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Determination of Total Particulate Phosphorus (TPP) and Particulate Inorganic Phosphorus (PIP) in Fresh/Estuarine/Coastal Waters

(Reference Method: EPA 365.1, Rev. 2.0)

Document #: NASLDoc-031

Revision 2023-1
Replaces Revision 2022-2
Effective May 1, 2023
I attest that I have reviewed this standard operating procedure and agree to comply with all procedures outlined within this document.

Employee (Print)	Employee (Signature)	Date
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Revised by: ______________________ Date: __________

Reviewed by: ______________________ Date: __________

Laboratory Supervisor: ______________________ Date: __________

Revisions 2023

Section 1.11: Updated the reference method to 365.1, Revision 2.0 (1993).

Section 9.4.2: Updated percent recovery limits from 80-120% to 90-110%.

Table 2: Updated acceptance limits for spikes and duplicates to +/-10%.

Section 12.3: Added "CRM" and noted the use of the 1N HCl average in the calculation.

Section 12.4: Updated percent recovery formula.

Section 13: Updated USEPA method reference.

1 SCOPE and APPLICATION

1.1 Total Particulate Phosphorus (TPP), Particulate Inorganic Phosphorus (PIP), and Phosphorus in algal and sediment samples are determined using this method.
1.2 Ammonium molybdate and potassium antimony tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex which is reduced to an intensely blue-colored complex by ascorbic acid. Color is proportional to phosphorus concentration. The method is used to analyze all ranges of salinity.
1.3 A Method Detection Limit (MDL) of 0.0010 mg P/L for Total Particulate Phosphorus was determined using the Student's *t* value (3.14) times the standard deviation of seven replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value.
1.4 A Method Detection Limit (MDL) of 0.0010 mg P/L for Particulate Inorganic Phosphorus was determined using the Student's *t* value (3.14) times the standard deviation of seven replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value.
1.5 A Method Detection Limit (MDL) of 0.009%P for Total Particulate Phosphorus sediment/algae was determined using the Student's *t* value (3.14) times the standard deviation of seven replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value.
1.6 The Quantitation Limit for TPP was set at 0.0030 mg P/L for filtered samples and 0.027%P for sediment and algal samples.
1.7 The Quantitation Limit for PIP was set at 0.0030 mg P/L.
1.8 The method is suitable for P concentrations 0.0010 to 3.72 mg PO_4-P/L.
1.9 This procedure should be used by analysts experienced in the theory and application of combusted, extractive particulate nutrient analysis. Three months experience with an analyst experienced in the analysis of combusted, extractive, particulate phosphorus analysis is required.
1.10 This method can be used for all programs that require analysis of particulate phosphorus.
1.11 The colorimetric portion of the procedure references EPA Method 365.1, Revision 2.0 (1993).

2 SUMMARY

2.1 Samples for the measurement of Total Particulate Phosphorus are combusted, then extracted in an acidic medium.
2.2 Samples for the measurement of Particulate Inorganic Phosphorus are extracted in an acidic medium.

2.3 Extracted samples are mixed with a sulfuric acid-antimony-molybdate solution, and subsequently with an ascorbic acid solution, yielding an intense blue color suitable for photometric measurement.

3 DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range – 0.05723 to 3.72 mg P/L in extract. The overall analytical range of extracted samples is comprised of three distinct, yet overlapping concentration ranges. A separate calibration is performed for each range. These ranges include 0.05723 to 0.744 mg P/L, 0.1488 to 1.488 mg P/L and 0.53143 to 3.72 mg P/L. Three sub-ranges are utilized so that extracted samples can be analyzed on the most appropriate scale possible. Final concentration of particulate phosphorus in the sample is dependent on volume filtered.

3.5 Batch –An analytical batch is composed of prepared environmental samples (extracts, digestates, concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor with the same process and personnel using the same lot(s) of reagents. A batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Blank – A volume of reagent water fortified with the same matrix as the calibration standards, without analyte added.

3.10 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.11 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.12 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

3.12.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.12.2 Initial Calibration Verification (ICV) – An individual standard, distinct from the Initial Calibration Standards (STD), analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.12.3 Continuing Calibration Verification (CCV) – An individual standard, distinct from the Initial Calibration Standards (STD), analyzed after every 18-23 field sample analyses.

3.13 Certified Reference Material (CRM) – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.14 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.15 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.16 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.17 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.18 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage external to the laboratory. (EPA-QAD)

3.19 External Standard (ES) – A pure analyte (Potassium dihydrogen phosphate (KH_2PO_4)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.20 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.21 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.22 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.23 Laboratory Reagent Blank (LRB) – A blank matrix (i.e. 1 N HCl) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument. (The LRB in this method is also used as a blank correction to calculate final results.)

3.24 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.25 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. This is also referred to as the MDL. (ACS)

3.26 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired. This is also referred to as the Quantitation Limit.

3.27 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.28 May – Denotes permitted action, but not required action. (NELAC)

3.29 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Standard Methods).

3.30 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.31 Photometer – Measures the absorbance of the solution in the cell in a multicell cuvette. Light passes from the lamp through the condensing lenses to the interference filter. The plane surface of the first condensing lens is coated with a material which reflects heat and infrared light. The filters are mounted on a filter wheel. There are 15 positions for filters. Each filter corresponds to a wavelength of interest. The 880 nm filter is specified by the test definition for particulate phosphorus. After passing through the filter the light is converted into a stream of light pulses by a chopper. Then the light is directed via a quartz fiber through a focusing lens and a slit to the beam divider. The beam divider divides the light into two parts. A specified portion is reflected to the reference

detector, which monitors the light level fluctuations. The remaining major portion of the light beam goes through the liquid in the cell to the signal detector, which measures the amount of light absorbed.

3.32 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.33 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.34 Quality Control Sample (QCS) – A sample of analyte of known and certified concentration. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also referred to as CRM.

3.35 Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.

3.36 Safety Data Sheets (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.37 Sample Segment – Bar-coded metal tray that holds up to fourteen four milliliter auto analyzer vials containing samples or standards. The user identifies each vial in the operating software.

3.38 Sample Segment Holder – An automated, temperature-controlled carousel that contains up to six sample segments. This carousel spins in clockwise or counterclockwise manner to move the sample segments into position for analysis. This carousel format allows for continuous processing.

3.39 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.40 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.41 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.42 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also referred to as CRM.

3.43 Test Definition – A photometric test consisting of a user defined testing sequence, reagent additions, calibration standards, incubations and absorption results.

3.44 Test Flow – Functions to define the parameter for reagent and sample dispensing, dilution, incubation and measurement.

4 INTERFERENCES

4.1 Suspended matter in the sample will scatter light as it passes through the cuvette to the detector. High blank responses will result. The identified sample will be reanalyzed. A sample result with high blank response will only be accepted if it is repeated in the reanalysis.

4.2 Blemishes in the cuvette, as result of the manufacturing process, will result in high blank responses. The identified sample will be reanalyzed.

4.3 Silicon (Si) at analysis temperature >40ºC and/or <2.2 N Sulfuric Acid in the Triple Reagent solution causes interference in the concentration range > 0.05 mg/mL Si in the extract. High silica concentrations cause positive interference. These conditions are avoided by maintaining an acid concentration of 2.45 N Sulfuric Acid in the reagents and analysis at 37ºC.

5 SAFETY

Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes must be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed. Inform the CBL Associate Director of Facilities and Maintenance of the incident and if additional treatment is required.

5.1 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.

5.2 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.3 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Safety Data Sheets (SDS).

Table 1

Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
Hydrochloric Acid	3	0	1	ACID, COR	
Sulfuric acid	4	0	2	ACID, COR	
Ammonium molybdate	4	0	1	Irritant	
Potassium antimonyl	2	0	0		

tartrate hemihydrate					
Ascorbic Acid	1	0	0	ACID	
Potassium dihydrogen phosphate	2	0	0		
Chloroform	3	0	0		
Bleach	3	0	0		

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)

HAZARD RATING

Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable if heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer = OXY

6 EQUIPMENT AND SUPPLIES

6.1 Filtering apparatus
6.2 Glass fiber filters. This laboratory uses Whatman GF/F (47 mm, 0.7 µm pore size) filter pads for water samples.
6.3 Foil pouches, labeled with sample identification and volume filtered.
6.4 Flat-bladed forceps.
6.5 Freezer, capable of maintaining -20° ± 5°C.
6.6 Drying oven. This laboratory uses Lindberg/Blue M Drying Oven
6.7 Crucibles and lids for combusting filter pads; a separate set of crucibles and lids for combusting sediments and algae
6.8 Muffle furnace. This laboratory uses a ThermoLyne F30428 combustion oven set at 500°C to obtain a true combustion temperature of 550°C.
6.9 Analytical balance accurate to 0.0001 g for weighing sediment and algae
6.10 AutoAnalyzer vial containers (sample cups) and racks to hold them
6.11 Lab ware: 50 mL plastic centrifuge tubes with screw caps
6.12 1 digital timer
6.13 1 10mL re-pipettor

6.14 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives. This laboratory cleans all lab ware related to this method with a 10% HCl (v/v) acid rinse. This laboratory cleans all lab ware that has held solutions containing ammonium molybdate with 10% NaOH (w/v) rinse.

6.15 Aqaukem 250 multi-wavelength automated discrete photometric analyzer. Aquakem 250 control software operates on a computer running Microsoft Windows 7 or higher operating system.

7 REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.

7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.

7.3 1 N Hydrochloric acid

Hydrochloric acid (concentrated) 172mL

In a 2000mL volumetric flask add approximately 1600 mL reagent water. Add 172 mL concentrated HCl to the reagent water, let cool, and bring to volume. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Store the flask at room temperature. Reagent is stable for one year.

7.4 9.8 N Sulfuric acid

Sulfuric acid (concentrated) 54.4 mL

In a 200 mL volumetric flask add approximately 120 mL reagent water. Add 54.4 mL H_2SO_4 to the reagent water, let cool, and bring to volume. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Store the flask at room temperature. Reagent is stable for one year.

7.5 Ammonium molybdate solution

Ammonium molybdate 8.0 g

In a 100 mL plastic volumetric flask, dissolve with immediate inversion 8.0 g Ammonium molybdate in approximately 90 mL reagent water. Bring flask to volume. Store the flask in the dark at room temperature. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance ID in the Analytical Reagent log book. Reagent is stable for one month. Discard if white precipitate appears in flask or on threads of cap.

7.6 Potassium antimonyl tartrate solution

Potassium antimonyl tartrate 0.6 g

In a 100 mL plastic volumetric flask dissolve 0.6 g Potassium antimonyl tartrate hemihydrate in approximately 90 mL reagent water. Bring flask to volume. Store the flask at room temperature. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance ID in the Analytical Reagent log book. Reagent is stable for one year.

7.7 Ascorbic acid solution

Ascorbic acid 3.6 g

In a 100 mL plastic volumetric flask dissolve 3.6g Ascorbic acid in approximately 90 mL reagent water. Bring flask to volume. Store the flask in the refrigerator. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance ID in the Analytical Reagent log book. Reagent is stable for two months.

7.8 Triple Reagent -

9.8 N Sulfuric acid	40 mL
Ammonium molybdate solution	12 mL
Potassium antimonyl tartrate solution	4.0 mL

Add 40 mL 9.8 N Sulfuric acid to a 60 mL reagent container. Carefully add 12 mL Ammonium molybdate solution to the reagent container. Carefully add 4.0 mL Potassium antimonyl tartrate solution to the reagent container. Cap. Invert six times to mix. Write name of preparer, preparation date, constituent solutions' preparation dates in the Analytical Reagent log book. Reagent is stable for two weeks.

7.9 Orthophosphate Stock Standard, 12,000 µM –

Potassium dihydrogen phosphate (KH_2PO_4), primary standard grade, dried at 45°C 0.816 g

In a 500 mL volumetric flask, dissolve 0.816 g of potassium dihydrogen phosphate in approximately 400 mL reagent water. Bring flask to volume with reagent water (1 mL contains 12 µmoles P). Add 1 mL chloroform as a preservative. Transfer to a brown bottle and store in refrigerator. Write name of preparer, preparation date, standard manufacturer, manufacturer lot number, and balance ID in the Analytical Standard log book. Make fresh every 6 months.

7.10 Working Low Orthophosphate in HCl Standard –

Stock Orthophosphate standard 0.20 mL

In a 100 mL volumetric flask, dilute 0.20 mL of Stock Orthophosphate Standard to volume with 1 N HCl to yield a concentration of 24 µM PO_4-P/L (0.744mg P/L). Write name of preparer, preparation date, Stock Standard preparation date in the Analytical Standard log book. Make fresh every two months.

7.11 Working Mid-Range Orthophosphate in HCl Standard –

Stock Orthophosphate Standard 0.40 mL

In a 100 mL volumetric flask, dilute 0.40 mL of Stock Orthophosphate Standard to volume with 1 N HCl to yield a concentration of 48 µM PO_4-P/L (1.488 mg P/L). Write name of preparer, preparation date, Stock

Orthophosphate Standard preparation date in the Analytical Standard log book. Make fresh every two months.

7.12 Working High Orthophosphate in HCl Standard –

Stock Orthophosphate Standard 1.00 mL

In a 100 mL volumetric flask, dilute 1.00 mL of Stock Orthophosphate Standard to volume with 1 N HCl to yield a concentration of 120 µM PO_4-P/L (3.72 mg P/L). Write name of preparer, preparation date, Stock Orthophosphate Standard preparation date in the Analytical Standard log book. Make fresh every two months.

7.13 Calibration Verification Low Orthophosphate in HCl Standard –

Stock Orthophosphate standard 0.15 mL

In a 100 mL volumetric flask, dilute 0.15 mL of Stock Orthophosphate Standard to volume with 1 N HCl to yield a concentration of 18 µM PO_4-P/L (0.558 mg P/L). Write name of preparer, preparation date, Stock Standard preparation date in the Analytical Standard log book. Make fresh every two months.

7.14 Calibration Verification Mid-Range Orthophosphate in HCl Standard –

Stock Orthophosphate Standard 0.30 mL

In a 100 mL volumetric flask, dilute 0.30 mL of Stock Orthophosphate Standard to volume with 1 N HCl to yield a concentration of 36 µM PO_4-P/L (1.116 mg P/L). Write name of preparer, preparation date, Stock Orthophosphate Standard preparation date in the Analytical Standard log book. Make fresh every two months.

7.15 Calibration Verification High Orthophosphate in HCl Standard –

Stock Orthophosphate Standard 0.80 mL

In a 100 mL volumetric flask, dilute 0.80 mL of Stock Orthophosphate Standard to volume with 1 N HCl to yield a concentration of 96 µM PO_4-P/L (2.976 mg P/L). Write name of preparer, preparation date, Stock Orthophosphate Standard preparation date in the Analytical Standard log book. Make fresh every two months.

7.16 Aquakem Cleaning Solution –

Bleach 55.0 mL

In a 100 mL volumetric flask, dilute 55.0 mL of bleach to volume with 45mL reagent water to yield a concentration of 55% bleach. Write name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Reagent is stable for six months.

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Total Particulate Phosphorus Water Samples

8.1.1 Water samples for total particulate phosphorus are filtered. If filtering is delayed more than one hour, the water samples are iced in a cooler or refrigerated until filtered.

8.1.2 For each sample, a recorded volume of water is filtered through a 47 mm Whatman GF/F filter pad that has been pre-combusted at 500ºC for 90

minutes. After filtering, the pad is folded in half using forceps. This folding maintains the integrity of the particulate matter concentrated on the pad.

8.1.3 The pad containing the sample is placed in a labeled foil pouch. The label identifies the sample, sampling date and volume filtered.

8.1.4 Freeze samples at -20° ± 5° C.

8.2 Particulate Inorganic Phosphorus Water Samples

8.2.1 Water samples for total particulate inorganic phosphorus are filtered. If filtering is delayed more than one hour, the water samples are iced in a cooler or refrigerated until filtered.

8.2.2 For each sample, a recorded volume of water is filtered through a 47 mm Whatman GF/F filter pad that has been pre-combusted at 500ºC for 90 minutes. After filtering, the pad is folded in half using forceps. This folding maintains the integrity of the particulate matter concentrated on the pad.

8.2.3 The pad containing the sample is placed in a labeled foil pouch. The label identifies the sample, sampling date and volume filtered.

8.2.4 Freeze samples at -20° ± 5° C.

8.3 Algae and sediment samples

8.3.1 Samples are dried overnight at 50°C, then ground to uniform powdery consistency and placed in labeled, capped vials.

8.4 Frozen samples may be stored up to 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.

9 QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Performance

9.2.1 The initial demonstration of capability (iDOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Linear Dynamic Range – LDR (Linear Calibration Range) should be established for phosphorus using appropriate seven-point calibration curve.

9.2.3 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ±10% of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either

proceeding with the initial determination of MDLs or continuing with analyses.

9.2.4 Method Detection Limits (MDLs) – MDLs should be established for particulate phosphorus using a low-level natural water sample, typically three to five times higher than the estimated MDL. The same procedure should be followed for sediments or other weighed samples. To determine the MDL values, analyze seven replicates and process through the entire analytical procedure. Perform all calculations defined in the procedure (Section 12) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = S\,t_{(n-1,1-\alpha=0.99)}$$

Where,

S = Standard deviation of the replicate analyses.
n=number of replicates
$t_{(n-1,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom (t=3.14 for 7 replicates.)

9.2.4.1 If the verified MDL is within 0.5 to 2.0 times the existing MDL then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the Initial MDL determination with six degrees of freedom.)

9.2.4.2 MDLs shall be determined yearly and whenever there is a significant change in instrument response or a significant change in instrument configuration.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of 1 N HCl treated the same as the samples. Analyte found in LRB indicates possible reagent or laboratory environment contamination. LRB data are used to assess and correct contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3s of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these QCS/SRM samples shall be used to determine batch acceptance.

9.3.3 The QCS are obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Control Charts – The Accuracy Control Chart for QCS/SRM and Reagent Blank samples are constructed from the average and standard deviation of sample measurements recorded annually. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter QCS/SRM and Reagent Blank results on the chart each time the samples are analyzed.

9.3.5 Initial and Continuing Calibration Verification (ICV/CCV) – Immediately following calibration (ICV) and following every 20 samples (CCV), one calibration verification of 18 µM PO_4-P/L (0.558 mg P/L) PPLOWCBL, 36 µM PO_4-P/L (1.116 mg P/L) PPCBL, 96 µM PO_4-P/L (2.976 mg P/L) PPHIGH is analyzed to assess instrument performance. The ICV/CCVs are made from the same material as calibration standards (KH_2PO_4), and are to be within the expected value ± 3*s*. Failure to meet the criteria requires correcting the problem, including reanalysis of any affected samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery - Percent Recovery

9.4.1 Analyte recovery is assessed through percent recoveries of laboratory spikes of samples.

9.4.2 Percent Recovery = ((Actual value-Original Value)/(Spike Conc. Added)) X 100. Percent recovery for each spiked sample should fall within 90-110%.

9.5 Assessing Analyte Precision – Relative Percent Difference

9.5.1 Analyte replication is assessed through duplicate analyses of samples – Relative Percent Difference (RPD).

9.5.2 RPD = (Laboratory Duplicate Result 1 – Laboratory Duplicate Result 2)/[(Laboratory Duplicate Result 1 + Laboratory Duplicate Result 2)/2] X 100.

9.6 Corrective Actions for Out of Control Data

9.6.1 Control limit – If one measurement exceeds Accuracy Control Chart CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.

9.6.2 Warning limit – If two out of three successive points exceed Accuracy Control Chart WL, analyze another sample. If the next point is within WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

9.6.3 Trending – If seven successive Accuracy Control Chart measurements are on the same side of the central line, discontinue analyses and correct the problem.

9.6.4 When external QCS samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.6.5 When external CCV samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.7 General Operation - To assure optimal operation and analytical results, the Reagent Blank, CCV and CRM are tracked daily in the raw data file and copied to Reagent Blank, CCV and CRM Control Charts.

Table 2

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Correlation Coefficient	≥ 0.995	If <0.995, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.	1 per batch if acceptable.
Initial Calibration Verification (ICV)	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Method Blank/Laboratory Reagent Blank (LRB)*	<0.08mg/L	If the LRB exceeds 0.08mg/L, results are suspect. Rerun the LRB. If the concentration still exceeds 0.08mg/L, reject or qualify the data. The LRB is also used as a blank correction to calculate final results.	Following the ICV and after every 18-23 samples following the CCV.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	± 10%	If QCS/CRM value is outside ± 10% of the target value reject the run, correct the problem and rerun samples.	Following the ICV and approximately every 18-23 samples following the CCV.
Continuing Calibration Verification (CCV)	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 20 samples.
Laboratory Fortified Sample Matrix Spike	± 10%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery	1/10 (spike OR duplicate)

		problem is judged matrix induced. Repeat the LFMS and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	
Laboratory Duplicate	± 10%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	1/10 (spike OR duplicate)

*New Whatman filter pad lots should be screened prior to use for shared TSS/PP projects. Prepare and analyze a few random filter pads from the new lot number as if they were samples. Record any significant issues in the 47mm lot check document found under the Quality System drive.

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Six- or seven-point calibrations are used with three sub-calibrations that cover the analytical range. Three working orthophosphate standards in HCl are used to produce the calibrators for each set of three calibration curves. The instrument performs serial dilutions of working standards to produce the calibrators defined for each curve. The following outlines the preparation of the working standards and the following table describes the subsequent serial dilutions the instrument performs to make each standard for each of the three calibration curves.

Orthophosphate Working Standards:

PPLOWCBL
Working Standard 0.744 mg P/L (0.20 mL stock to 100 mL)
Working CCV 0.558 mg P/L (0.15 mL stock to 100 mL)
PPCBL
Working Standard 1.488 mg P/L (0.4 mL stock to 100 mL)
Working CCV 1.116 mg P/L (0.3 mL stock to 100 mL)
PPHIGH
Working Standard 3.720 mg P/L (1.0 mL stock to 100 mL)
Working CCV 2.976 mg P/L (0.8 mL stock to 100 mL)

Table 3. Orthophosphate Calibrators:

Test Name	Working Standard	Dilution Factor	Concentration mg P/L
PPLOWCBL	0.744 mg P/L	1+12	0.0572
	0.744 mg P/L	1+9	0.0744
	0.744 mg P/L	1+6	0.1063
	0.744 mg P/L	1+3	0.186
	0.744 mg P/L	1+2	0.248
	0.744 mg P/L	1+1	0.372
	0.744 mg P/L	1+0	0.744
PPCBL	1.488 mg P/L	1+9	0.1488
	1.488 mg P/L	1+4	0.2976
	1.488 mg P/L	1+3	0.372
	1.488 mg P/L	1+2	0.496
	1.488 mg P/L	1+1	0.744
	1.488 mg P/L	1+0	1.488
PPHIGH	3.72 mg P/L	1+6	0.531
	3.72 mg P/L	1+4	0.744
	3.72 mg P/L	1+3	0.93
	3.72 mg P/L	1+2	1.24
	3.72 mg P/L	1+1	1.86
	3.72 mg P/L	1+0	3.72

10.2 The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met the entire curve can be reanalyzed or individual standards can be reanalyzed. One standard value (original or reanalyzed) for each and every standard is incorporated in the curve. The coefficient of determination (Pearson's r value) for the calibration curve as well as the calculated concentration of each calibrator is reviewed. The calculated value of each calibrator must be within ten percent of the expected value. The coefficient of determination (Pearson's r value) for the calibration curve must be greater than 0.995.

11 PROCEDURE – DAILY OPERATIONS QUALITY CONTROL

11.1 Total Particulate Phosphorus Combustion and Extraction

11.1.1 Remove samples on pre-combusted filter pads from freezer. Open aluminum foil pouches containing the samples slightly to allow air

circulation and dry in drying oven overnight. Samples for PP only are dried at 45°C. Samples that are also analyzed for TSS have been previously dried at 103-105°C in accordance with the TSS method.

11.1.2 Place dried filter pads in labeled Coors crucibles, recording crucible number, sample identification number and volume filtered. Cover with lids. Weigh approximately 15-20 mg of PACS-3 (CRM/SRM) into a labeled Coors crucible, recording crucible number, reference material name and sample weight. (Prepare two separate CRMs for this procedure.) Cover with lids. Combust at setting of 500ºC for 90 minutes. For this laboratory's muffle furnace, this setting has been determined to produce 550 ºC.

11.1.3 Cool to room temperature. Transfer combusted pads to numbered 50 mL plastic screw cap centrifuge tubes whose numbers correspond to Coors crucible numbers.

11.1.4 Using re-pipettor, add 10 mL 1 N HCl to each centrifuge tube. Screw on cap.

11.1.5 After a minimum of 24 hours, shake each sample. Check that each pad remains in the 1 N HCl after shaking. Wait an additional 24 hours before analyzing. Samples remain stable for 35 days. If analysis is performed after 21 days, it may be necessary to filter the 1 N HCl to remove filter pad particulates.

11.1.6 To analyze, transfer an aliquot of each sample to a labeled AutoAnalyzer vial container (sample cup) for analysis that day.

11.2 Particulate Inorganic Phosphorus Extraction

11.2.1 Remove samples on pre-combusted filter pads from freezer. Open aluminum foil pouches containing the samples slightly to allow air circulation and dry in drying oven overnight at 45ºC.

11.2.2 Transfer dried pads to numbered 50 mL plastic screw cap centrifuge tubes, recording sample identification number and volume filtered.

11.2.3 Using re-pipettor, add 10 mL 1 N HCl to each centrifuge tube. Screw on cap.

11.2.4 After a minimum of 24 hours, shake each sample. Check that each pad remains in the 1 N HCl after shaking. Wait an additional 24 hours before analyzing. Samples remain stable for 35 days. If analysis is performed after 21 days, it may be necessary to filter the 1 N HCl to remove filter pad particulates.

11.2.5 To analyze, transfer an aliquot of each sample to a labeled AutoAnalyzer vial container (sample cup) for analysis that day.

11.3 Total Algal or Sediment Phosphorus Combustion and Extraction

11.3.1 Place vials containing ground algae or sediment samples in drying oven at 50ºC overnight with their screw caps loosened slightly.

11.3.2 Remove from drying oven, tighten screw caps.

11.3.3 After samples reach room temperature, weigh approximately 15-20 mg of each sample into labeled Coors crucibles, recording crucible number, sample identification number and sample weight. Cover with lids. Weigh approximately 15-20 mg of PACS-3 (CRM/SRM) into a labeled Coors

crucible, recording crucible number, reference material name and sample weight. (Prepare two separate CRMs for this procedure.) Cover with lids. Combust at setting of 500ºC for 90 minutes. For this laboratory's muffle furnace, this setting has been determined to produce 550 ºC.

11.3.4 Cool to room temperature. Transfer combusted samples to numbered 50 mL plastic screw cap centrifuge tubes whose numbers correspond to Coors crucible numbers. Using re-pipettor, add 10 mL 1 N HCl to each crucible and pour quantitatively into centrifuge tube. Again, using re-pipettor, add 10 mL 1 N HCl to each crucible and pour quantitatively into centrifuge tube. Screw on cap. Sample is in a total of 20 mL 1 N HCl.

11.3.5 After a minimum of 24 hours, shake each sample. Wait an additional 24 hours before analyzing. Samples remain stable for 35 days.

11.3.6 To analyze, transfer an aliquot of each sample to a labeled AutoAnalyzer vial container (sample cup) for analysis that day.

11.4 Turn on computer. Computer will automatically initiate Konelab software. Once software is running, turn on instrument and allow connection between instrument and computer to complete.

11.5 Place cuvette waste box into cuvette waste sliding drawer. Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh reagent water.

11.6 Begin daily bench sheet documentation.

11.7 Once water reservoir is full, use instrument software to click More, Instrument Actions, and Perform Water Wash. – complete at least five wash cycles.

11.8 After performing water washes, clean the dispensing needle by performing test washes. Click More, Instrument Actions, More, Adjustment Program. Once in the Adjustment Program, click 4-Dispensing Unit, 1-Dispenser, 8-Test Wash. Perform 8 to 10 Test Washes. When complete, press "Q" to quit until you are able to bring up the Main Page.

11.9 Perform Start Up operations by clicking Start Up at the bottom of the Main Page.

11.10 Gather working standards from the bench top and reagents from refrigerator during startup. Assess standards and reagents. Remake anything that has exceeded the time over which it is considered stable.

11.11 Once startup is complete, check the instrument water blanks by clicking More, Instrument Actions, More, Check Water Blank. If any of the instrument blanks are outside their predefined and software-controlled limits, the user will be notified on the main menu page. User takes corrective action to return instrument functions to controlled limits.

11.12 Load reagents in specified position in reagent carousel and place in refrigerated reagent compartment. Reagent positions can be found by clicking reagents at the top of the main page.

11.13 Load working standards in a sample segment, identify the standards in their positions from the drop-down menus at the individual segment positions, and load into instrument. (Click Samples from the top of the Main Page, then click desired segment number.)

11.14 Select the methods to be calibrated by clicking Calibr./QC Selection at the bottom of the main page. Select PPLOWCBL, PPCBL and PPHIGH as the three methods to be calibrated then click Calibrate at the bottom of the page. The three methods will now show as pending. Return to the main page.

11.15 Start instrument and calibration by clicking Page Up (or a green button) on the keyboard. See test flow below for stepwise instrument functions for the analysis of standards and samples.

Test Flow – Method of Analysis, Stepwise

- 150 µL reagent water to cuvette with mixing
- 15 µL sample to cuvette with mixing
- Blank response measurement at 880 nm
- 14 µL Triple Reagent to cuvette with mixing
- 7 µL Ascorbic Acid Reagent to cuvette with mixing
- Incubation, 600 seconds, 37ºC
- End point absorbance measurement, 880 nm
- Software processes absorbance value, blank response value and uses calibration curve to calculate analyte concentration (mg P/L as PO_4)
- User is notified if any measured values used to calculate final concentration are outside preset limits. If so, analyst has options to accept result, rerun the sample or rerun the sample diluted to a user or software specified factor.
- User is notified of each blank response value. Blank response >0.001 absorbance units indicates a scratched cuvette or turbid sample. If the blank response value exceeds 0.001 absorbance units, the analyst specifies that the sample is reanalyzed. If the blank response value of the reanalyzed sample is <0.001 absorbance units, the reanalyzed result is accepted. If the same concentration and blank response value >0.001 absorbance units is again obtained, the results are accepted.

11.16 Organize samples, reagent blanks, filter blanks, check standards and all quality control samples while instrument performs calibrations.

11.17 As calibration curves are produced by the instrument, review them for acceptability. The instrument software prepares a standard curve for each set of calibrators. A graph plotting measured absorbance against standard concentration is presented for review and approval. If acceptance criteria are not met, either the entire curve shall be reanalyzed or individual standards shall be reanalyzed, depending on the violation. One standard value (original or reanalyzed) for each and every calibrator is incorporated in the curve.

11.18 The first sample analyzed during calibration should be an ICV (initial calibration verification) sample. There should be one ICV sample for each calibration curve, of a concentration close to the middle of each range. The following are the recommended ICV samples for each curve: 0.558 mg P/L for PPLOWCBL, 1.116 mg P/L for PPCBL and 2.976 mg P/L for PPHIGH.

11.19 Samples are loaded into the segments and analyzed. CCV (Continuing Calibration Verification) samples (one for each of the three calibration ranges) follow every 20 samples. Laboratory Reagent Blanks (LRB) and Standard Reference Material (SRM) samples are analyzed after the ICV samples and

checked continuously throughout the run. Samples are chosen throughout the analytical bath as Laboratory Duplicates and Laboratory Spikes to assess analyte precision and analyte recovery, respectively. The total number of duplicates and spikes performed will be equal to or greater than ten percent of the total number of samples in the analytical batch.

11.20 As sample analysis is complete, results must be reviewed and accepted manually. If results fall outside acceptance limits, the sample should be reanalyzed. If sample result exceeds the highest standard of the highest calibration range, the samples can be automatically diluted by the instrument and reanalyzed. If the result is such that it will fall within a higher calibration range, it should be reanalyzed in the higher range. If the result is such that it will fall within a lower calibration range, it should be reanalyzed within the lower range.

11.21 Upon completion of all analysis, results are saved to a daily report file. Click Report on the bottom of the Main Page, More, Results To File, and select one row per result. The file is named by the run date. The daily report file for analytical batch of July 1, 2017 would be named 070117. The file is converted to Microsoft Excel for data work up. Remaining samples are discarded.

11.22 All reagents are removed from the reagent chamber and returned to the refrigerator. Reagents that have exceeded their stability period are discarded.

11.23 Click on Stand By on the bottom of the main page and insert Aquakem Cleaning Solution into the instrument; shut down procedures are initiated. Daily files are cleared from the instrument software by clicking More, Management, Clear Daily Files. The software is exited and the instrument is turned off. The computer is shut down.

11.24 The waste is flushed down the drain with copious amounts of tap water. The waste cuvette box is moved to the fume hood and covered.

12 DATA ANALYSIS AND CALCULATIONS

12.1 Upon completion of all analysis, results are saved to a daily report file. The file is named by the run date. The daily report file for analytical batch of July 1, 2017 would be named 070117. The file is converted to Microsoft Excel for data work up. The instrument software has calculated final "Raw" sample concentration (uncorrected for sample volume filtered or mass weighed, and uncorrected for filter pad or 1 N HCl Blank) in mg P/L from the designated standard curve, and also correcting each concentration for its associated blank response and any user or instrument specified dilution. Dilution by the instrument is noted by software as analysis ensues and documented in the Excel data report file. The analyst examines each row of data. Results are eliminated that are outside the limits of the calibration range, or have an unrepeated blank response measurement greater than 0.001 absorbance units.

12.2 Calculate concentration of Total Particulate Phosphorus or Particulate Inorganic Phosphorus on filter pads from "Raw" sample concentration in mg P/L, normalizing for volume filtered and extraction in 10 mL 1 N HCl:

$$\text{mg P/L} = \frac{(\text{"Raw" Sample mg P/L-Filter Pad Blank mg P/L}) \text{ X } 0.01 \text{ L}}{(\text{Volume Filtered mL/1000 mL})}$$

12.3 Calculate % Phosphorus in Algae or Sediment Samples or CRM from "Raw" sample concentration, normalizing for sample weight and extraction in 20 mL 1 N HCl:

$$\% \text{ P} = \frac{[(\text{"Raw" Sample mg P/L-1 N HCl Blank mg P/L}) \text{ X } 0.02 \text{ L}] \text{ X } 100}{\text{Sample weight in mg}}$$

*Use the 1 N HCl Blank average in this calculation.

12.4 Calculate spike concentrations and recoveries of Total Particulate Phosphorus or Particulate Inorganic Phosphorus on filter pads from "Raw" sample concentration in mg P/L taking blank correction into effect. Do not normalize for volume filtered, sample weight or extraction 1 N HCl.

Spike Value = ("Raw" Sample spike mg P/L-Filter Pad Blank mg P/L)

Original Value = ("Raw" Sample mg P/L-Filter Pad Blank mg P/L)

$$\text{Expected Value} = \frac{(\text{Spike conc. x spike vol.}) + \text{Original Value}}{(\text{sample volume + spike volume})}$$

$$\text{Spike Conc. Added} = \frac{(\text{spike concentration x spike volume})}{(\text{sample volume + spike volume})}$$

Percent Recovery = ((Spike Value-Original Value)/(Spike Conc. Added)) X 100.

13 REFERENCES

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