Standard Operating Procedure for the Digestion and Analysis of Fresh Water Samples for Total Phosphorus and Total Dissolved Phosphorus CCAL 35B.3

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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 - 0.200 mg P/L. Sample concentrations greater than 0.200 mg P/L can be analyzed by dilution of the sample prior to digestion, or by dilution of the digested sample at the time of analysis.

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 an oven with alkaline persulfate; the sodium hydroxide is consumed causing sample pH to become acidic, for the acid hydrolysis of organic phosphorus. 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.

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.

4.3 Silica at concentrations greater than 10 mg SiO_2/L causes positive interference.

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

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 Balance measuring to at least 0.1 mg

6.2 Volumetric flasks and pipettes as required

6.3 Technicon Auto-Analyzer II

- 6.3.1 Multichannel proportioning pump
- 6.3.2 Colorimetric detector
- 6.3.3 Data system
- 6.3.4 Alpkem manifold and method 155-71W (modified)
- 6.3.5 Inert sample probe

6.4 1.0 mL pipette

6.5 10.0 mL pipette

6.6 Oven capable of maintaining 100°C

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

6.12 40 mL borosilicate vials with screw on caps

7.0 Reagents and Standards

7.1 Preparation of Reagents

Solutions 7.1.1 & 7.1.4 need to be filtered through a prewashed GF/F filter after preparation

7.1.1 *Persulfate Reagent:*

Add 19.2 g of sodium hydroxide (NaOH) to approximately 1 L of DI water in a clean 2 L volumetric flask and mix until the NaOH is dissolved. Add 80.4 g of low-nitrogen potassium persulfate ($K_2S_2O_8$) to the NaOH Solution and adjust volume to 2 L. The mixed reagent contains 0.296 eq/L of $K_2S_2O_8$ and 0.24 eq/L of NaOH. This solution is stable. Refilter if crystallization occurs.

7.1.2 *Sulfuric acid solution, 5 N*

Slowly add 140 ml of concentrated H_2SO_4 to 700 mL of DI water over an ice bath. Equilibrate to room temperature and bring up to 1000 mL with DI water.

7.1.3 *Antimony potassium tartrate solution*

Dissolve l.37l5 g antimony potassium tartrate ($C_8H_4K_2Sb_2O_{12}$. 3 H_2O) 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 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 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 0.9 g of ascorbic acid to a 50 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 *Combined color reagent*

To prepare 160 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.

80 ml 5N H2SO4
8 ml Antimony potassium tartrate solution
24 ml Ammonium molybdate solution
48 ml Ascorbic acid

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

7.1.7 *SLS Water*

Dissolve 0.353 g of sodium lauryl sulfate in 2.0 mL of DI water. Add this solution to 200 mL of DI water.

7.2 Preparation of Standards

7.2.1 *Calibration Standards:*
Standards are prepared by dilution of a standard purchased from a vendor that provides traceability to NIST standards. A stock standard is prepared by dilution of the purchased reagent to an intermediate concentration. A mixed stock standard is prepared by dilution of the purchased reagent to an intermediate concentration. The stock standard with various concentrations of nitrate and phosphorus is used to prepare working standards in the table below.

	mg PO4P/L
1	0.010
2	0.050
3	0.100
4	0.200

7.2.2 *Second Source Check Standard:*
Standards are prepared by dilution of a standard purchased from a vendor that provides traceability to NIST standards; this reagent is from a source other than that of the calibration standards. A stock standard is prepared by dilution of the purchased reagent to an intermediate concentration. Check standard concentrations are the same as calibration standard 3.

7.3.3 *TP Digest Efficiency Check Standard:*
To prepare a 500 mg P/L stock solution, add 0.2966 g Adenosine 5'-triphosphate disodium salt (Na_2ATP) which has been oven dried at 100ºC for 1 hour and cooled in a dessicator, to a 100 mL volumetric flask and dilute to volume with DI water. Dilute 4.00 mL of the stock solution to 500 mL with DI water to prepare the 4.0 mg P/L working standard.

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 -15ºC.

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 after the calibration and before and after each check standard.

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 ten samples.

9.6 An ATP digest standard and a Quality Control Check Sample are digested each digest batch to verify TP recovery. Two DI water blanks are digested to determine the phosphorus present in the mixed persulfate digestion reagent.

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.

10.3 Calibration curve with $r^2 \geq 0.995$. (See 17.3 for calibration data set-up.)

10.4 Calibration verification with check standards, monitored throughout the run. If measurement exceeds +/- 10% of the theoretical value, the analysis should be terminated and the instrument recalibrated. The calibration must be verified before continuing analysis.

11.0 Procedure

11.1 Digest Procedure

11.1.1 Preparation of samples and reagents for digestion

11.1.1.1 Pipette 20 mL of sample into a clean, labeled, 40 mL vial. (Note: Bring samples to room temperature and shake well just prior to subsampling to minimize pipetting and sampling errors.)

11.1.1.2 Add 0.5 mL TP Digest Standard and 19.5 mL DI water to a clean, labeled, 40 mL vial. Final tube concentration after addition of Persulfate Reagent, 0.08 mg P/L. Alternatively, for combined TN/TP Digest Standard, add 0.5 mL TP Digest Standard, 0.5 mL TN Digest Standard (see CCAL SOP 33A) and 19.0 mL DI water to a clean, labeled, 40 mL vial. Final tube concentrations after addition of Persulfate Reagent 0.08 mg P/L and 0.185 mg N/L.

11.1.1.3 Add 20 mL of check sample (collected in bulk; an aliquot is digested once each digest batch) to a clean, labeled, 40 mL vial.

11.1.1.4 Add 20 mL of DI water to each of two clean, labeled, 40 mL vial.

11.1.2 Pipette 5 mL of mixed persulfate digestion solution into each vial, cap and swirl immediately after addition.

Note: For low volume samples, dilute to 20 mL total sample volume.

11.1.3 Weigh vials and record weight.

11.1.4 Place vials in plastic coated wire racks and place the racks in a forced air oven set at 100ºC. Digest for 12 – 16 hours. Remove racks from the oven and cool to room temperature.

11.1.5 Reweigh the vials and record the weight. Analytical results will be corrected for weight loss; see section 12.0 Data Analysis and Calculations.

11.2 Calibration and Analysis Procedure

11.2.1 Prepare reagents and standards as outlined in Section 7.

11.2.2 Set up manifolds as shown in Section 17.1.

11.2.3 Samples are injected into the reaction path at a fixed time interval, determined by the cam timing set in the auto-sampler. Setup or confirm data system parameters as detailed in Section 17.3.

11.2.4 Pump DI water through all reagent and sample lines. Check for leaks and stable bubble pattern (smooth flow). Pump reagents through all lines until the system equilibrates (minimum of one hour).

11.2.5 Record sample id's in the data template.

11.2.6 Calibrate the instrument with standards. Calibration regression equations must have $r^2 \geq 0.995$.

11.3 System Notes

11.3.1 If the baseline is excessively noisy, clean the manifold using the following procedure:
- Place all reagent and carrier lines in rinse water and pump to clear reagents.
- Place reagent lines in contrad reagent for several minutes.
- Place lines in DI water and pump until thoroughly rinsed.

11.3.2 If baseline has a large number of air spikes, check pump tubes for excessive wear and replace as necessary.

11.3.3 Poor sensitivity or insufficient color development may be the result of old ammonium molybdate solution. Prepare fresh and flush system thoroughly.

12.0 Data Analysis and Calculations

12.1 The data system prepares a calibration curve by plotting response of injected standards versus known standard concentration. The resulting regression equation is used to calculate the sample concentration.

12.2 All results and sample information are filed in the analysis data system by analysis run. Details specific to the instrumental analysis are noted in the Instrument Run Log created and maintained for the AAII. Analytical results are entered into electronic format and entries are verified by a second person.

12.3 Correct analytical results for weight loss due to evaporation; subtract the average persulfate reagent blank from the sample concentration and then correct the sample concentration for dilution by the persulfate digestion solution.

Note: "For low level samples the variability in the reagent blanks determines the limit of detection, not the error associated with the NO_3 and PO_4 analyses" Qualls (1989).

13.0 Method Performance

13.1 This method was validated through inter-laboratory studies. The CCAL Water Analysis Laboratory participates in the USGS Standard Reference Water QA program and the National Water Research Institute's (NWRI) Environment Canada Proficiency Testing (PT) 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 14.3 For further information on pollution prevention, consult *Less is Better: Guide to Minimizing Waste in Laboratories*, available from the American Chemical Society at www.acs.org.

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 *Less is Better: Guide to Minimizing Waste in Laboratories*, available from the American Chemical Society at www.acs.org, and *Environmental Management Guide For Small Laboratories* (233B00001) from the US Environmental Protection Agency at https://nepis.epa.gov.

16.0 References

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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.

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16.14 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.

16.15 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.

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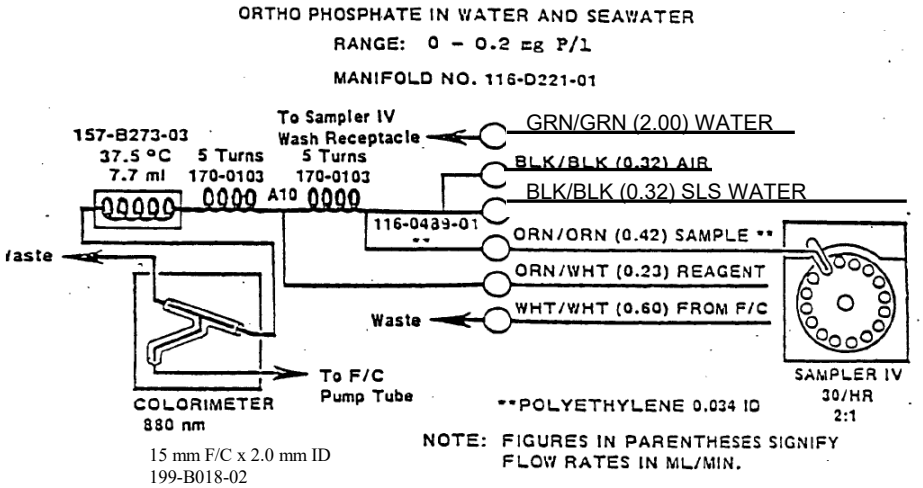
17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Orthophosphorus Reaction Manifold



17.2 Orthophosphorus Manifold Specifications

Carrier is SLS water

Interference filter is 880 nm

Pump tubing is Tygon

Manifold tubing is 0.030 mm i.d.

Mixing coils are 5 turn with an injection “T” fitting between coils.

15 mm x 2 mm flow cell

Inert sample probe

17.3 Data System Parameters

Cycle throughput: 30 samples/hr
Cycle Period: 120 s

Analyte Data:
Concentration Units mg PO_4-P/L

Calibration Data:

Level	1	2	3	4
Concentration mg/L	0.010	0.050	0.100	0.200

Calibration Fit Type: linear regression

Sampler Timing:
Minimum Probe in Wash Period: 30 s
Probe in Sample Period: 90 s

18.0 Document Revision History

Original Document: April 2006
Version: 41A.0
Title: Standard Operating Procedure for the Digestion and Analysis of Fresh Water Samples for Total Phosphorus and Total Dissolved Phosphorus

Edit Date: March 2010
New Version: 41A.1
Address update
Section 4.3: add silica interference statement
Section 11.4: correct section numbering
Section 13.1: add Environment Canada Proficiency Testing Program participation

Edit Date: May 2010
New Version: 35B.0
Update documentation to reflect change to Automated Colorimetric Analysis Method

Edit Date: July 2011
New Version: 35B.1

Updates to reflect procedural, standard and reagent modifications for combined TN/TP digestion and analysis.

Edit Date: April 2014
New Version: 35B.2
Section 1: change working range of method for appropriate significant figures.
Section 7.1: update filtering, stability and refiltering for persulfate reagent.
Section 7.2: change preparation of standards to include a mixed intermediary standard solution and provide a table of final standard concentrations.
General editing.

Edit Date: July 2019
New Version: 35B.3
Address update
General edits and updates throughout.