Arikaree Environmental Laboratory
Revision 1
Date: Aug. 28, 2018

TP/TDP by FIA Acid Persulfate Digestion SOP

ANALYTES: Total Phosphorus/Total Dissolved Phosphorus

1) Applicable Matrices

1) This method is applicable to ground, surface and waste waters**.**

2) Scope and Application

1) This method covers the determination of Total Phosphorus, or if the sample is filtered through a 0.45µm membrane filter, Total Dissolved Phosphorus.

2) The method is applicable in the range from 2 ppb P to 100 ppb P.

3) Interferences

1) Silica forms a pale blue complex which also absorbs light at 880nm. Since a silicate concentration of ~30 mg/L is needed to produce a 5 ppb P positive error the interference is deemed insignificant.

2) Ferric iron concentrations greater than 50 ppm cause negative error, as the iron causes orthophosphate to precipitate. Samples with high iron can be pretreated with bisulfite will eliminate this source of error.

3) Glassware contamination is a concern with low level P analysis. Glassware should be washed with 10% HCl and triple rinsed the ultrapure water (ASTM Type I). Do not used detergents to clean glassware as most contain phosphate.

4) Equipment and Supplies

1) Analytical balance capable with 0.0001g sensitivity.

2) Top loading balance with 0.01g sensitivity.

3) 1-10mL, 20-200µL, and 200-1000µL adjustable pipettes with corresponding pipette tips.

4) Repeating Pipette with 5 mL tips.

5) Class A volumetric flasks and pipets for reagent and standard preparation.

6) Lachat 8500 Autoanalyzer with orthophosphate manifold and 880 nm filter.

7) Aries Gemini High Purity water system for preparation of ASTM Type I water AKA “ultrapure.”

8) 16x125mm glass culture tubes

5) Reagents and Standards

5.56M Sulfuric Acid

1.) In a 1000mL volumetric flask with a stir bar, add ~500mL of ultrapure water. Place a tub of cold water on a stir plate. Place flask into water. Turn on stir plate to a reasonable speed. Measure 311mL of concentrated sulfuric acid (18M) in a graduated cylinder. Pour acid into flask slowly**. Flask contents will get hot!** Allow to stir and cool. When water in tub around flask gets warm, remove the flask carefully, discard water in tub, and refill with cold water. Once the acid mixture cools to room temp, remove flask from water and dilute to the 1L mark. Invert to mix. Store in a glass 1L flask in acid cabinet.

Digestion Solution

1.) In a 100mL volumetric flask, dissolve 12.8g ammonium persulfate, along with 32mL of 5.6M sulfuric acid. Dilute to 100mL mark with ultrapure water. Prepare fresh each day samples are digested.

Stock Ammonium Molybdate Solution

1.) In a 1000mL volumetric flask with a stir bar, dissolve 40.0g ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in ~500mL of ultrapure water. Remove the stir bar, dilute to the mark and invert to mix. Store in 1L Nalgene container, record date prepared and refrigerate. Stable for 6 months.

Stock Antimony Potassium Tartrate Solution

1.) In a 1000mL volumetric flask with a stir bar, dissolve 3.0g antimony potassium tartrate $K(SbO)C_4H_4O_6\cdot 1/2H_2O$ in ~500mL of ultrapure water. Remove the stir bar, dilute to the mark and invert to mix. Store in a brown 1L Nalgene container and refrigerate. Stable for 6 months.

Molybdate Color Reagent

1.) In a 1000mL volumetric flask, add ~ 500mL of ultrapure water. Add 21mL of concentrated sulfuric acid. The solution will get warm. Be sure it can be handled before moving on.

2.) Using a graduated cylinder, add 72mL of the antimony potassium tartrate stock solution.

3.) Then, also with a graduated cylinder, add 213mL of ammonium molybdate stock solution. Dilute to the mark and invert to mix. Pour solution carefully into its marked container. Prepare weekly.

Ascorbic Acid Reducing Solution

1.) In a 1000mL volumetric flask, dissolve 60.0g ascorbic acid. Dilute to the mark and invert to mix. Transfer solution into bottle. Degas solution for 5 minutes with helium. Add 0.5g sodium lauryl sulfate (dodecyl sulfate) to solution. Discard after 1 week. Discard if yellow color develops

1000ppm P Stock Standard

1.) Dry ~5g of anhydrous potassium phosphate monobasic primary standard at 105°C for 1 hour.

2.) In a class A 1000mL volumetric flask, dissolve precisely 4.3936g of the now dry anhydrous potassium phosphate monobasic primary standard. Do not use a stir bar in the flask. Swirl the flask by hand to dissolve the standard. After the solid has dissolved, pipet 1mL of concentrated sulfuric acid for preservation. Dilute carefully to the mark, and invert to mix. Store in a 1L Nalgene bottle.

10 ppm P Working Standard

1.) In a class A 500mL volumetric flask, dilute 5.00mL, using a class A volumetric pipet, of the 1000ppm stock standard. Dilute carefully to the mark, and invert to mix. Preserve with 0.5mL sulfuric acid.

0.8mg/L P Matrix Spike Solution

1.) In a class A 100mL volumetric flask, dilute 8.00mL of the 10ppm P working standard using a calibrated, adjustable pipet. Preserve with 0.1mL of concentrated sulfuric acid. Dilute to 100mL mark, cap, and invert. Store in a 125mL Nalgene. Refrigerate.

6) Sample Collection, Preservation, Shipment, and Storage

1) Refrigerate samples and analyze as soon as possible after collection. Preserve (<pH 2) samples with sulfuric acid if analyzing within 28 days. If samples will be stored long term (>1 month), store the samples frozen.

7) Quality Control

1) Calibrate instrument before each analytical run. Analyze Initial Calibration Verification Standard (ICVS) from a second source standard after calibration. Calibration r-value is required to be > 0.995, if not re-calibrate.

2) Run a blank (ICVB) at the beginning of each run and then once every 20 samples. ICVB must be <LOD. If not, re-analyze, if still out of control qualify data. Subsequent blanks (CCVB) must be <LOD, or less than ten percent of the measured concentration in the samples from the adjacent set.

3) Analyze a Continuing Calibration Verification Standard (CCVS) with every 20 samples.

4) Analyze PT sample once per year.

5) Analyze and calculate MDL at least once per year using EPA method. Analyze MDL spikes quarterly to check for MDL changes.

8) Calibration

1) Prepare TP/TDP calibration standards as listed below using the 10ppm P working standard, class A volumetric pipets and class A volumetric flasks. Preserve all standards with conc. sulfuric acid (1mL/1000mL, 0.5mL/500mL).

Standard	P Concentration (mg/L)	mL 10mg/L	Total volume
A	0.100	5.00	500mL
B	0.050	5.00	1000mL
C	0.020	2.00	1000mL
D	0.005	50.00mL of B Std	500mL
E	0.002	50.00mL of C Std	500mL

9) Sample Set-up and Digestion

1) Locate and organize the samples, calibration standards and instrument performance checks/quality control standards (IPC/QCS) to be digested and analyzed. Organize the samples by placing them in numerical order according to sample set.

2) Once the samples are organized, begin filling out a TP/TDP benchsheet. These sheets are located on the Arikaree Share drive. Print a few if needed. Fill in the date, and analyst information. Decide how many calibration standards, check standards, and IPC/QCS you will need and record them in the sample ID column. Write the sample names/numbers in the sample ID column. Make sure there are enough clean digestion vials for the amount of standards and samples to be digested.

3) Every 20 samples include a duplicate and matrix spike. In the appropriate column on the digestion prep sheet, record the volume of sample used. In addition, record the volume of matrix spike added to the sample in the standards concentrations column. For this analysis, it will be 0.1mL of 0.8mg/L P for the matrix spike in 8.0mL of sample.

4) Once all of the standard/sample information has been recorded on the prep sheet, the pipettes shall be calibrated. Calibrate the 1-10mL pipette to 8.0mL, the 200µL-1000µL pipette to 1000µL, and the repeating pipette set at 5 using a 5mL pipette tip (0.5mL) by pipetting ultrapure water @ 20°C at the volume specified. Record the masses of the water dispensed on the top of the prep sheet. Use the Pipette Calibration Table located on the wall next to the analytical balance to compare the dispensed water masses to the ±0.5% control limits. The pipette must dispense 3 consecutive aliquots within the control limits before it can be used for analytical setup.

5) Using the repeating pipette, dispense 0.5mL of digestion solution into each digestion tube. The syringe tip will have to be refilled multiple times depending on how many standards and samples are being prepared.

6) Begin dispensing standards and samples into digestion tubes, keeping track of what sample is in which tube number. If the numbers do become out of order, try to back-track and figure out where it went wrong.

7) Once all the tubes are filled with samples and digestion solution, cap the tubes tightly, and invert each tube to mix. Then, loosen the cap of each tube slightly. Conversely, each tube can be mixed after the sample has been pipetted. Just be sure the caps are loose after mixing.

8) Place rack of filled tubes into autoclave. Make sure autoclave reservoir is full of water. If not, fill with deionized water up to the bottom of the relief valve. Turn the bottom knob on the control panel to "FILL WATER." This will fill the chamber with water from the reservoir. Allow the water to flow into the chamber until it reaches the line at the front of the chamber. Turn the knob from "FILLWATER" to "STE". Close the autoclave door and tighten the handle until it is very tight. Turn the autoclave switch to "ON" and turn the timer knob to 50 min.

9) After the autoclave cycle is completed, allow the chamber to cool below 90°C to avoid the samples boiling over in the tubes. Open the chamber and, using thermally protective gloves, remove the rack from the autoclave. Place the rack on the bench and allow the samples to cool to room temperature.

10) After the samples have cooled, tighten the caps. Refrigerate the samples if they will not be analyze immediately.

11) Before analysis on the Lachat 8500, the samples must be poured into plastic test tubes and placed into their correct cup# in the autosampler rack. Use the benchsheet to ensure each sample ends up in the correct cup.

10) Procedure for Lachat 8500 operation

1) Turn on the Lachat autoanalyzer by moving the switch on the power strip behind the autosampler to the on position. The instrument will go through its normal startup routine (autosampler probe moves, switching valves on the instrument turn, etc.).

2) Place reagent tubing into water and start the peristaltic pump. Clamp each pump tube clamp onto the rollers. Push the tensioner cam lever back one click.

3) Hit the space bar on the computer keyboard to "wake-up" the computer. Double-click on the Omnion icon on the desktop.

4) Click the open button on the top toolbar. Find the correct template for the TDP analysis and double-click to open.

5) Make sure the concentrations for the IPC/QCS sample are correct by clicking on the sample in the worksheet. If the values are incorrect, change them by highlighting all of the samples in the DQM set (most likely DQM1) and clicking "clear DQM set" from the menu. Change the concentration in the "Run Properties" window in the upper right of the screen. Highlight the DQM samples again and right click. Select define DQM set. Set the frequency to once.

6) Start inputting sample names/numbers into the worksheet. To add more spaces for samples, highlight a row and right click. Select “Insert many” and input how many rows to add. Remember to hit “enter’ after each sample is input. Use the benchsheet to enter the sample identifiers. To enter multiple samples quickly, select multiple rows and right click. Go to “Columns” and select “Auto SampleID.” Enter the part of the sample identifier that will not change in the “Fixed Part of Sample ID” then the number or letter to start. Click “Accept.” It is helpful if the number of rows highlighted matches the number of samples to be input automatically.

7) Once finished, highlight the entire worksheet and right click. Go to “Columns” and select “Auto-number cups.” Make sure the number of cups in the worksheet matches what is on the benchsheet. If the numbers do not match, check the duplicates and matrix spikes to see if they match. Once you find a matching duplicate and spike set, you will know where the discrepancy is located.

8) Remove the reagent lines from the water and begin placing them in their corresponding reagent bottles. Allow reagents to flow through manifold for ~10 minutes.

9) Click on the “Configuration” menu at the top of the Omnion window. Go to “Autosamplers” and click the button “Intialize Autosampler.” This will put the sampler probe into the wash well to begin drawing water through the sample line.

10) After reagent has been pumping for ~10 min. click the preview button at the top of the Omnion window. This will let you see the baseline before starting the run. The baseline is flat and the signal is where it is expected, click “Stop” next to the “Preview” button and then click “Start.”

11) During the beginning of the run, watch to make sure there is good Gaussian peak shape and the peak expection window is integrating the entire peak correctly.

12) After the calibration standards are integrated, check the calibration curve by clicking on the bottom button on the left side of the channel window. Make sure calibration coefficient is ≥0.995 and %RSD for each standard is under 10%. If either of these limits are exceeded begin by excluding peaks that may skew the curve. If this does not correct the problem, recalibrate.

13) Next, be sure the initial calibration check standards are within 10% of the expected value. It not, reanalyze the standards. If this does not correct the issue, recalibrate.

14) After everything in the beginning is in control, the rest of the run can be monitored for duplicate RPD’s and matrix spike recoveries. If these are out of control, try reanalyzing the samples. Also, a continuing calibration verification blank (CCVB) and continuing calibration verification standard will be analyzed every 20 samples. Make sure these are within 10% of known value.

(Procedure cont’d next page)

15) If everything in the run goes to plan, the instrument will stop after all of the samples in the worksheet have been analyzed. The reagent lines can be removed and placed into ultrapure water for a few minutes then into an EDTA cleaning solution to remove any phosphate deposits left in the Lachat tubing. Let this solution run through the tubing for a few minutes. Remove tubes and place back into water and allow to flush for ~10min. After the 10min., remove tubes from water and hang them over the edge of the bench, and allow air to be pumped through the manifold. Pump air until all water has been evacuated from the manifold lines.

16) Release the pump tubes on the pump and stop the pump. Turn off the Lachat by flipping the switch on the power strip behind the autosampler.

17) Samples in the tubes can be disposed by dumping the sample into the waste drum. Tubes will be cleaned and used again.

11) Corrective Action for Out-of-Control Data

a) Any CCV blank that exceeds the LOD, the analyst must inspect the concentration of the previous sample. If the blank is greater than 10% of the previous sample, reanalyze the blank immediately following the CCVS. Samples cannot be run until the blank meets requirements.

b) CCV and LCS standards must fall within 10% of true value (90%-110% Recovery). If not re-mix and re-analyze, if still out of range re-calibrate.

10) Contingencies for Handling Out-of-Control Data

a) Samples that fail the CCVB or ICVS will have to be qualified back to the last sample that the quality control met the above conditions.

11) Waste Management

a) All waste must be collected and disposed of following Colorado University EHS guidelines.

12) References

a) Lachat QuikChem Method 10-115-01-1-F. Determination of Total Phosphorus by Flow Injection Analysis Colorimetry (Acid Persulfate Digestion Method) D. Diamond revision date 14 October 1994