

Standard Operating Procedure for the
Digestion and Analysis of Fresh Water Samples
for Total Phosphorus and
Total Dissolved Phosphorus
CCAL 41A.0

Cooperative Chemical Analytical Laboratory
Forestry Sciences Laboratory
Oregon State University
3200 SW Jefferson Way
Corvallis, Oregon

Prepared by Kathryn Motter and Cameron Jones
April 2006

**Standard Operating Procedure for the
Digestion and Analysis of Fresh Water Samples for
Total Phosphorus and Total Dissolved Phosphorus
CCAL 41A.0**

Table of Contents

1.0	Scope and Application	3
2.0	Summary of Method	3
3.0	Definitions	3
4.0	Interferences	4
5.0	Safety	4
6.0	Equipment and Supplies	5
7.0	Reagents and Standards	5
7.1	Preparation of Reagents	5
7.2	Preparation of Standards	7
8.0	Sample Handling and Storage	7
9.0	Quality Control	7
10.0	Calibration and Standardization.....	8
11.0	Procedure.....	8
11.1	Sample Setup and Digestion.....	8
11.2	Titration	9
11.3	Analysis	9
11.3	System Notes.....	10
12.0	Data Analysis and Calculations	10
13.0	Method Performance	11
14.0	Pollution Prevention	11
15.0	Waste Management	11
16.0	References	11
17.0	Tables, Diagrams, Flowcharts, and Validation Data	12

Standard Operating Procedure for the Digestion and Analysis of Fresh Water Samples for Total Phosphorus and Total Dissolved Phosphorus CCAL 41A.0

1.0 Scope and Application

- 1.1 This method covers the determination of total phosphorus and total dissolved phosphorus in fresh waters in the range of 0.001 - 0.2 mg P/L. Sample concentrations greater than 0.2 mg P/L can be analyzed by dilution of the sample prior to digestion.

2.0 Summary of Method

- 2.1 Phosphorus occurs in fresh waters almost solely in the form of various phosphates. Common forms of phosphates in fresh waters include orthophosphates, condensed polyphosphates and organically bound phosphates. Phosphates may exist in solution, in particles or debris, or in the bodies of aquatic organisms. Analysis of phosphate involves two general procedural steps: (a) conversion of phosphates to dissolved orthophosphate, and (b) the subsequent colorimetric determination of dissolved orthophosphate. For total dissolved phosphorus determination, samples are filtered through GF/F or GF/C glass fiber filters; total phosphorus is determined on an unfiltered sample. Samples are digested in a microwave by acid hydrolysis with sulfuric acid and potassium persulfate. Orthophosphorus is determined colorimetrically by reaction of ammonium molybdate and antimony potassium tartrate to form phosphomolybdic acid, which is then reduced to intensely colored molybdenum blue by ascorbic acid.

3.0 Definitions

- 3.1 DI water: Water that has been through a deionization system to produce water similar to ASTM Type I reagent with 16.7 Mohms resistivity (ASTM) (Reference 16.3).

- 3.2 Method Detection Limit (MDL): The minimum concentration of an analyte that can be measured and reported with 99% confidence, based on a one-sided 99% confidence interval (t -value at a significance level of 0.01 and $n-1$ degrees of freedom) from at least seven repeated measurements of a low concentration standard measured within an analysis run.

$$\text{MDL} = ts$$

Where,

t = Student's t value at a significance level of 0.01 and $n-1$ degrees of freedom

s = standard deviation of at least seven repeated measurements of a low level standard

4.0 Interferences

- 4.1 Arsenates at concentrations as low as 0.1 mg/l, react with molybdate reagent to produce a blue color resulting in positive interference in colorimetric analysis at 880 nm.
- 4.2 Nitrite and hexavalent chromium interfere to give low analytical results at concentrations as low as 1.0 mg/l.

5.0 Safety

- 5.1 The toxicity or carcinogenicity of each reagent has not been precisely determined; however, each chemical should be regarded as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. Cautions are included for known extremely hazardous materials.
- 5.2 The following chemicals have the potential to be highly toxic or hazardous. For detailed explanations, consult the MSDS.
- 5.2.1 Sulfuric acid
 - 5.2.2 Antimony potassium tartrate
 - 5.2.3 Sodium hydroxide
 - 5.2.4 Potassium persulfate
 - 5.2.5 Ethanol

6.0 Equipment and Supplies

Note: *Brand names, suppliers and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

- 6.1 Spectrophotometer suitable for measurements at 880 nm with a 10 cm pathlength cell
- 6.2 125 mL Erlenmeyer flasks calibrated to 50 mL
- 6.3 50 mL glass Griffin beakers
- 6.4 5.0 ml plunger-type repipette
- 6.5 5.0 mL Repipette for addition of strong digestion acid
- 6.6 Microwave with stainless steel sides and bottom, with a glass tray covered with a clean paper towel or a paper bag to reduce hot and cold spots
- 6.7 Safety glasses
- 6.8 Nitrile gloves
- 6.9 Lab coat or apron
- 6.10 Laboratory exhaust fume hood
- 6.11 High Density Polyethylene (HDPE) bottles

7.0 Reagents and Standards

7.1 Preparation of Reagents

Solution 7.1.4 needs to be filtered through a prewashed GF/F filter after preparation

7.1.1 Sulfuric acid solution, 5 N

Slowly add 140 ml of concentrated H₂SO₄ to 700 mL of DI water over an ice bath. Equilibrate to room temperature and bring up to 1000 mL with DI water.

7.1.2 Strong H₂SO₄ Digest Solution, 11.2 N

Slowly add 310 mL of concentrated H₂SO₄ to 600 mL of DI water over an ice bath. Equilibrate to room temperature and bring up to 1000 mL with DI water.

7.1.3 *Antimony potassium tartrate solution*

Dissolve 1.3715 g antimony potassium tartrate (C₈H₄K₂Sb₂O₁₂ · 3 H₂O) in 400 mL DI water in a 500 ml volumetric flask. Bring to volume with DI water. Store at room temperature. Stable at least six months.

7.1.4 *Ammonium molybdate solution*

Add 20 g (NH₄)₆Mo₇O₂₄ · 4H₂O to a 500 mL volumetric flask and fill to the mark with DI water. Swirl until dissolution complete. Store in a brown glass bottle in the dark at room temperature. Good until precipitate forms, but no longer than one month.

7.1.5 *Ascorbic acid, 0.1 M*

Transfer 2.0 g of ascorbic acid to a 100 mL volumetric flask and dilute to the mark with DI water. The solution is stable for two days if stored refrigerated. Bring to room temperature before use.

7.1.6 *Phenolphthalein solution*

1.) Dissolve 100 mg phenolphthalein in 100 ml 95% ethanol.

7.1.7 *Combined color reagent*

To prepare 200 mL of color reagent, mix reagents in the following proportions and in the order given. All reagents must be at room temperature before they are mixed. The reagent will be clear with a yellow-green tint. Total volume may be adjusted proportionately.

100 ml	5N H ₂ SO ₄
10 ml	Antimony potassium tartrate solution
30 ml	Ammonium molybdate solution
60 ml	Ascorbic acid

The reagent must be prepared fresh daily. Make only as much reagent as will be needed for that days analyses. Discard solution if the color deteriorates (i.e., gets darker or precipitates). The reagent must be stored in the dark.

7.1.8 *Potassium persulfate salt, analytical grade*

Grind with mortar and pestle to remove large crystals and to homogenize consistency; transfer to a small, labeled, air-tight container for bench use.

7.2 Preparation of Standards

7.2.1 *Standard KH₂PO₄ Stock Solution, 50.0 µg PO₄-P/mL:*

a) Dissolve 0.2197 g of oven-dried (105°C) KH₂PO₄ (anhydrous) in 800 mL DI water. When dissolution complete, bring to 1000 mL volume with DI water. Store in the refrigerator in a brown polyethylene bottle. Bring to room temperature before use. Remake stock solution every 12 months or as needed.

7.2.2 *KH₂PO₄ working solution, 1.00 µg PO₄-P/mL:*

10.0 mL of stock solution brought to 500 mL with DI water. Store solution in a clear polyethylene bottle at room temperature.

7.2.3 *Calibration and Check Standards:*

Prepare calibration and check standards from the working solution as follows:

0.000 mg/l (blank) = 50 mL DI water

0.050 mg/L PO₄-P = 2.5 mL of std. + 47.5 ml DI water

0.100 mg/L PO₄-P = 5.0 mL of std. + 45.0 ml DI water

0.200 mg/L PO₄-P = 10.0 mL of std. + 40.0 ml DI water

8.0 Sample Handling and Storage

8.1 If required, unfiltered samples are filtered upon receipt through glass fiber filters into clean HDPE bottles and stored at 4°C in the dark. Samples are digested within 28 days to ensure sample integrity. If samples must be held prior to analysis, they are stored frozen at -18°C.

8.2 Acid-washed glassware: All new glassware used in the procedure should be soaked in 0.5 N hydrochloric acid overnight, and then rinsed with DI water. Soak in DI water overnight. Glassware treated in this manner should be used only for the determination of phosphorus. After analytical use it should be rinsed with DI water, run through a glassware washer, soaked in dilute HCl overnight, rinsed four times with DI water and drained upside down. When the glassware has dried it is ready for use. Commercial detergents should never be used.

9.0 Quality Control

9.1 Preparation of stock standards is recorded on worksheets and documented by weight of standard added to a given flask before dilution to volume

with DI water. All records of certification are kept on file at CCAL Laboratory.

- 9.2 Blank: DI water run before the calibration.
- 9.3 Quality Control Check Sample: Run once per analysis batch.
- 9.4 Method Detection Limit (MDL): Established for each analyte. Based on a one-sided 99% confidence interval (t-value) from at least seven repeated measurements of a low concentration standard. The t-distribution value is multiplied by the standard deviation of the population (n-1) to obtain the MDL.
- 9.5 Analytical Duplicate: Separate analysis from the same sample aliquot. Run a minimum of once every analysis set.
- 9.6 Standard recoveries are tracked over time to monitor overall performance.

10.0 Calibration and Standardization

- 10.1 Balances: calibrated yearly by external vendor.
- 10.2 Pipette delivery checked by weight to within 2% of theoretical weight of aliquot volume.

11.0 Procedure

- 11.1 Sample Setup and Digestion
 - 11.1.1 There are 30 flasks in a normal sample set. Each set must contain a DI water blank, three standard concentrations of 0.050, 0.100, and 0.200 mg/L, a check standard and at least one duplicate.
 - 11.1.2 If samples are added to an existing partial sample set, then an additional standard and an additional duplicate sample must also be setup that day.
 - 11.1.3 Put 50 ml of filtered or unfiltered sample in a 125 ml Erlenmeyer flask which has been properly marked to identify the sample, and cover with a 50 mL beaker.
 - 11.1.4 Add potassium persulfate using the glass scoop calibrated to deliver 0.5 g, and 1.0 mL of 11.2 N H₂SO₄ acid solution to each flask.
 - 11.1.5 Samples can sit undigested up to 14 days if they have been fixed with persulfate and acid.

- 11.1.6 Place the samples in the microwave oven and digest for 60 minutes at full power.
- 11.1.7 Allow to cool to room temperature before removing from the oven.
- 11.1.8 Digested samples should be analyzed as soon as possible but can sit unanalyzed for seven days.

11.2 Titration

- 11.2.1 The color reaction is pH dependent, so samples must be titrated to the proper pH range.
- 11.2.2 To each sample flask add 5 drops of phenolphthalein.
- 11.2.3 Titrate each flask with 10 N sodium hydroxide to a pink end point, then back titrate with 5 N sulfuric acid dropwise to discharge the pink color.
- 11.2.4 The titration process for digested samples, once initiated, must be completed and the samples analyzed the same day.
- 11.2.5 Bring all flasks to 50 mL volume with DI water to adjust for volume loss during digestion.

11.3 Analysis

11.3.1 Add Color Reagent:

- a) Use a 5.0 ml repipette to add 4.0 ml of the mixed color reagent to each flask. Swirl each flask upon addition of color reagent to mix.
- b) Add color to the first 4 flasks in the set noting the time.
- c) 10 minutes later add color reagent to the next 8 flasks; 5 minutes later add color to the next 6 flasks; after 5 more minutes the next 6; and the final 6 after 5 more minutes.
- d) It is important that samples are all analyzed immediately after 30 minutes color development. The reaction is time and temperature dependent. Standards, blanks and samples must all have the same color development time.
- e) High room temperatures will hasten color development; increase/decrease development times as recommended below:

14 - 18°C	35 minutes;
18 - 23°C	30 minutes;
23 - 27°C	25 minutes;
27 - 32°C	20 minutes.

11.3.2 Spectrophotometer Setup

- a) Turn on the Milton-Roy 601 spectrophotometer. Wait for the internal diagnostic audio prompt (a single beep) and then turn on the accessory control module (ACM). Set to 880 nm.
- b) Use a 10 cm pathlength cell. The sample cell must be clean and free of lint or smudges. Do not use sample cells that have scratched, discolored or etched cell end windows.

- c) Rinse the sample cell with a small amount of DI water before filling with DI water. The sample cell must be filled so that there are no air bubbles within the cell.
- d) Wipe the cell dry with a Kimwipe, making sure that the end windows are clean and dry.
- e) Place the cell in the cell holder. Make certain that the cell holder is aligned properly with the light path.
- f) Close the sample compartment door and zero the instrument.
- g) The spectrophotometer must be allowed to stabilize at the appropriate wavelength for 30 minutes prior to analysis, with the cuvette full of DI water in place.

11.3.3 Spectrophotometer Calibration and Sample Analysis

- a) Rezero with fresh DI water immediately before analysis.
- b) Rinse the sample cell with a small amount of the reagent blank; fill the cell with the blank, clean cell, and align in the instrument. Record results and rezero the instrument.
- c) Calibrate the instrument with the 0.05, 0.10 and 0.20 standards.
- d) Analyze each of the samples in the set; record concentration and absorbance.
- e) Rinse flasks as soon as possible following analysis to avoid adsorption of the characteristic blue color to the flask walls.

11.3 System Notes

- 11.3.1 Molybdate reagent may be good beyond one month. Monitor blank signal to track degradation.
- 11.3.2 The reagent blank reading checks reagent purity and procedural integrity. A high blank can indicate analytical problems, especially precipitation or degradation of the ammonium molybdate reagent.
- 11.3.3 Standard concentrations as high as 0.300 mg/l can be used for calibration with the 10 cm cell. If a smaller pathlength cell is used, standard concentrations as high as 0.600 mg/l can be used to calibrate the instrument. Samples exceeding this concentration range must be diluted before analysis.

12.0 Data Analysis and Calculations

- 12.1 The software for the Milton Roy 601 spectrophotometer provides sample data calculated using the calibration standards in a linear regression equation. Results are expressed in mg/l and no further calculation is necessary for sample data (other than in-run sample dilutions or prediluted samples where the dilution factor must be applied to the analysis results to calculate the actual sample concentration).

- 12.2 If samples have been added to a pre-existing set then there will be an additional standard. Check recovery of digest check standards to monitor procedural performance.

13.0 Method Performance

- 13.1 This method was validated through inter-laboratory studies. The CCAL participates in the USGS Standard Reference Water QA program.

14.0 Pollution Prevention

- 14.1 The chemicals used in this method pose little threat to the environment when properly managed.
- 14.2 All standards and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of disposable waste.
- 14.3 For further information on pollution prevention consult *Less is better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington D.C. 20036, (202) 872-4477.

15.0 Waste Management

- 15.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, and to protect the environment by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is required.
- 15.2 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel", and "Less is Better: Laboratory Chemical Management for Waste Reduction", both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington DC, 20036.

16.0 References

- 16.1 Standard Methods For The Examination of Water and Wastewater, Method 4500-P Phosphorus; 4500-P B. Sample Preparation; 4500-P E. Ascorbic Acid Method. American Public Health Association. 21st Edition, 2005.
- 16.2 Code of Federal Regulations. Protection of Environment. Section 40, Appendix B to Part 136. Definition and procedure for the determination of the method detection limit. Revision 1.11. Revised July 1, 1990. Office of the Federal Register, National Archives and Records.
- 16.3 ASTM. American Society for Testing and Materials. Standard Specifications for Reagent Water. D1193-77 (Reapproved 1983). Annual Book of ASTM Standards, Vol. 11.01. ASTM: Philadelphia, PA, 1991.
- 16.4 FWPCA Methods for Chemical Analysis of Water and Wastes.
- 16.5 Murphy J., and J. Riley. "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." Anal. Chem. Acta. 27, 31. 1962.
- 16.6 Gales, M. E., Jr., E. C. Julian, and R. C. Kroner. "Method for Quantitative Determination of Total Phosphorus in Water." J. AWWA 58:1363. 1966.
- 16.7 American Water Works Assoc. 1958. Committee Report. "Determination of Ortho Phosphate, Hydrolyzable Phosphate and Total Phosphate in Surface Waters." J. Am. Water Works Assoc. 50:1563.
- 16.8 Strickland, J. D. H. and T. R. Parsons. 1965. A Manual of Sea Water Analysis, 2nd Ed. Fish Res. Bd., Ottawa, Canada.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

- 17.1 NA