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| **TITLE:** | **Hach Method 8190 – PhosVer 3TM Spectrophotometric Measurement of Total P** |
| **ANALYTE:** | **Total Phosphorus** |
| **FACILITY:** | **Acme WWTP** |
| **REFERENCE METHOD:** | **Standard Methods 4500-P E – 1999** |
| **DATE OF ISSUE:** | **7/18/18** |

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. Phosphates are converted to reactive orthophosphate. Orthophosphates react with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. The color is proportional to the phosphorus concentration and is measured at 650 for colorimeters or 880 for spectrophotometers.
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
   2. COD Reactor block capable of heating to 150°C
   3. Class A pipets or verified mechanical pipettors
   4. Acid-rinsed glassware: graduated cylinders and 50 mL volumetric flasks
   5. Funnel, micro
   6. Test tube rack
   7. pH paper, narrow range (0 – 6.0); used to confirm pH of <2 for preservation
   8. pH paper, narrow range (5.5 – 8.0); used to confirm preserved samples are neutralized to 6.0 to 8.0
5. Reagents and Standards
   1. Distilled water
   2. Hach Company Total Phosphorus Test ‘N Tube Reagent Set, Cat. No. 274645
      1. Cuvette test tubes
      2. PhosVer 3TM Phosphate Reagent Pillows
      3. Potassium persulfate powder pillows
      4. Sodium hydroxide standard solution, 1.54 N
      5. Sulfuric acid standard solution, 1.54 N
   3. 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.”
   4. ICV (initial calibration verification standard), second source phosphate stock standard, 50 mg/L as P. This solution expires according to manufacturer’s expiration date.
   5. 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.
   6. Sulfuric acid solution, 5 N (for preserving samples)
   7. Sodium hydroxide solution, 5 N (for neutralizing preserved samples)
   8. Proficiency Testing samples an approved provider
6. Interferences
   1. There are no interferences caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in seawater. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.
   2. The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.
   3. Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in seawater, it does not interfere.
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) 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. Note: if a batch of 20 samples contains two different lot numbers of vials, run a method blank with each lot number.
      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.) Note: if a batch of 20 samples contains two different lot numbers of vials, run a CCV/LCS with each lot number.
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. (The pre-programmed vendor calibration 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 digesting all of the calibration standards just as samples are digested.
  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. Turn on the COD reactor block and heat to 150°C. Refer to the User Manual for instructions on temperature programs or temperature adjustments.
   2. If the samples were preserved with acid, neutralize to a pH of 6 - 8 with 5 N sodium hydroxide solution. Use either a pH meter or pH paper.
      1. The amount of 5 N sodium hydroxide will be about the same as the amount of 5 N sulfuric acid used to preserve the sample.
      2. Correct the test result for the amount of acid and base used if the addition represents more than 2% of the total sample volume.
   3. Warm all samples and reagents to room temperature.
   4. Label all vials that will be needed including vials for QC samples.
   5. Pipet 5 mL of samples and QC samples to the appropriate labeled vials.
   6. Add one potassium persulfate powder pillow to each vial.
   7. Cap vials and shake to mix.
   8. Verify the temperature of the COD reactor reached 150°C, and record the temperature on the bench sheet.
   9. Set a timer and heat the vials for 30 minutes in the COD reactor at 150°C.
   10. Remove the vials from the reactor immediately after 30 minutes, and allow the vials to cool to room temperature.
   11. Pipet 2 mL of the 1.54 N NaOH solution to each vial, cap, and shake.
   12. Turn on the spectrophotometer, and press the program # (make sure the wavelength is set to 880 nm).
   13. Zero the spectrophotometer with a method blank sample **without PhosVer 3TM added** (this is the instrument blank). Insert the vial, close the cover, and press “ZERO” to zero the meter.
   14. Remove the vial and add the content of one PhosVer 3TM powder pillow to the method blank vial. One at a time, add the PhosVer 3TM powder pillow to all of the vials.
       1. From the time you add the PhosVer 3TM packet until you take the reading, no more than 8 minutes may pass (about 15 vials can be done in that time).
   15. Cap and shake the vial for 20-30 seconds to mix (the powder will not dissolve completely).
   16. Set the timer for 2 minutes. Try to be consistent for all samples.
   17. After the timer goes off, wipe the outside of the vial, insert into the meter and record the absorbance reading on the phosphorus bench sheet.
       1. To see the absorbance reading, press “shift ABS,” then “READ.”
   18. Insert each vial into the meter, push “READ,” and record absorbance.
       1. From the time you add the PhosVer 3TM packet until you take the reading, no more than 8 minutes may pass.
   19. 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 LOD (about 0.02) to 1.10 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 E, 1999.
   2. Hach Company PhosVer 3TM Phosphorus – Spectrophotometric Measurement of ortho- and Total Phosphorus in Water and Wastewater, Method 8190, Hach Company, edition 11, 2003.
4. Disclaimer:
   1. The mentioning of company or product names does not constitute endorsement by the Wisconsin Department of Natural Resources or the authors.