

Phosphorus, Total, Persulfate Digestion

(EPA/600/R-93/100, Method 365.1)

1. Application (analytes and matrices)

- 1.1 This procedure is applicable to the determination of total phosphorus in drinking, ground and surface water and soil, sludges and wastes.

2. Method Sensitivity

- 2.1 The approximate working range is 0.005 to 1 mg P/L. This range might be extended by diluting the sample prior to analysis.
- 2.2 The method detection limit is 0.005 mg/L.

3. Summary of Methods

- 3.1 Samples are digested in an autoclave for 30 minutes at 121C and 15-20 psi with ammonium persulfate and sulfuric acid to convert all phosphorus to orthophosphate. The orthophosphate ion (PO₄)⁻³ reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. The complex is reduced with ascorbic acid to form a blue color which absorbs light at 880 nm. Lachat method 115-01-1-F is used for analysis on QuikChem 8000 flow injection analyzer.

4. Safety and Waste Management

- 4.1 Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.
- 4.2 All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations.
- 4.3 Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

5. Sample Collection, Preservation and Handling

- 5.1 Samples are collected by clients in clean containers and submitted to the laboratory for analysis.
- 5.2 A chain-of-custody form is submitted with the samples. If samples were not preserved by client they will be preserved immediately at the lab.

- 5.3 Samples are preserved by addition of 1 mL of concentrated sulfuric acid per liter of sample and kept in a fridge at 0-4C until analyzed.
- 5.4 Maximum holding time after preservation is 28 days.

6. Potential Interferences

- 6.1 A list of interferences is documented in Method 365.1 section 4 of EPA Methods for Chemical Analysis of Water and Wastes (1993). These include high concentration of ferric iron (negative error), silica (positive error)...etc.

7. Equipment and Analytical Instruments

- 7.1 20X150 mm disposable digestion tubes (screw cap).
- 7.2 Autoclave.
- 7.3 10-mL adjustable electronic pipet.
- 7.4 Culture tubes: 13X100 mm disposable glass.
- 7.5 Polypropylene caps for disposable digestion tubes.
- 7.6 Vortexer.
- 7.7 Lachat Flow injection analyzer 8000 system
- 7.8 XYZ sampler.

8. Consumable Supplies, Reagents and Standards

- 8.1 All reagents and standards should be stored in the appropriate bottles and labeled with the following information: identity, supplier, lot number, date, preparer's initials and concentrations. Use de-ionized water for all solutions.
- 8.2 Stock acid solution, 5.6M Sulfuric Acid: Dilute 310 mL of concentrated H₂SO₄ to 1 L with Double de-ionized water.
- 8.3 Working digestion acid solution: Dissolve 12.8 g ammonium persulfate and 32 mLs of 5.6M H₂SO₄ in a 100-mL volumetric flask. Dilute to mark with double de-ionized water. Prepare daily.
- 8.4 Stock Ammonium Molybdate Solution: In a 1 L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate in approximately 800 mL of DDI water. Dilute to mark and invert to mix. Store in plastic container and refrigerate. Stable for 6 month.
- 8.5 Stock Antimony Potassium Tartrate Solution: In a 1 L volumetric flask dissolve 3.4 g antimony potassium tartrate trihydrate in approximately 800 mL DDI water. Dilute to mark and invert to mix. Store in a dark bottle and refrigerate. Stable for 6 months.
- 8.6 Molybdate Color Reagent: To a 1 L volumetric flask add about 500 mL DDI water, then add 21.0 mL concentrated sulfuric acid, then add 72.0 mL Stock Antimony Potassium Tartrate Solution and 213 mL Ammonium Molybdate Solution. Dilute to mark. Degas using helium gas for 1 minute prior to use. Stable for 1 week.
- 8.7 Ascorbic Acid Reducing Solution: In a 1 L volumetric flask dissolve 60.0 g Ascorbic Acid in about 700 mL of DDI water. Dilute to mark and invert to mix.

- Add 1.0 g dodecyl sulfate wetting agent. Stable for a1 week when refrigerated. Discardd if becomes yellow.
- 8.8 Carrier: Sulfuric Acid 0.13M. In a 1 L volumetric flask add 500 mL DDI water and 7.2 mL concentrated sulfuric acid. Dilute to mark and invert to mix. Use this carrier solution to make any dilutions for analysis.
 - 8.9 NAOH-EDTA Solution: in a 1 L volumetric flask, dissolve 65.0 g of NaOH and 6.0 di-sodium EDTA in about 500 mL DDI water. Dilute to mark and invert to mix. Store in a dark plastic bottle. Stable for 6 months. Use this solution if during analysis a blue color appears in tubing. Place color reagent and ascorbic acid transmission lines into this reagent and pump solution for 5 minutes or until color disappears. Rinse lines with DDI water afterwards.
 - 8.10 Stock phosphorus standard: In a 1 L volumetric flask dissolve 0.4393 g potassium phosphate monobasic (KH₂PO₄) (dried at 105C for an hour) in 900 mL DDI water. Add 1 mL of concentrated H₂SO₄ and dilute to mark. Refrigerate at 4C. Stable for 6 months.
 - 8.11 Working standard solutions: the following working standard solutions are prepared from Stock standard solution in DDI water. 0, .05, .25, .50, 1.00 mg P/L and 30.00 mg P/L spike solution and .04 mg P/L quality control solution is prepared from a different P standard source.

9. Procedure of Extraction and Analysis

- 9.1 Load rack with disposable test tubes.
- 9.2 Transfer 8 mL of sample, blank or standard per tube using a 10-mL electronic pipet.
- 9.3 Add 0.5 mL of working digestion acid to each tube.
- 9.4 Vortex and cover with caps. Keep tubes loosely capped.
- 9.5 Autoclave the digestion tubes for 30 minutes at 121C and 15-20 psi.
- 9.6 Remove tubes from autoclave, cool, and securely cap tubes.
- 9.7 Allow any particulate matter to settle overnight.
- 9.8 The sample preparation procedure for analysis is taken from Lachat QuikChem Method 115-01-1-F

10 Calibration, Standardization and Calculations

- 10.1 The instrument is calibrated using a set of 5 calibration standards prepared and digested with the samples. Date of preparation, lot number, initials of the person who prepared the standards and expiration date of standards are marked on the standards containers. Working standards are preserved in concentrated sulfuric acid and kept in a fridge at 0-4°C.
- 10.2 Correlation coefficient of standard curve of calibrators must be at least 0.995 for the calibration test to pass. Sample concentration is calculated from a regression equation by plotting response versus standard concentration

11. Quality Control

- 11.1 Method Blank (MB) – At least one MB must be analyzed with each batch of samples in order to assess contamination from the laboratory environment. If MB values exceed the method detection limit, laboratory or reagent contamination should be suspected, take correction action before continuing the analysis.
- 11.2 Laboratory Control Sample (LCS) – At least one LCS must be analyzed with each batch of sample. Calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90-110%, the analyte is judged out of control, take corrective action before continuing analysis.
- 11.3 Replicates and Spikes_ At least 10% of samples of a sample batch are replicates and spikes. If spikes and replicates do not fall within the specified limits of $\pm 10\%$ of true value then re-digest and analyze sample batch including QC samples.
- 11.4 Continuing Calibration Verification (CCV) – For all determinations, a mid-range check standard must be analyzed immediately after daily instrument calibration, after every tenth sample, and at the end of the sample run. This process verifies that the instrument is within 10% of calibration. If the CCV standard indicates that the calibration is outside of present limits, take corrective action before continuing analysis.

12. Data Assessment and Reporting of Results

- 12.1 Data are accepted when quality control samples fall within the set limits. Corrective actions of re-digestion and/or re-analyzing of samples and quality control samples will take place to handle out of control data.
- 12.2 Data are reported as mg/l of TP for water and solids.

13. References

- 13.1 US EPA method 600. Methods for Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100. Revised August 1993.