TOTAL PHOSPHORUS (TP) AUTOCLAVE SAMPLE PREPARATION

1. SCOPE AND APPLICATION

1.1. This method details the preparation of aqueous samples for total phosphorus (TP) determination.

1.2. All other required information about the analysis, such as method detection limits (MDLs), interferences, quality control, data assessments and acceptance criteria for quality control measures, can be found in DEP SOPs NU-082 and NU-090.

2. SUMMARY OF THE METHOD

2.1. This method is based on EPA method 365.1, Rev. 2.0 (1993).

2.2. Both organic and inorganic phosphorus in a sample are converted to orthophosphate through a digestion with sulfuric acid and ammonium persulfate. The total phosphorus is then analyzed as orthophosphate by a colorimetric method (see DEP SOPs NU-082 and NU-090). For a discussion of interferences refer to DEP SOPs NU-082 and NU-090.

3. EQUIPMENT AND SUPPLIES

3.1 3870 ELB Tuttnauer Autoclave or Consolidated Sterilizer Autoclave

3.2. 25 mL graduated autoclave tubes with trays

3.3. Top loading balance and analytical balance

3.4. 2 L volumetric flask (Class A)

3.5. 100 mL and 50 mL graduated cylinders (Class A)

3.6. Eppendorf pipette with tips

3.7. Rainin pipette with tips

3.8. Weighing boat

3.9. 50 mL beakers

3.10. 2 inch stir bars

3.11. Optional: MadgeTech Temperature Data Logger, Model *Temp1000S* with USB Interface cable.

3.12 ChamberBrite- powdered autoclave cleaner

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

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 prep worksheet in LIMS. The unique ID and expiration date of the carrier solution (4.2.4) is documented in the analysis folder (see DEP SOP NU-082). If available, a Certificate of Analysis for each purchased reagent, standard or chemical is also maintained in the REAGENT_PREP folder on the Nutrients

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drive at \\FLDEP1\DEAR\labs\Lab3\Chem_Data\Inorganic\Nutrients\. All containers holding standards or reagents must be labeled with their unique IDs and expiration date.

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

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. Reagent Grade Concentrated Sulfuric Acid

4.1.2. Ammonium Persulfate

4.1.3. Potassium Dihydrogen Phosphate

4.1.4. Glycine

4.1.5. β-Glycerophosphoric Acid, Disodium Salt, Pentahydrate

4.1.6. Standard Reference Material (ALTQC)

4.2. Reagents:

4.2.1. **Sulfuric Acid Digestion Solution.**

CAUTION! The mixing of concentrated sulfuric acid with water generates much heat and can result in serious injury if not performed carefully. The dilution needs to be done under a fume hood with proper protective equipment (eye protection, gloves, lab coat). The concentrated acid should be added to water slowly with cooling between each addition.

Pour about 1.2 L deionized (DI) water into a 2 L beaker under the fume hood. Slowly and carefully add 620 mL of concentrated sulfuric acid (4.1.1.). Leave under the fume hood to allow the solution to cool down to room temperature. Once at room temperature, transfer the solution to a 2 L volumetric flask, dilute to volume with DI water, cap, and invert several times until the solution is thoroughly mixed. Transfer into a clean 2 L Nalgene bottle and close with the pump dispenser. Label the bottle. Digestion solution holding time 90 days.

4.2.2. **Ammonium Persulfate Solution**. Dissolve 30 g ammonium persulfate (4.1.2.) in 60 mL water; mix well until all the persulfate has dissolved. Prepare volume of reagent as needed. Do not change the persulfate to water ratio. This solution is prepared daily.

4.2.3. **Diluent Solution, 0.2 % H_2SO_4** (to be digested and used for method blanks (MBLKs), laboratory fortified blanks (LFBs), blanks (MDLs, continuing calibrations blanks (CCBs)), and sample dilutions) - Add 4 mL of reagent grade sulfuric acid (4.1.1.) to a 2 L volumetric flask containing 1 L of DI water. Dilute to volume with DI water, cap and invert several

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times to mix. Make a total of 10 L and combine in a Nalgene carboy with mixing. Label the carboy. Holding time, 30 days.

Note: To clean the 10 L carboy add 500 mL of 50 % hydrochloric acid and fill to the top with DI water. Let stand for 15 min and drain through the spigot. Refill the carboy 3 times with DI water and drain it the same way again each time. Finally, let DI water run through the spigot for about 10 min**.**

4.2.4. **Carrier Solution** (used for Bran & Luebbe (B&L) analyses only). Add 40 mL of ammonium persulfate digestion solution (4.2.2.) and 40 mL of sulfuric acid digestion solution (4.2.1.) to a 2 L volumetric flask containing 1 L of DI water. Make up to volume with DI water and mix thoroughly. Transfer the solution to a 2 L Nalgene bottle, cap loosely, and autoclave. Add carrier solution to the digestion sheet. Extra labels with the unique ID and expiration date may be printed for documentation in the analysis folder. Holding time, 30 days.

4.3. Standards and Spiking Solutions:

4.3.1. **TP/orthophosphate stock standard 1000 mg P/L.** To a 1 L volumetric flask containing approx. 400 mL DI water, add 4.394 g anhydrous potassium dihydrogen phosphate (4.1.3.) which has been dried at 105 ^{o}C overnight. Dilute to the mark and invert 12 times. Refrigerate. Holding time, 180 days.

4.3.2. **TP/orthophosphate 10 mg P/L standard.** Into a 250 mL volumetric flask containing approx. 100 mL DI water, pipette 2.5 mL of the 1000 mg P/L stock standard. Bring to volume. Refrigerate. Holding time, 30 days.

4.3.3. **TP working calibration standards.** Prepare standards by pipetting the amounts of the 10 mg P/L working standard (4.3.2.) specified in the table below into 500 mL volumetric flasks. Add 1 mL conc. sulfuric acid (4.1.1.) to each flask and make up to volume with DI water. This makes the acid content of the standards the same as that of the preserved samples (0.2 %). The following standard solutions must be digested with each tray of samples. Holding Time, 30 days.

Std ID (Final volume: 500 mL)	Conc. (mg P/L)	Volume of 10 mg P/L (4.3.2.) (mL)	Comments
BL CAL2	0.003	0.15	
BL CAL3	0.005	0.25	B&L PQCK1
BL CAL4	0.008	0.4	
BL CAL5	0.01	0.5	
BL CAL6	0.02	1.0	
BL CAL7	0.05	2.5	AQ2 PQCK1
BL CAL8	0.10	5.0	(B+L high standard)
AQ2 CAL STD	1.0	50.0	(AQ2 high standard)

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4.3.4. **TKN/TP Spiking Stock Solution (TKN - 150 mg N/L, TP - 50 mg P/L).** Dry approx. 1.1 g glycine (4.1.4.) overnight at 60 ºC. Add 0.804 g glycine and 0.4941 g β-glycerophosphoric acid (4.1.5.) to a 1 L volumetric flask containing approx. 400 mL DI water. Bring to volume with DI water. Invert 12 times. This solution is used to prepare LFBs, matrix spikes (MSs), and matrix spike duplicates (MSDs). This solution is also used for the analysis of TP and TKN in soils (DEP SOPs NU-084 and NU-081, respectively), and TKN in water (DEP SOPs NU-092 and NU-098). Refrigerate. Holding time, 180 days.

4.3.5. **ALTQC Standard (ERAQC#).** The quality control standard (4.1.6.) is prepared from the ampule according to the manufacturer's instructions. The supplier (Environmental Resource Associates) differs from the supplier of the calibration standards. The stock ALTQC standard may be diluted appropriately so that the concentration falls within the calibration range. For the concentration and expiration date of the ampule please see the manufacturer's certificate.

Holding time stock standard, 90 days.

Holding time working standard, 30 days.

4.3.6. **Working Check Standards.** For the Seal AQ2 instrument, the check standard (CCV) concentration is 0.40 mg P/L - In a 500 mL volumetric flask add approx. 250 mL of DI water and 1 mL conc. H_2SO_4 (4.1.1.). Pipette 20 mL of the 10 mg P/L TP/PO4 standard (4.3.2.) and invert 12 times. For the B&L instrument, the check standard (CHCK1) concentration is 0.04 mg P/L - In a 500 mL volumetric flask add approx. 250 mL of DI water and 1 mL conc. H_2SO_4 (4.1.1.). Pipette 2 mL of the 10 mg P/L TP/PO4 standard (4.3.2.) and invert 12 times. Holding time, 30 days.

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

5.1. Sample preservation and holding times: Samples are preserved with 0.2 % sulfuric acid at collection. They are stored refrigerated (≤ 6 ºC, not frozen) in the custody room C301. The samples have a 28-day holding time.

5.2. Fresh and ground water samples may be batched together. Effluent samples are batched separately. A batch may contain up to 10 samples.

5.3. All Samples having conductivities above 5000 µmhos/cm (equivalent to about 3.0 ppth salinity) are batched separately. They are diluted two-fold prior to digestion unless notified otherwise by a supervisor. (Check the conductivity of the samples in your backlog. If a sample conductivity exceeds 5000 umhos/cm or salinity of 3 PPTh, it should be digested in a salt batch even if it is not labeled as a salt matrix.)

5.4. Samples with TP concentrations above 5 mg P/L must be prediluted to a concentration of less than 5 mg P/L and re-digested.

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5.5. Log the samples out following the proper protocol to preserve chain of custody and allow samples to come to room temperature before digestion. Arrange samples in batch order.

5.6. Remove the ALTQC, the spiking solution and the calibration standards from the refrigerator and allow them to come to room temperature.

5.7. Select clean tubes for the digestion (see DEP SOP NU-046 for glassware cleaning). Print the tube labels on a Sato printer using the Prep Worksheet Manager module in LIMS. Label tubes accordingly and rinse once with DI water.

5.8. Shake each sample and rinse tube with ~ 3 mL of sample then pour 25 mL of sample into the respective tubes (pour sample to the mark if using calibrated glass tubes, otherwise use a pipet). MBLKs, LFBs, CHCK, ALTQC and calibration standards are prepared the same way. Four extra tubes with diluent solution are also prepared as blanks. These are used as CCBs. If a sample needs to be diluted, use the following table for dilutions made directly in the tube.

Dilution	Pipette Sample Volume (mL)	Pipette Diluent Volume (mL)	Total Volume in Tube (mL)
1:2	12.5	12.5	25
1:5	5.00	20.0	25
1:10	2.50	22.5	25
1:20	1.25	23.75	25
1:25	1.00	24.0	25
1:50	0.50	24.5	25

Dilutions that are 100 times or greater must be made via volumetric flask, then added to the tube. Add a note in the sample comments section on the digestion worksheet with the exact dilution made in the volumetric flask.

5.9. Spiking procedure - The spike level is 0.4 mg P/L. LFBs and MS's are prepared by adding 200 µL of 50 mg P/L spiking solution (4.3.4.) to a 25 mL aliquot of sample for MS's or to a 25 mL aliquot of diluent solution (4.2.3.) for LFBs.

5.10. Add 500 µL of ammonium persulfate solution (4.2.2.) and 500 µL of the sulfuric acid digestion solution (4.2.1.) to each tube.

5.11. Cap the digestion tubes loosely and place the tubes in the autoclave. Label each tray with the tray ID.

5.12. The Tuttnauer autoclave reservoir must be fully drained and refilled with fresh DI water weekly. Add fresh DI water to the autoclave daily if the level on the dipstick is low.

5.13. One spot in the tray is chosen at random to place tubes with 25 ml of water containing the Tuttnauer autoclave temperature probes. Alternatively, MadgeTech data loggers may be used. One spot in the tray is chosen at random to place the data logger (3.11.). The temperature probes (or data logger device) are used for

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temperature recording during the autoclave process. The temperature probes record temperature readings throughout the entire cycle.

The data logger program is started in advance using the MadgeTech4 software, as follows:

5.13.1. Plug data logger into the USB cable at the PC with the MadgeTech4 software installed.

5.13.2. 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.

5.13.3. In the Device tab menu bar, click Quick Start (this will use the most recent device settings, visible in the Connected Devices panel). When the data logger has been properly started, the status will read "Running".

5.13.4. Unplug the data logger from the USB interface cable, and place it in one spot of the autoclave sample tray.

5.14. Press and hold door closed for 10 seconds. The autoclave will lock securely. An error message will appear if closed incorrectly. Select the "TP digestion" cycle. This cycle is preset to 121^{o} C for 45 minutes.

5.15. Place the ALTQC, spiking and calibration standard solutions back into the refrigerator. Return samples to receiving room.

5.16. Once the digestion is complete, remove the tubes from the autoclave and allow them to cool to room temperature.

Note: The Tuttnauer autoclave will not open if the temperature is above 85 ^{o} C or if the pressure gauge is above zero! Autoclave contains steam under pressure. Release of pressurized steam could cause injury if autoclave opened too soon.

Note: The Consolidated Sterilizer Autoclave may serve as a backup autoclave. The autoclave must be turned on and the jacket pressure must reach 20 psi. The chamber can then be opened and trays inserted. MadgeTech data loggers must be used to collect temperature readings throughout the cycle. Close the chamber door securely. Adjust temperature and time if needed. The cycle should be 121^{o} C for 45 minutes. Press the start button. The cycle time begins once the Chamber pressure reaches 20 psi. The autoclave will beep when the cycle is complete. Press the start button to stop. Open the chamber and remove trays. Close the chamber securely and turn the autoclave off.

5.17. Once the cycle is completed, press the up and down arrow simultaneously to access the data transfer menu. Insert a flash drive into the USB port located under the printer. Select "transfer last 10 cycles". Once download is complete, remove the flash drive and open the report on the PC.

If utilizing the data logger: Remove the data logger from the tray, and repeat steps 5.13.1 and 5.13.2., above. Click "Stop" in the Device tab. Click "Download" to open the temperature report. Close the MadgeTech4 software, and recharge the data logger battery if necessary.

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Use the worksheet ID/tray number (see section 5.18.) to name either temperature file. Save the report at \\FLDEP1\DEAR\labs\Lab5\Chemistry\InorganicPrep\WRKSHTS\Templates\CURRENT\Nutrients\TP\Temperature Log . Print the report for the analyst to include with the run folder. Check carefully to make sure 120 ºC was reached and maintained for 30 min. Acknowledge the acceptability with a check mark (✓) and initials, and include the temperature report in the analysis folder.

5.18. Create a new prep worksheet in the Prep Worksheet Manager module in LIMS (this step may be done before or during the sample preparation process):

5.18.1. 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 "365.1 (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.

5.18.2. 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-049), prep analyst name, prep start date/time and prep completion date/time, equipment ID (e.g., Tuttnauer 1)), and the spike standard solution serial number and reagent serial numbers (ensure that the corresponding identities and expiration dates are correct).

5.18.3. 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 '_L3_' 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.

5.19.4. If a sample is re-digested for confirmation the bottle's label must be checked against the field label. Add a comment in the worksheet confirming that the label was checked.

5.18.5. Once the information in the worksheet is complete and correct (see sections 5.3. and 5.8. regarding dilution factors and sample volumes), ask a supervisor to review the worksheet. 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 for convenience when setting up the analysis.

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6. SAMPLE ANALYSIS/QUANTITATION

See DEP SOPs NU-082 and NU-090 for the sample analysis procedure and quantitation information.

7. QUALITY CONTROL/ DATA ANALYSIS (SEE DEP SOPS NU-038 AND NU-043)

Note: For method performance and for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to the following DEP SOPS; NU-038, NU-043, NU-082, and NU-090.

7.1. One digested PQL for analysis on the B&L and one for the AQ2 per tray.

7.2. One digested ALTQC per tray for analysis on the AQ2 and B&L (diluted into the calibration range on the analytical run).

7.3. One digested LFB per batch of 10 samples or less spiked at 0.40 mg P/L.

7.4. One method blank (MBLK) per batch, and 4 extra tubes with diluent solution (4.2.3.) digested for CCBs, blank standards, and sample dilutions.

7.5. One sample spiked per batch at 0.40 mg P/L. This spiked sample is digested in duplicate or a sample duplicate is digested to obtain precision for the batch.

7.6. One digested check standard (CCV) for the AQ2 at 0.40 mg P/L, and one for the B&L at 0.04 mg P/L.

8. Autoclave Maintenance

8.1. **After each digestion**. Clean the autoclave by wiping the inside of the chamber. Check for and remove any debris that may be left on the autoclave walls or on the trays. Record in the maintenance log.

8.2 **Monthly**. The autoclave should be cleaned using the ChamberBrite monthly. Sprinkle the powder inside the chamber,and run a “Sterilization cycle”. Completely drain the reservoir and refill with DI water after the sterilization cycle. Run another sterilization cycle, and repeat the draining and refilling of the reservoir.

8.2. **Additional maintenance and logbook entries**. Record PMs, repairs, or phone troubleshooting performed by the manufacturer in the maintenance log. If the autoclave was used for purposes other than TP digestion, the description should also be recorded in the maintenance log.

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.

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9.4. Neutralize the sulfuric acid digestion solution (4.2.1.) using sodium bicarbonate before disposal through the sink.

9.5. Residual concentrated acid must be neutralized using sodium bicarbonate before disposal in the sink.

9.5. Any sample waste, Ammonium persulfate reagent waste, and diluent waste can be discarded through the sink with plenty of water.

10. 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.

10.1. EPA METHOD 365.1, Rev. 2.0 (1993).

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

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

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

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

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

10.7. DEP SOP NU-046, Procedure for TKN/TP/LLTP Glassware Cleaning.

10.8. DEP SOP NU-101, Determination of Total Kjeldahl Nitrogen in Soils Using the Seal AQ2 Discrete Analyzer.

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

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

10.11. DEP SOP NU-090, Determination of Total Phosphorus in Water 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 NU-098, Determination of Total Kjeldahl Nitrogen in Water by Gas Diffusion Segmented Flow Analysis.

10.14. Quality Manual, Bureau of Laboratories, DEP.

10.15. Laboratory Health and Safety Plan.

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Appendix of Significant Changes

02/25/2008 Updated SOP to comply with the latest revision of method: 365.1, *Revision 2.0*, August 1993.

02/10/2010 Changes were made in section 5 describing the use of an electronic data logger to collect temperature data from the autoclave.

02/03/2012 A description of the preparation of standard and spiking solutions was added. A new section was also added describing how to complete prep worksheets in LIMS and digestion sheets for each digestion procedure.

09/20/2012 The range for TP analysis on the Bran & Luebbe instrument was changed to 0.005-0.1 mg P/L. Two spike levels are now used per batch for LFBs and matrix spikes: 0.04 mg P/L and 0.4 mg P/L. The check standard was changed to 0.04 mg P/L for the TP analysis on the Bran & Luebbe instrument, and 0.4 mg P/L for TP analysis on the Seal AQ2 instrument.

02/06/2013 One spike level is prepared for the LFB and matrix spikes at 0.40 mg P/L per preparation batch. These are analyzed on the AQ2.

08/12/2014 Implement use of the electronic preparation worksheet (LIMS module). See section 5 for new instructions.

08/20/2015 Documentation of the digestion reagents in the LIMS prep worksheet was added to section 4.

12/13/2017 Documentation of the carrier solution in the LIMS prep worksheet was added to section 4. Preparation instructions for sample dilutions were added to section 5.

12/18/2018 Added a section for autoclave maintenance (section 8). Added discard details for reagents and samples to the waste management section (section 9). Added requirement to document bottle label checks in the digestion sheet (5.19.4). Section 4 and 5 were edited for clarity. Updated the reference for the Determination of TKN in soils using the AQ2 Discrete Analyzer.

12/12/2019 Updated sections 3, 5, and 8 to include instructions for use and maintenance of the 3870 ELB Tuttnauer Autoclave and Consolidated Sterilizer Autoclave.

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