ANALYSIS OF LOW LEVEL TOTAL PHOSPHORUS USING THE BRAN+LUEBBE SEGMENTED FLOW ANALYZER

1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of total phosphorous (TP) in drinking, surface, saline waters and wastewaters. The calibration range is from the method detection limit (MDL) 0.002 to 0.10 mg P/L. Higher concentrations may be measured by using sample dilution. The practical quantitation limit (PQL) is 0.005 mg P/L.

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. The described procedure is based on EPA Method 365.1, Rev. 2.0 (1993) and Seal Method G-146-95 Rev. 3.

2.2. Prior to analysis samples are prepared by autoclave digestion (DEP SOP NU-049) in which all phosphate containing compounds, both organic and inorganic, are hydrolyzed to generate orthophosphate ion (PO_4^{3-}). During analysis orthophosphate forms a complex with molybdenum and antimony in an acid medium. This phosphoantimony/molybdenum complex is reduced with ascorbic acid and generates a blue colored solution. The intensity of this color is measured at 880 nm for total phosphate analysis.

Interferences - High iron concentrations can cause precipitation and subsequent loss of phosphorus. High conductivity can cause interferences in matrix spike recoveries.

3. EQUIPMENT AND SUPPLIES

3.1. Bran+Luebbe microcomputer controlled segmented flow analysis system:

3.1.1. Bran+Luebbe XYZ-3 autosampler, sample time/wash time 10/30 (set using software).

3.1.2. AA3 pump control via the AACE software. The red switch must be in position “Run” and the black switch in position “Normal”.

3.1.3. AA3 analytical manifold, with heater temperature set at 37 ºC, (see Bran+Luebbe method for manifold setup).

3.1.4 AA3 Digital Colorimetric detector. The AA3 Digital Colorimetric detector contains a 10 mm flow cell and 880 nm wavelength filter. The flow cell size can be changed.

3.2. Pump tubing:

Orange/White	Sample
Orange/White	Ascorbic Acid

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Orange/White	Sulfuric Acid Reagent
Black/Black	Air
Red/Red	Working Color Reagent
Green/Green	Wash

3.3. Glassware, bottles and pipettes:

Note: All volumetric glassware listed in this SOP is Class A.

Glass pipettes

5-500 mL Nalgene bottles with caps for carrier and reagents.

50 and 25 mL graduated cylinders

6-250 mL Nalgene bottles with caps

2-100 mL volumetric flasks

3-1 L volumetric flasks

2-500 mL volumetric flasks

1-2L volumetric flask

3.4. Miscellaneous:

2 spatulas for weighing out reagents

Variable Eppendorf pipettes (100-1000 µL and 1-10 mL)

Weighing boats

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) submitted with each analysis folder. If available, a Certificate of Analysis for each purchased reagent, standard, or chemical is maintained in the REAGENT_PREP folder on the Nutrients drive at \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

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

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4.1. Chemicals:

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

4.1.2. Antimony Potassium Tartrate, $K(SbO)C_4H_40_6*1/2H_20$

4.1.3. Ascorbic Acid, $C_6H_8O_6$

4.1.4. Sodium Dodecylsulfate, SDS

4.1.5. Sulfuric Acid, concentrated, H_2SO_4

4.1.6. Sodium Hypochlorite, NaOCl (Generic Bleach, 8.25 %, conc. may vary)

4.1.7. Hydrochloric Acid, HCl

4.2. Reagents:

4.2.1. Color Reagent - To a 1 L volumetric flask containing approximately 500 mL DI water, first add 0.10 g of antimony potassium tartrate (4.1.2.) followed by 2.4 g ammonium molybdate (4.1.1.). Slowly, with swirling, cautiously add 22 mL of concentrated sulfuric acid (4.1.5.). Add 1.4 g of SDS (4.1.4.) and mix well. The order in which the reagents are added is important. Bring to volume with DI water. Store in a refrigerator using an airtight amber container. Holding time, 5 days.

4.2.2. Sulfuric acid reagent - To a 500 mL volumetric flask containing approx. 250 mL DI water add 5.5 mL of concentrated H_2SO_4 (4.1.5.) and 0.8 g of SDS (4.1.4) and dilute to the mark. Mix thoroughly. Store in the refrigerator. Holding time, 5 days.

4.2.3. Ascorbic acid reagent - To a 500 mL volumetric flask containing approx. 250 mL DI water add 5.5 g ascorbic acid (4.1.3.). Bring to volume and mix thoroughly. Store in a dark bottle in the refrigerator. Holding time, 5 days.

4.2.4. Bleach wash (to clean instrument) - Prepare a 1 % v/v bleach solution by adding 1 mL Bleach (4.1.6.) to 100 mL DI water. The bleach solution is made fresh when needed.

4.2.5. SDS wash - Add 2 g of SDS (4.1.4.) to 1 L DI water and mix thoroughly. Holding time, 180 days.

Note: Carrier solution (see section 6.1.5.) is digested for use in this analysis (see DEP SOP NU-049, section 4.2.4.). Document the unique ID and expiration date of the carrier solution on the reagent and standard logsheet in the analysis folder.

4.3. Calibration Standards and Quality Control Standard - Refer to DEP SOP NU-049 for the preparation of all standard solutions. The prepared ALTQC (designated as ERAQC#) is diluted 1:10 for this analysis.

5. SAMPLE PREPARATION, PRESERVATION AND HANDLING

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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/QUANTITATION/CALIBRATION

Note: Read the manufacturer operation manual before proceeding. Follow DEP SOP NU-033.

6.1. Bran+Luebbe AA3 Detector and Pump Start-Up Procedure:

6.1.1. Turn on the instrument computer. Next, turn on the autosampler and allow needle to return home. Then turn on the AA3 pump followed by the AA3 Digital Colorimetric detector (heater will take approximately 15 minutes to come to temperature). Then secure platens for the tubing being used.

6.1.2. Double click on the AACE icon on the desktop to start the main program. Click on the "Charting" icon and make sure that the pump's two rocker switches are set to Run and Normal (speed) respectively. Close out Channel 2, since only Channel 1 will be used for the run.

6.1.3.
- Perform the daily wash - Flush the lines with DI water for 10 to 15 min until the baseline is stable (normally 5 %).
- Alternatively, the lines are flushed with the following reagents to maintain a stable baseline. The analyst may decide whether this step needs to be performed depending on the baseline noise and the number of samples analyzed daily. This step is typically performed every other day. The lines are flushed sequentially as follows:
    a. 1:10 Hydrochloric acid (5 minutes)
    b. DI water (5 minutes)
    c. 1 N Sodium Hydroxide (5 minutes)
    d. DI water (10 – 15 minutes)
    e. SDS wash (see section 4.2.5.) for 15 minutes.
- The system is also flushed with 1 % v/v bleach solution (4.2.4.) once a month or if contamination is suspected.

6.1.4. While proceeding with the wash procedure (6.1.3.), type up the run. Click on Set Up → Analysis → LLTP → New Run → Tray Protocol and save the new run as "TPYYMMDDB1". The file name follows the TPYYMMDDB1 data format, where TP stands for Total Phosphorus, YY is the year, MM is the month, DD is the day of the month, and B indicates the B+L instrument B. Postscripts 1, 2 etc. are used to denote the first or second run of the day. For example, the first run on Sept. 1, 2015 would be named TP150901B1.TXT.

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.

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Type the tray number beside the PQCK1 at cup # 18 (e.g. PQCK1_TP3287), and the prep batch ID number with each MBLK sample line. Also enter the SRM name with no spaces between characters but a space for the dilution factor (e.g., for ERAQC17 with a dilution factor of 10, type ERAQC17 10). Print the run; this will be the bench sheet for the run.

An example run sequence includes:

- **Baseline**
- **Primer** - the B+L high standard (see DEP SOP NU-049, section 4.3.3.)
- **Drift -** the B+L high standard (see DEP SOP NU-049, section 4.3.3.)
- **2 BLKs**
- **Calibrants** - (see DEP SOP NU-049, section 4.3.3.)
- **High/Low/Low sequence** - to check carryover (the B+L high standard followed by two blank cups)
- **PQCK1 (PQL and MDL verification spike)_tray #** (See DEP SOP NU-049, section 4.3.3.)
- **ERAQC# dilution factor** (See DEP SOP NU-049, section 4.3.5.)
- **CCB and CHCK1** - these checks bracket every 10 cups on the run (see DEP SOP NU-049, sections 4.3.6. and 4.2.3.), and may be followed by a Drift cup and a Baseline check
- **Batched samples,** including the method blanks (MBLKs) (indicate prep batch ID)
- **Final CCV/CCB bracket with a Drift cup and a Baseline check** (see section 6.1.8.)

Note: LLTP samples are normally run initially on the AQ2 (see DEP SOP NU-090). 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. Check carefully against the backlog or prep worksheet to make sure that no samples are missed in the process. Matrix spikes and laboratory fortified blanks (LFB's) are analyzed on the AQ2.

Note: Multiple trays can be run on the same run sequence using one calibration. Indicate on the report which tray the BLK, CCV, and calibration standards came from. Identify the tray QC in the sequence. The first ALTQC should be identified as the initial calibration verification (ICV). A failing ICV renders the calibration unusable.

6.1.5. Place all reagent tubing in their respective reagent bottles.

- Sulfuric acid reagent (Orange/White tubing)
- Ascorbic acid reagent (Orange/White tubing)
- Working color reagent (Red/Red tubing)

Place the sample wash line (Green/Green tubing) in the digested carrier solution container (see DEP SOP NU-049, section 4.2.4.). Allow the reagents to run through the tubing for approximately 20-30 minutes, while adjusting the baseline every 5 minutes until the baseline is stable (5.0 % –

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5.1 %). To view the current baseline click on 'Charting' and check that the baseline is stable and that the complete system is ready. To adjust the baseline, right-click on 'Set Base' and select 'Auto Base' from the pop-up menu (normally 5 %).

6.1.6. While waiting for the baseline to stabilize (6.1.5.), pour up the run. Use the printed bench sheet to pour up the QC and samples in order. Use clean 4 mL autosampler cups and label them with QC/sample ID to avoid confusion. Rinse each cup with a small amount of sample before filling the cup and place it in the correct spot on the analysis tray. Remove the old calibration standard solutions from the standards rack on the autosampler and replace them with fresh standards.

6.1.7. After pouring up the samples and checking the baseline for stability, start the run. Click on Run → Start → LLTP → Current Run (scroll down). Double click on "Current Run" and enter the analyst's initials next to the Operator field followed by Instrument B next to Instrument. Click on "Start". Monitor the run for bad peaks (peak shape, air spikes etc.) and failing QC. Rerun any samples with air spikes or poor peaks at the end of the run.

6.1.8. When you are ready to stop the run, go to Setup → Analysis → LLTP and double click on Run → Tray Protocol. Add the label "END" at the cup number where you wish the run to stop. Make sure that all samples are bracketed by passing CCVs and blanks. There should be a Blank, CCB, CHCK1, Drift and Baseline (in that order) prior to the cup labeled "END". After the run is complete, flush the system tubing for 10 minutes with DI water or SDS wash solution (4.2.5.) as needed.

6.1.9. Turn the pump off. Release the pump's platen cover to release the pressure on the pump's tubing. The instrument is turned off in the opposite way it was turned on (6.1.1.).

6.2. Quantitation/Calculations:

6.2.1. After completion of the analysis print a summarized analysis report containing the peaks, calibration graph and values. Also print the raw data. The instrument reports analysis results in mg P/L.

6.2.2. The individual calibration standards (seven plus a calibration blank) are prepared according to section 4.3.3. in DEP SOP NU-049. 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.

6.2.3. 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 the FDEP Quality Manual (Section 5.9.).

6.6.4. Convert the excel file into Raw data in the QC Manager. The Instrument used is 'Nutrients: Bran Luebbe B'.

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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 (W-S-A-TP, W-S-A-TP-F), filename/s (TPYYMMDDB1, see section 6.1.4 for a detailed description of the file naming convention), 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 Bran+Luebbe run - analysis report, bench sheet, raw data sheet(s), 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. Bran+Luebbe 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\SFA\BL\LLTP_Data\ .

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations .

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

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

8.2. A check standard (CHCK1; 0.04 mg P/L; see DEP SOP NU-049, section 4.3.6.) and CCB (blank) are run at the beginning, every 10 samples, and at the end of each run. Acceptance limits for recovery of the CHCK1 standard are 90 - 110 %. Acceptance limits for the MBLK and CCB are > -0.002 and < 0.002 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 on the AQ2 instrument (see DEP SOP NU-090) 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 practical quantitation limit level standard, PQCK1 (0.005 mg P/L; see DEP SOP NU-049, section 4.3.3.), is analyzed on every analytical run. Acceptance limits for recovery of the PQCK1 standard are 70 – 130 %. The PQCK1 standard also acts as the MDL spike for ongoing MDL and PQL 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 recovery of the ALTQC standard are 90 – 110 %.

8.6. 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. Acceptance limits for the percent error of calibration standards above the PQL are ±10%. Acceptance limits for the percent error of the PQL level standard is ±30%. Acceptance limits for the percent error of the 0.003 mg P/L calibrant are -33 to 50%. The acceptance limits for the blank calibration point result are below the MDL and above the negative value of the MDL (-MDL < blank < MDL). Every day samples are analyzed a new calibration must be performed and verified at the beginning of the analysis. Consecutive runs on one day may use the same calibration.

8.7. Annual MDL and PQL verification is required, (see NU-043, 2.3.7). Method blank (MBLK) and PQCK1 data is collected and utilized for the ongoing MDL and PQL verification. Perform annual verifications using the LIMS statistics tracker module.

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. MANIFOLD CLEANING, MAINTENANCE

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

10.2. Flush the instrument on a regular basis as described in section 6.1.3.

10.3. Change the pump tubing every 3 weeks or more often if needed.

11. REFERENCES/DEFINITIONS

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.

11.1. EPA method 365.1, Rev. 2.0 (1993).

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11.2. Bran+Luebbe™ Analyzer Series AA3 System Operation Manual.

11.3. Bran+Luebbe method for Total Phosphorous G-146-95 Rev.3.

11.4. Quality Manual, Bureau of Laboratories, FDEP.

11.5. Laboratory Health and Safety Plan.

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

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

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

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

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

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

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

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

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

Appendix of Significant Changes

02/22/2008 Updated SOP to comply with the latest revision of method: 365.1, *Revision2.0,* August 1993.

02/23/2010 Updated section 6 of the SOP by including greater detail on the daily operation of the instrument.

02/15/2012 Removed the standard preparation section and added it to the sample digestion SOP (NU-049).

09/17/2012 The MDL and calibration range were changed. The MDL is 0.002 mg P/L. The calibration range is from 0.005 mg P/L (PQL) to 0.10 mg P/L.

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

12/20/2018 Ascorbic acid reagent preparation description was corrected (4.3.2.). MDL (blank) is discontinued in the run sequence. Ongoing MDL verifications are based on MBLK's and MDL verification spikes. The PQCK1 serves as the MDL and PQL verification spike. The daily wash procedure in section 6.1.3. was modified.

12/10/2019 Section 6 was modified to clarify the procedure for running multiple trays on one run. Section 8 was expanded to include the percent error acceptance limits of the calibration standards. In Section 12 technical support information was removed.

02/13/2020 Added acceptance limits for the calibration points below PQL.

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