

ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA  
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-115r1  
Rev. 2 (5/07))

**EXTRACTION OF PARTICULATE PHOSPHORUS FOR  
SEDIMENTS AND SUSPENDED PARTICULATE MATERIAL**

Prepared By: Paul Kiry

Approved By: \_\_\_\_\_ Date: \_\_\_\_\_  
Robin S. Davis  
Quality Assurance Unit



## **Extraction of Particulate Phosphorus for Sediments and Suspended Particulate Material**

### 1. SUMMARY OF METHOD.

- 1.1. Suspended particulate samples (on glass fiber filters) or sediments are combusted at elevated temperatures to oxidize and release P from most solid phases. The P is then solublized into a weak HCl solution and analyzed using the ascorbic acid method as ortho-phosphate (OP). OP reacts with molybdenum(VI) and antimony(III) in an acid medium to form a phospho-antimonylmolybdenum complex. This complex is subsequently reduced by ascorbic acid to a heteropolyblue with an absorbance maximum at 880 nm.

### 2. INTERFERING SUBSTANCES.

- 2.1. Ferric iron up to 70 mg/L, copper up to 10 mg/L and silica up to 10 mg/L do not interfere. Filter turbid samples prior to analysis (1). Silica at analysis temperatures > 40°C and/or <2.2 N sulfuric acid in the mixed reagent solution causes interference. Maintaining a temperature of 37 °C and an acid concentration of 2.45 N sulfuric acid avoids these conditions.

### 3. SAMPLE HANDLING AND PRESERVATION.

- 3.1. Water samples are filtered and the filter is frozen. Sediment samples are frozen for storage.

### 4. PROCEDURE FOR SUSPENDED PARTICULATE P AND SEDIMENTS.

- 4.1. Muffle 47-mm, 0.7- $\mu$ m pore size Whatman GF/F glass fiber filters or equivalent at 550°C for 90 minutes. Filters should not be brittle after pre-heating. If the filters are brittle, do not use and muffle new filters at a lower temperature. Check actual muffle furnace temperature.
- 4.2. Carefully filter a known volume of water through the pre-heated filter and record the volume. Rinse filter with a minimum amount of DIW; approximately 2 ml.
- 4.3. Carefully remove the filter from the filtration apparatus and place in a 47-mm plastic Petri dish or Al foil packet, and freeze until analysis.

- 4.4. Carefully weigh a small amount of dried sediment (0.05g) to the nearest mg (0.000 g) into a 125-ml Erlenmeyer flask or equivalent glass tube (i.e., 25 ml glass test tube with Teflon top).
  - 4.5. For analysis place the filter into a 125-ml erlenmeyer flask or equivalent container (i.e., 25 ml glass test tube with Teflon top), for sediment into a glass tube, and add 1 ml of 25% magnesium nitrate to wet the filter and/or sediment, cover tube with Al foil (i.e., not Teflon top). Muffle tube (without top) at 550°C for 2 hr. After removing from muffle furnace add 20 ml of 1 N HCl and allow to stand for 24 hours. Shake the flasks periodically during the 24-hour period.
  - 4.6. Transfer 100 or 50 µl depending on the expected phosphorus concentration to a 2-ml sampler cup and dilute with 2.0 ml of deionized water and then analyze on the Alpkem Analyzer. Alternatively, take between 100 to 200 µl and dilute to 10 ml; analyze this solution for P (see below).
5. Ortho-Phosphate Method Summary (A303-S203) RFA™ Methodology Alpkem Corp. (1987).
    - 5.1. Reagents: The components of the procedure are listed below.
      - 5.1.1. Hydrochloric Acid (1N): 86 ml of concentrated acid to 1000 ml of deionized water (DIW).
      - 5.1.2. Diluent: 0.3 ml of Levor IV to 1000 ml of DIW. This is used only if online dilution is done.
      - 5.1.3. Stock Sulfuric Acid, 5N H<sub>2</sub>SO<sub>4</sub> (500 ml): Cautiously add 70 ml of concentrated sulfuric acid to 300 ml of deionized water contained in a 1000-ml erlenmeyer flask. Cool to room temperature and transfer to a 500-ml volumetric flask. Dilute to the mark with deionized water. **Caution: The dilution of sulfuric acid in water generates a great amount of heat.**
      - 5.1.4. Stock Antimony Potassium Tartrate (100 ml) (APT; (K(SbO)C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>·1/2H<sub>2</sub>O; FW 333.94). Dissolve 0.3 g of antimony potassium tartrate in 80 ml of deionized water contained in a 100-ml volumetric flask. Dilute to the mark with deionized water. Store at 4°C in a dark bottle.
      - 5.1.5. Stock Ammonium Molybdate (100 ml) (AMMO: (NH<sub>4</sub>)<sub>6</sub>MO<sub>7</sub>O<sub>24</sub> \* 4H<sub>2</sub>O (FW 1235.95)): Dissolve 4 g of ammonium molybdate in 70 ml of deionized water contained in a 100-ml volumetric flask. Dilute to the mark with deionized water. Store at 4°C in a polyethylene bottle.

- 5.1.6. Stock Ascorbic Acid (100 ml) (AA:  $C_6H_8O_6$  (FW 176.13): Dissolve 1.8 g of ascorbic acid in 70 ml of deionized water contained in a 100-ml volumetric flask. Dilute to the mark with deionized water. Stable for 1 week if stored at 4°C.
- 5.1.7. Working Reagent (100 ml); Stock Sulfuric Acid (50 ml): Stock Antimony Potassium Tartrate (5 ml): Stock Ammonium Molybdate (15 ml): Stock Ascorbic Acid (30 ml).
- 5.2. Add reagents in the order stated above and mix after each addition. Prepare reagent daily.
- 5.3. NOTE: Alternate Working Reagents (prevents excessive baseline drift and improves resolution).
  - 5.3.1. Ascorbic Acid Reagent: Dissolve 0.54 g of ascorbic acid in 100 ml of deionized water in a 100-ml volumetric flask. Transfer to a 125-ml erlenmeyer flask, add two drops of Levor IV and mix.
- 5.4. NOTE: Alternate Combined Reagent: Combine and mix in this order. 45 ml 5N  $H_2SO_4$ , 15 ml DI, 5 ml APT, 15 ml AMMO.
- 5.5. P Standards.
  - 5.5.1. Stock 100 mg P/L (1L): Potassium Dihydrogen Phosphate ( $KH_2PO_4$  FW 136.09): Dissolve 0.4393 g of dry potassium dihydrogen phosphate in approximately 900 ml of deionized water contained in a 1-L volumetric flask. Dilute the solution to the mark with deionized water. Alternatively purchase a stock 1000 mg/L stock standard that is NIST traceable.
  - 5.5.2. Working Standards: Use adjustable, microliter pipets to add the designated volumes of stock standard, tabulated below, to the required number of volumetric flasks that each contain approximately 85 ml of deionized water and mix well. Keep these solutions tightly sealed. Prepare working standards daily. If samples are preserved with sulfuric acid, add acid in the same proportion to each volumetric flask.
  - 5.5.3. Prepare standards in the desired range as suggested below. From a 1000 mg/L stock prepare a 10.0 mg/L working stock by diluting 1 ml of the 1000 to 100 ml with DIW.

Nominal Concentration (mg L <sup>-1</sup> )	Stock Standard Volume (ml)
---	----------------------------

0.01	200 $\mu$ l/200 ml DI
0.05	1000 $\mu$ l/200 ml DI
0.1	2000 $\mu$ l/200 ml DI
0.2	2000 $\mu$ l/100 ml DI
0.3	3000 $\mu$ l/100 ml DI
0.5	5000 $\mu$ l/100 ml DI
1.0	10000 $\mu$ l/100 ml DI

6. REFERENCES.

- 6.1. Standard Methods for the Examination of Water and Wastewater, 14<sup>th</sup> Ed.1975, p. 625.
- 6.2. Methods for Chemical Analysis of Water and Wastewater, March 1984, EPA-600/4-79-020, “Sample Preservation”, p. xvii, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH. 45286.
- 6.3. Methods for Chemical Analysis of Water and Wastewater, March 1984, EPA- 600/4-79-020, “Phosphorous, All Forms”, Method 365.1 (Colorimetric, Automated Ascorbic Acid).
- 6.4. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples, November 1992, EPA/600/R-92/121, Method 365.5.
- 6.5. Nutrient Analytical Services Laboratory, Chesapeake Biological Laboratory, University of Maryland, Center for Environmental Science, Solomons, Maryland 20688-0038, Standard Operating Procedures, Technical Series No. 158-97.
- 6.6. Aspila, I., H. Agemian and A.S. Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst* 101:187-197.