at least 7 blank "C" cups at the end of the run to avoid a known timing issue affecting the final CCB/CCV results (see the Seal AQ2 Method EPA-111-A, Rev. 4 for more information).

An example run sequence includes:

- **Calibrants** - auto-diluted from the high standard, 6.2.1.
- **CCV/CCB** - these checks bracket every 10 cups on the run, 6.3.3.
- **PQCK1_tray #** (See DEP SOP NU-049, section 4.3.3.)
- **ERAQC#** (See DEP SOP NU-049, section 4.3.5.)
- **Batched samples,** including MBLKs (indicate prep batch ID), LFBs, and spiked samples
- **CCV/CCB bracket**
- **7 blank "C" cups**
- **Final CCV/CCB bracket**

Next, highlight the typed lines and select the **LLTP2** 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.

Note: LLTP samples are normally initially ran on the AQ2. Sample results above 0.05 mg P/L are reported from the AQ2, and samples with concentrations below 0.05 mg P/L are run on the Bran+Luebbe (see DEP SOP NU-082). Check carefully against the backlog or

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prep worksheet to make sure that no samples are missed in the process. In rare cases (instrument out of commission, large sample backlogs, etc.) the entire tray may be ran on the Bran+Luebbe instrument.

6.4.7. Pouring the analytical run - cup positions 1 and 2 are reserved for the calibration standards. 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. Here check the auto-standardize box and click 'ok'. If the previous calibration is to be used for the run do not check the auto-standardize option. Select 'ok' on the following dialog box.

6.4.9. The reagent levels are checked by the instrument and are displayed on the upper right corner of your 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.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for TP in mg P/L.

6.5.2. The QC-Manager calculates 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 Sections 5.9 of the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to section 4.3.3. in DEP SOP NU-049. The standards (seven plus a calibration blank) used in the calibration are auto-diluted by the instrument from the high standard. The linear calibration curve, including the correlation coefficient and the % error between the target value of each calibrant and its calculated value, is documented by the instrument software and is part of the analysis report.

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), see section 7.4.

6.6.4. 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

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calibration and all the data. Select the chronological order report function to print your report.

6.6.5. Convert the excel file created in 6.6.3. into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 2'.

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 (W-S-A-TP, W-S-A-TP-F), filename/s (TPYYYYMMDD001Q), and initials of the analyst. Submit the folder to a supervisor for review and authorization.

7.2. Each report folder should contain the following items for the AQ2 run - report summary, calibration curve, method parameters, digestion temperature log, Reagent and Standard Logsheet, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

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 at, \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\AQ2\LLTP\.

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

8.1. For method performance and acceptance criteria for QC measures and corrective action for handling out-of-control or unacceptable data, refer to DEP SOPs NU-038 and NU-043.

8.2. The 0.40 mg P/L standard (CCV; see DEP SOP NU-049, section 4.3.6.) and the MBLK/MDL (digested blank) will be run at the beginning of the run, every 10 samples, and at the end of each run. Acceptance limits for recovery of the CCVs are 90-110 %. Acceptance limits for the MBLK and MDL are < 0.005 and > -0.005 mg P/L.

8.3. A laboratory fortified blank (LFB; 0.4 mg P/L; see DEP SOP NU-049, section 5.9.) and duplicate samples and/or duplicate matrix spikes are analyzed for every batch of 10 samples. Acceptance limits for the LFB and matrix spike recoveries are 90-110 %. The sample/matrix spike reproducibility should be less than 20 % RPD for results above the PQL.

8.4. One PQL level standard, PQCK1 (0.05 mg P/L; see DEP SOP NU-049, section 4.3.3.), is analyzed on every analytical run. Acceptance limits for recovery for PQCK1 are 70-130 %. The PQCK standard also acts as an LOQ verification (see DEP SOP LB-031).

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8.5. One certified standard reference material, ALTQC, is analyzed on every analytical run. The supplier for the QC stock standard differs from the supplier of the calibration standards. Acceptance limits for the ALTQC are 90-110 %.

8.6. Linear Calibration Range (LCR) - See 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. Recoveries for calibration standards above the PQL must be 90-110 %, and 70-130 % for the PQL-level standard.

8.7. Every day samples are analyzed a new calibration has to be performed at the beginning of the analysis and the LCR must be verified (See 8.6.). 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 internet 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. Sample waste, excess reagents, and waste exiting the flow cell may be poured down the sink while flushing with water.

10. 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.

10.1. AQ2 Operator Handbook and AQ Software guide.

10.2. EPA Method 365.1, Rev. 2.0 (1993)

10.3. SEAL Analytical AQ2 METHOD NO: EPA-119-A Rev. 5

10.4. DEP SOP LB-031, Limit of Detection Verification.

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

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

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

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

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

10.10. DEP SOP NU-049, Total Phosphorus (TP) Autoclave Sample Preparation.

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10.11. DEP SOP NU-082, Analysis of Low Level Total Phosphorus Using the Bran+Luebbe Segmented Flow Analyzer.

10.12. Quality Manual, Bureau of Laboratories, FDEP.

10.13. Laboratory Health and Safety Plan.

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

11. TECHNICAL SUPPORT CONTACT

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Appendix of Significant Changes

07/15/2010 Section 1.1 and 8.2: Changed MDL from 0.020 mg P/L to 0.005 mg P/L (effective 12/29/2009).

04/14/2011 Section 8: Verification of the LCR was clarified.

03/28/2012 The standard preparation section was removed and added to the sample digestion SOP (NU-049).

08/20/2015 Data archival information added to section 7.

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