ESS Method 310.2:
Phosphorus, Total, Low Level
(Persulfate Digestion)

Environmental Sciences Section
Inorganic Chemistry Unit
Wisconsin State Lab of Hygiene
465 Henry Mall
Madison, WI 53706

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(Persulfate Digestion)

1.0 Scope and Application

This method is applicable to the determination of total phosphorus in surface waters in the range of 0.002 to 0.200 mg P/L.

2.0 Summary of Method

Samples are digested in an autoclave for 30 minutes at 121°C with ammonium persulfate and sulfuric acid to convert all phosphorus to orthophosphate. The orthophosphate is then analyzed with the Technicon AAII using the ascorbic acid procedure (Method 310.1).

3.0 Sample Handling and Preservation

Samples are preserved in the field by the addition of 2 mL of 12.5% H_2SO_4 per 250 mL sample. They are refrigerated at 4°C until analysis is performed.

4.0 Apparatus

4.1 Digestion tubes, 20 x 150 mm, disposable borosilicate glass.

4.2 Autoclave.

4.3 Technicon AutoAnalyzer II system consisting of:

4.3.1 Sampler IV with a 30/h (2:1) Cam

4.3.2 Analytical manifold (Orthophosphate in Seawater) with internal heating bath at 37.5°C

4.3.3 Proportioning pump III

4.3.4 Colorimeter equipped with 50 mm flow cells and 880 nm interference filters

4.3.5 Recorder/Printer

4.4 8 mL and 4 mL volumetric pipettes.

4.5 Culture tubes: 15 x 85 mm disposable glass.

4.6 Caps, Polypropylene, for disposable culture tubes (4.5).

5.0 Reagents

5.1 Stock acid solution, 6 N Sulfuric Acid: Dilute 166 mL of concentrated H_2SO_4 to 1 L with Milli-Q water. This is equivalent to the procedure used in Methods for Chemical Analysis of Water and Wastes, p. 365.3 (Section 10.0), if 1 mL/L sulfuric acid is used to preserve the samples.

5.2 Stock persulfate solution: Dissolve 32 g ammonium persulfate ($(NH_4)_2S_2O_8$) in Milli-Q water and dilute to 100 mL (Stable two weeks at 4°C).

5.3 Working digestion acid solution: Combine equal volumes of stock acid (Section 5.1) and stock persulfate (Section 5.2) solutions. Prepare daily.

5.4 Color reagent:

5.4.1 Stock Solution A; Sulfuric acid solution, 4.9 N: Add 136 mL concentrated H_2SO_4 to 800 mL Milli-Q water. Cool and dilute to 1 L with Milli-Q water.

5.4.2 Stock Solution B; Ammonium molybdate solution: Dissolve 40 g of $(NH_4)_6Mo_7O_{24}•4H_2O$ in 900 mL Milli-Q water and dilute to 1 L. Store at 4°C.

5.4.3 Stock Solution C; Ascorbic acid: Dissolve 9 g of ascorbic acid ($C_6H_8O_6$) in 400 mL Milli-Q water and dilute to 500 mL. Store at 4°C. Keep well stoppered. Prepare fresh monthly or as needed.

5.4.4 Stock Solution D; Antimony potassium tartrate: Dissolve 3.0 g of $K(SbO)C_4H_4O_6•½H_2O$ in 800 mL Milli-Q water and dilute to 1 L. Store at 4°C.

5.4.5 Combined color reagent: Combine the following solutions in order, mixing after each addition: (Prepare fresh daily)

Stock A, 5.4.1 (4.9 N H_2SO_4)	50 mL
Stock B, 5.4.2 (Ammonium molybdate solution)	15 mL
Stock C, 5.4.3 (Ascorbic acid solution)	30 mL
Stock D, 5.4.4 (Antimony-tartrate solution)	5 mL

5.5 Sampler wash solution: Dilute 6 mL of concentrated sulfuric acid to 1 L with Milli-Q water.

5.6 Diluent water solution: Add 4.0 g sodium lauryl sulfate and 5 g NaCl per L of Milli-Q water.

5.7 Stock phosphorus standard: Dissolve 0.4394 g of potassium phosphate monobasic (KH_2PO_4) (dried at 105°C for 1 h) in 900 mL Milli-Q water. Add 2.0 mL of concentrated H_2SO_4 and dilute to 1 L. 1.0 mL = 0.100 mg P (100 mg P/L).

5.8 Standard phosphorus solution: Dilute 10.0 mL of stock phosphorus standard (5.7) to 1 L. 1.0 mL = 0.001 mg P (1.0 mg P/L).

5.9 Working standard solutions: Prepare the following standards by diluting suitable volumes of standard solution (5.8) to 200.0 mL with Milli-Q water (Add 20 mL of 1% H_2SO_4 before diluting to 200.0 mL):

mg P/L	mL of standard solution (5.8)/200.0 mL
0.005	1.0
0.050	10.0
0.100	20.0
0.150	30.0
0.200	40.0

5.10 Stock Adenosine 5'-Monophosphate (AMP) solution: Dissolve 0.2242 g of AMP (dried at 105°C for 1 h) in 900 mL of Milli-Q water. Add 2 mL of conc. H_2SO_4 and dilute to 1 L. 1.0 mL = 0.02 mg P (20 mg P/L).

5.11 Working AMP solution: Dilute 5 mL of stock AMP (5.10) to 1 L. (0.100 mg P/L).

6.0 Procedure

6.1 Load test tube racks with disposable digestion tubes and add samples, standards, duplicates, spikes and blanks according to CFDA Tray Protocol.

6.1.1 Prepare a standard curve by pipetting 8 mL of standards and blanks (Milli-Q water) using a Class A volumetric pipet.

6.1.2 Transfer 8 mL of each sample to a digestion tube using an 8 mL cut-off (large bore) volumetric pipet.

6.1.3 A 0.100 mg P/L standard with a following Milli-Q water blank should be inserted after every 20 samples.

6.1.4 Prepare a minimum of 10% of the samples in duplicate, and spike 5% or at least two samples per digestion. Spikes are prepared by mixing 4 mL of a sample with 4 mL of AMP solution (5.11) or 4 mL of 0.050 mg P/L working standard solution.

6.2 All digestion tubes should have 8 mL of liquid before the addition of digestion acid. Add 0.5 mL of working digestion acid solution (5.3) to each tube, mix and cover with Caps.

6.3 Autoclave the digestion tubes for 30 minutes at 121°C, 15-20 psi (specify *manual* autoclave).

6.4 Remove the tubes from the autoclave, cool, mix, transfer to the 15 x 85 mm disposable glass culture tubes and cover with parafilm.

6.5 Allow any particulate matter to settle overnight.

6.6 Sample analysis

6.6.1 Set up manifold as shown in Figure 1.

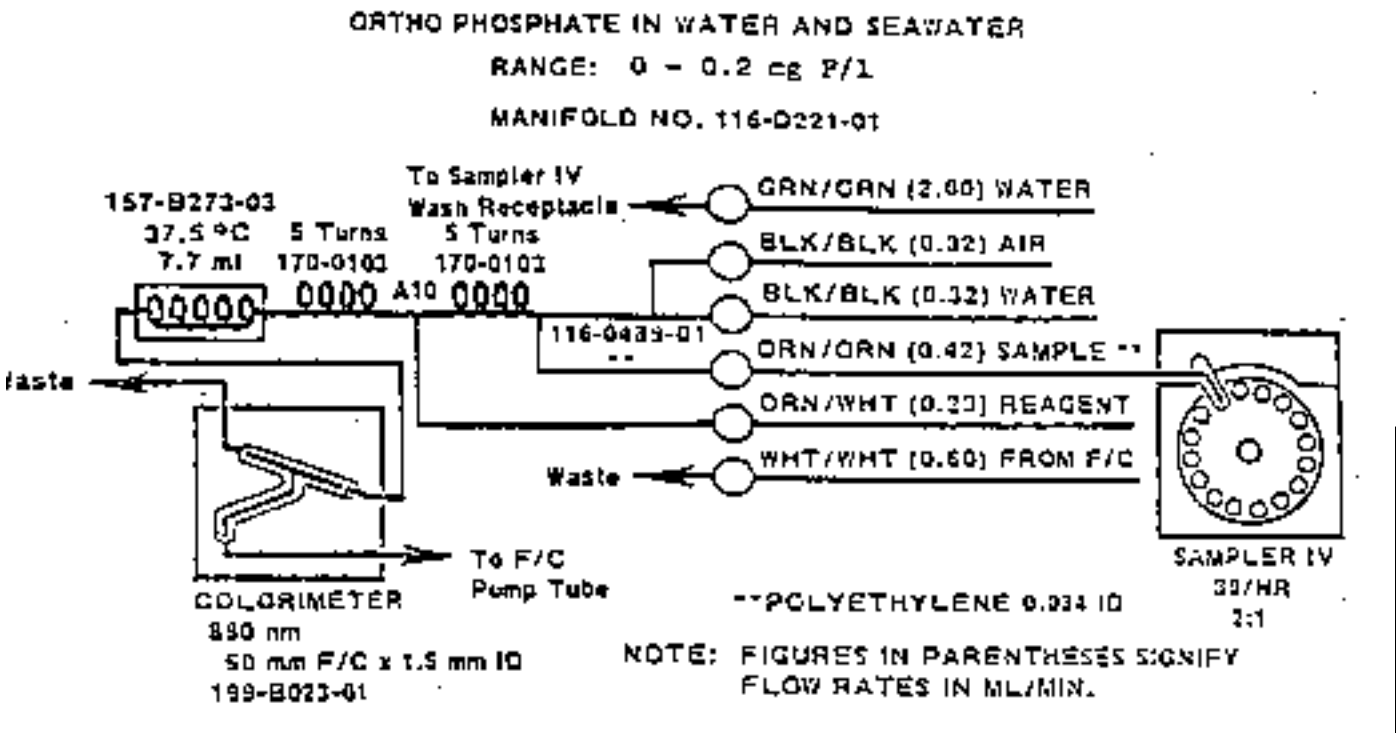

Figure 1. Manifold Set Up

6.6.2 Allow the colorimeter, recorder and printer to warm up for 30 minutes.

6.6.3 Obtain a stable baseline with all reagents, feeding Milli-Q water through the sample line.

6.6.4 Place culture tubes in the sampler and remove the parafilm.

6.6.5 Analyze according to procedures described in General AutoAnalyzer Procedures and LIMS-CFDA Methods manual.

7.0 Precision and Accuracy

Precision and accuracy data are available in the Inorganic Chemistry Unit Quality Assurance Manual.

8.0 References

8.1 Central Regional Laboratory Procedure for the Analysis of Total Phosphorus, U.S. Environmental Protection Agency, Region V, 4 p., (1978).

8.2 Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency, EPA 600/4-79-020, p. 365.1, (1983).

8.3 Ortho Phosphate in Water and Seawater, Industrial Method 155-71W, Technicon Instruments Corporation, Tarrytown, NY (1973).