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| **TITLE:** | **Total Phosphorus – Persulfate Digestion and Colorimetric Ascorbic Acid Analysis** |
| **ANALYTE:** | **Total Phosphorus** |
| **FACILITY:** | **Acme WWTP** |
| **REFERENCE METHOD:** | **Standard Methods 4500-P B – 1999 and 4500-P E – 1999** |
| **DATE OF ISSUE:** | **7/18/21** |

1. Applicable Matrices
   1. This method is applicable to drinking water, surface and saline waters, and domestic and industrial wastes matrices.
2. Summary of the Test Method
   1. Phosphorus samples are digested to release phosphorus as orthophosphate. Ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.
3. Safety
   1. Proper personal protection equipment, such as a lab coat, safety glasses, and nitrile gloves should be worn when performing any laboratory test.
   2. When using acid solutions, wear appropriate protective clothing: rubber apron, protective sleeves, gloves, and safety goggles or glasses.
   3. Always have adequate ventilation when working with acids, bases, and solvents to minimize exposure to vapors.
   4. Refer to the Safety Data Sheet for information on a specific chemical.
4. Equipment and Supplies
   1. Spectrophotometer, ABC Company Model 123 for use at 880 nm, providing a light path of 2.5 cm or longer.
   2. Digestion equipment:
      1. Hot plate; or
      2. Autoclave – Capable of developing 98 to 137 kPa
   3. Class A pipets or verified mechanical pipettors
   4. Acid-rinsed glassware: Erlenmeyer flasks, graduated cylinders and volumetric flasks
   5. Glass scoop; used to hold required amount of persulfate crystals
   6. pH paper, narrow range (0 – 6.0); used to confirm pH of <2 for preservation
5. Reagents and Standards
   1. Distilled water
   2. Ammonium persulfate, (NH4)S2O8, solid, or potassium persulfate, K2S2O8, solid
   3. Antimony potassium tartrate solution. Dissolve 1.3715 g K(SbO)C4H4O6 • ½H2O in 400 mL distilled water in a 500 mL volumetric flask and dilute to volume with distilled water.
   4. Ammonium molybdate solution. Dissolve 20 g (NH4)Mo7O24 • 4H2O in 500 mL distilled water.
   5. Ascorbic acid, 0.1 M. Dissolve 1.76 g ascorbic acid in 100 mL distilled water. This solution is stable for about 1 week at 4°C.
   6. Combined reagent. Let all reagents reach room temperature before they are mixed, and mix in the following order: 50 mL of 5 N H2SO4, 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. Mix after addition of each reagent. This reagent is stable for 4 hours.
   7. Phenolphthalein indicator aqueous solution
   8. Phosphate stock standard, 5 mg/L as P. This solution expires according to manufacturer’s expiration date. Note: use standards “as P” instead of “as PO4.”
   9. ICV (initial calibration verification standard), second source phosphate stock standard, 50 mg/L as P. This solution expires according to manufacturer’s expiration date.
   10. CCV (continuing calibration verification standard)/LCS (lab control standard) phosphate solution, 0.5 mg/L P. Add 5.0 mL of 5 mg/L phosphate stock standard (5.3) to a 50 mL volumetric flask using a pipette, and bring to volume with distilled water.
   11. Sulfuric acid solution, 5 N
   12. Sodium hydroxide solution, 1 N
   13. Proficiency Testing samples from an approved provider
6. Interferences
   1. Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg/L As interfere with the phosphate determination. Hexavalent chromium and NO2- interfere to give results about 3% low at concentrations of 1 mg/L and 10% to 15% low at 10 mg/L. Sulfide (Na2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.
7. Sample Collection, Preservation, and Storage
   1. Resistant-glass or plastic bottles may be used for sample collection. Containers should be cleaned with a non-phosphate detergent and water, and rinsed thoroughly with tap water. Bottles should then be rinsed with 1% hydrochloric acid (HCl) and followed by tap water and deionized or distilled water.
   2. If samples cannot be analyzed within 15 minutes of collection, samples must be preserved by acidifying with sulfuric acid (H2SO4) or hydrochloric acid (HCl) to a pH <2 immediately after collecting.
      1. Generally, 0.5 mL of 5 N H2SO4 per 200 mL of sample is sufficient to reduce the pH to <2.
      2. Samples must be tested periodically (at least quarterly) with narrow range pH paper (0-6 pH) to confirm the pH is <2 and documented on the bench sheet or on a separate log.
   3. Store the preserved samples at ≤6°C but above freezing.
   4. Hold times may not exceed 28 days from the time of collection.
8. Quality Control
   1. Initial Quality Control
      1. All analysts shall perform an IDC once for each lab technician. An IDC is required of all new lab technicians.
   2. Initial and On-going Quality Control
      1. Method Detection Limit (MDL): every thirteen months, calculate and verify the MDL. See the “Method Detection Limits and Reporting” section.
      2. Calibrate the spectrophotometer annually or whenever the calibration check verification (CCV) standard fails (see “Calibration and Standardization” section).
      3. Verify the calibration immediately following the calibration curve by analyzing an initial calibration verification (ICV) standard. The ICV must be from a second source standard.
      4. Annually a Proficiency Testing (PT) sample must be obtained and analyzed from one of the Wisconsin DNR approved providers.
   3. On-going Quality Control
      1. Verify the calibration on non-calibration days with each analysis and before any samples or other QC by analyzing a continuing calibration verification (CCV) standard. Also run a CCV after every batch of 20 samples.
      2. Analyze a method blank (MB) every run of 20 samples after the ICV or CCV.
      3. Analyze a laboratory control sample (LCS) with every run of 20 samples. (If the calibration standards are processed the same as samples and the CCV, the LCS will be the same as the CCV; in this case, a separate LCS does not need to be analyzed.)
9. Calibration and Standardization
   1. The ABC Company Model 123 spectrophotometer is used. Refer to the User Manual for additional information.
   2. Calibration must be performed whenever the ICV or CCV fails, after non-routine maintenance, the spectrophotometer leaves the direct control of the lab, or there is a change in expected behavior. (Pre-programmed vendor calibrations must not be used.)
   3. The linear calibration curve must be generated with a calibration blank and at least 3 standards.
   4. Prepare the calibration standards from the 5 mg/L phosphorus stock standard. Using a class A pipet, add the following amounts of 5 mg/L phosphorous stock standard into a 50-mL volumetric flask. Then fill the volumetric flask to the line with distilled water.

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| Standard Concentration (mg/L) | Volume of 5 mg/L standard (mL) | Final total volume (mL) | Calibration standard final concentration (mg/L as P) |
| 5 | 0 | 50 | 0 |
| 5 | 2 | 50 | 0.2 |
| 5 | 4 | 50 | 0.4 |
| 5 | 6 | 50 | 0.6 |
| 5 | 8 | 50 | 0.8 |
| 5 | 10 | 50 | 1.0 |

* 1. Follow the “Procedure” section for digestion and analysis of all of the calibration standards just as samples are digested and analyzed.
  2. The following are the instructions for creating a user-defined calibration curve on the Model 123 meter to calculate all sample results directly on the instrument (refer to the instrument manual for further instruction on how to use this feature):

After the timer goes off, insert the 0 standard (calibration blank) and enter the concentration (0.00), press enter, press read to get the absorbance value, and record absorbance on the bench sheet. Insert the 0.2 standard and enter the concentration (0.2), press enter, press read, and record the absorbance. Do this with each of the prepared standards. After the 1.0 standard concentration was entered, press exit, display will show “Force Zero on/off,” and select force Zero off. Press down, display will show Calibration Formula, press enter, and select formula for r2=, press enter. Press down arrow to Store Program, and press enter.

* 1. Alternatively, use the WI DNR supplied spreadsheet to record all absorbances and calculate calibration results.
  2. The calibration r-value must be ≥0.995. If not, re-calibrate.
  3. Document all of the calibration results and information on the bench sheet.

1. Procedure
   1. Warm all samples, standards, and reagents to room temperature.
   2. Label all beakers or flasks that will be needed including flasks for QC samples.
   3. Using a graduated cylinder, add 50 mL or a suitable portion of thoroughly mixed sample and QC samples to the appropriate labeled flasks.
   4. Add 1 drop phenolphthalein indicator solution. If a red color develops, add H2SO4 solution dropwise to just discharge the color.
   5. Add 1 mL H2SO4 solution.
   6. Add either 0.4 g solid ammonium persulfate or 0.5 g solid potassium persulfate. A scoop may be used to measure this amount.
   7. Hot plate method:
      1. Boil gently on a preheated hot plate for 30 – 40 minutes or until about 10 mL is left in the flask. **Do not let go to dryness.**
      2. Cool to room temperature, and dilute to 30 mL with distilled water.
      3. Add 1 drop phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH.
      4. In a volumetric flask, bring up to a final volume of 50 mL with distilled water.
   8. Autoclave method:
      1. Alternatively, heat for 30 minutes in an autoclave at 98 to 137 kPa. Refer to the User Manual for instructions on the autoclave use.
      2. Verify the pressure of the autoclave reached 98 – 127 kPa, and record on the bench sheet.
      3. Cool to room temperature, add 1 drop phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH.
      4. In a volumetric flask, bring up to a final volume of 100 mL with distilled water.
   9. Using a graduated cylinder, pipet, or volumetric flask, add 50.0 mL of the digested sample into a clean, dry Erlenmeyer flask.
   10. Add 1 drop phenolphthalein indicator. If a red color develops, add 5 N H2SO4 solution dropwise to just discharge the color.
   11. Turn on the spectrophotometer, and press the program # (make sure the wavelength is set to 880 nm).
   12. Zero the spectrophotometer with a method blank sample **without the combined reagent** (this is the instrument blank). Insert the cuvette, close the cover, and press “ZERO” to zero the meter.
   13. Add 8.0 mL combined reagent to each sample and QC sample and mix thoroughly.
   14. After at least 10 minutes but no more than 30 minutes, measure the absorbance of each sample, and record the absorbance on the bench sheet.
   15. If samples have color or turbidity, prepare a sample blank by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. Insert the cuvette, and zero the spectrophotometer (similar to zeroing with the instrument blank but with a portion of unreacted sample).
   16. If any sample has an absorbance reading higher than the highest standard in the calibration curve, the sample must be diluted and reanalyzed.
2. Calculations
   1. Results are calculated from the linear calibration curve (y = mx + b) generated by the lab. Include any applicable dilution factors. The WDNR supplied spreadsheet may be used to do all calculations.
   2. Report results as mg/L P.
3. Method Detection Limits and Reporting
   1. This method has an applicable range from the MDL (about 0.01) to about 6 mg/L P. All samples with absorbances above the absorbance of the highest calibration standard must be diluted. The lowest value that can be reported is no lower than the MDL: report as less than (<) the MDL value.
   2. The MDL must be less than the permit limit.
   3. The MDL is calculated annually by following the EPA 40 CFR Part 136 Appendix B protocol.
      1. All method blanks associated with reported results are entered into the WDNR supplied spreadsheet.
      2. Two spiked blanks are analyzed each quarter and entered into the WDNR supplied spreadsheet.
4. QC Data Assessment, Acceptance Criteria, and Corrective Actions and Contingencies for Out-of-Control QC Measures
   1. Quality control samples summary:

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| QC Test | Criteria |
| Calibration curve | r≥0.995 |
| ICV | 90 – 110% true value |
| Method Blank | ≤ Highest of:  LOD,  5% permit limit, or  10% sample conc’n |
| CCV/LCS | 90 – 110% true value |

* 1. If the calibration curve does not have an r value ≥0.995, recalibrate. Corrective actions may also include preparing new calibration standards.
  2. If the ICV is not 90-110% of the true value, re-make the ICV solution and reanalyze. If the ICV still does not pass, re-calibrate.
  3. If the method blank is not ≤LOD (or 5% of the permit limit or 10% sample concentration), reanalyze. If it is still out of range, qualify the data.
  4. If the CCV/LCS is not 90-110% of the true value, take corrective action such as re-make the CCV/LCS solution and reanalyze. If the CCV still does not pass, re-calibrate.
  5. Qualify all sample results with method blank or CCV/LCS exceedances on the bench sheet and the eDMR.
     1. Samples that fail the Quality Control will have to be qualified back to the last date that the quality control met the above conditions. Include a lab comment on the DMR.
  6. The Proficiency Testing (PT) sample must be within the criteria of the provider. If the criteria limits are not met, the technician must immediately order another sample to be analyzed.
  7. For any of the above items or if there are any other obvious errors or deviations from the standard operating conditions, complete the Corrective Actions Log and resolve the problem. Notify the Supervisor.
     1. If results are unacceptable, take appropriate corrective action. This may include acid washing all containers, checking the water source, checking expiration dates, and documenting any changes or adjustments made.
     2. Complete the Corrective Actions in the log sheets. Enter in all information as completely as possible, even if the short-term reasons for failures are not clear.
     3. Seek help from an outside source if specific QC issues cannot be resolved. These sources may be another lab, the Wisconsin Rural Water Association wastewater trainer, or the facility’s lab auditor.

1. Pollution Prevention
   1. Consider environmental impact when purchasing materials, handling chemicals, and disposing of wastes.
   2. Prevent pollution at the source whenever possible.
2. Waste Management
   1. All laboratory waste, excess reagents, and samples must be disposed of in a manner that is consistent with applicable rules and regulations.
3. References
   1. Standard Methods for the Examination of Water and Wastewater, Method 4500-P, 1999.
4. Disclaimer:
   1. The mentioning of company or product names does not constitute endorsement by the Wisconsin Department of Natural Resources or the authors.