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Standard Operating Procedure for Determination of Total Dissolved Phosphorus (TDP) and Total Phosphorus (TP) in Fresh/Estuarine/Coastal Waters Using Alkaline Persulfate Digestion of Phosphorus to Orthophosphate (PO4) with Colorimetric Analysis by Random Access Discrete Photometric Analyzer (References Standard Methods #4500-P.B.5, #4500 P.E, and EPA Method 365.1)

Document #: NASLDoc-023

Revision 2025-1
Replaces Revision 2023-1
Effective May 15, 2025

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
Employee (Print)	Employee (Signature)	Date
Employee (Print)	Employee (Signature)	Date
Employee (Print)	Employee (Signature)	Date

Revised by: _____ Date: _____

Reviewed by: _____ Date: _____
Laboratory Supervisor: _____ Date: _____

Changes affecting Revision 2025

3.29 Changed “Material Safety Data Sheets (MSDS)” to “Safety Data Sheets (SDS)”
5.4 Changed “Material Safety Data Sheets (MSDS)” to “Safety Data Sheets (SDS)”
7.6 Updated reagent stability to one week.
13.1 Removed “and then to a Lotus 123 file.”

Determination of Total Dissolved Phosphorus (TDP) and Total Phosphorus (TP) in Fresh/Estuarine/Coastal Waters Using Alkaline Persulfate Digestion of Phosphorus to Orthophosphate (PO4) with Colorimetric Analysis by Random Access Discrete Photometric Analyzer

1. SCOPE and APPLICATION

1.1 Potassium persulfate is used to oxidize organic and inorganic phosphorus to orthophosphate under heated acidic conditions.
1.2 Ammonium molybdate and potassium antimony tartrate react in an acid medium with dilute solutions of orthophosphate to form an antimony-phosphomolybdate complex which is reduced to an intensely blue-colored complex by ascorbic acid. Color is proportional to orthophosphate concentration. The method is used to analyze salinities under 34 ppt.
1.3 A method detection limit (MDL) of 0.0015 mg TDP as PO_4-P/L was determined using the MDL method as specified in the EPA Federal Register 821-R-16-006, titled Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.
1.4 The Quantitation Limit/Reporting Limit for TDP as PO_4 was set at 0.0045 mg TDP as PO_4-P/L.
1.5 This procedure should be used by analysts experienced in the theory and application of aqueous organic and inorganic analysis. A three month training period with an analyst experienced in the analysis of TDP in aqueous samples is required.
1.6 This method can be used for all programs that require analysis of TDP, and is both digested and analyzed in conjunction with Total Dissolved Nitrogen (TDN) samples.
1.7 This procedure references Standard Methods #4500-P.B.5, #4500 P.E, and EPA Method 365.1 (1979).

2. SUMMARY

2.1 An exact amount of filtered sample (whole water for TP) is placed in test tubes where an exact amount of Potassium Persulfate Digestion Reagent is added. Under initially alkaline conditions and heat, nitrate is the sole nitrogen product. As the potassium persulfate continues to oxidize, conditions become acidic and orthophosphate becomes the sole phosphorus product.
2.2 The now digested samples are buffered, then 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 – There are multiple analytical ranges/standard curves used for determination of TDP. See Appendix 1 for all analytical ranges used.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical 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 meter or other device, or the correct value for each setting of a control knob. 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 the 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, 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 ICV, which is analyzed after every 18-23 field sample analysis.

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 internal to the laboratory. (EPA-QAD)

3.19 External Standard (ES) – A pure analyte (potassium 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 Field Reagent Blank (FRB) – An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine

if method analytes or other interferences are present in the field environment.

3.22 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.

3.23 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.

3.24 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., reagent water) 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.

3.25 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)

3.26 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 MDL. (ACS)

3.27 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.

3.28 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).

3.29 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.

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

3.31 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)

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

3.33 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 orthophosphate. 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.

3.34 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)

3.35 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.

3.36 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.

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

3.38 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.

3.39 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.

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

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

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

3.43 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.

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

3.45 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.

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 High silica concentrations cause positive interferences. Silicon at a concentration of 100µM Si causes interferences equivalent to approximately 0.04 µM P.

5 SAFETY

5.1 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 and also inform the CBL Associate Director of Administration and Facilities Maintenance of the incident. Contact the CBL Associate Director of Administration and Facilities Maintenance if additional treatment is required.

5.2 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.

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

5.4 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	
Sodium Hydroxide	3	0	1	ALK, COR	
Sulfuric Acid	4	0	2	ACID, COR	
Ammonium molybdate	4	0	1	Irritant	
Ascorbic Acid	1	0	0	ACID	
Potassium antimonyl tartrate hemihydrate	2	0	0		
Potassium dihydrogen phosphate	2	0	0		
Chloroform	3	0	0		
Hydrochloric Acid	3	0	2	ACID, COR	
Clorox	3	0	0		
Potassium Persulfate	2	0	2	OXY	
Boric Acid	2	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 is heated, 0 - stable

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

6 EQUIPMENT AND SUPPLIES

6.1 Aquakem 250 multi-wavelength automated discrete photometric analyzer. Aquakem 250 control software operates on a computer running Microsoft Windows NT, XP or 7 operating system.
6.2 Freezer, capable of maintaining -20 ± 5° C.
6.3 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, except test tubes, 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.
6.4 Pressure Cooker with pressure regulator and pressure gauge.
6.5 Hot plate with variable heat settings.

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 9.8 N Sulfuric Acid

Sulfuric Acid (concentrated H_2SO_4)	54.4 mL
Reagent water	up to 200 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 the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. The reagent is stable for one year.
7.4 Ammonium molybdate solution

Ammonium molybdate	8 g
Reagent water	up to 100 mL

In a 100 mL plastic volumetric flask, dissolve, with immediate inversion, 8 g of ammonium molybdate, in approximately 90 mL reagent water.

Bring flask to volume. Store flask in dark at room temperature. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance number in the Analytical Reagent log book. The reagent is stable for one month. Discard if white precipitate appears in flask or on threads of cap.

7.5 Potassium antimonyl tartrate solution

Potassium antimonyl tartrate	0.6 g

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

7.6 Ascorbic acid solution

Ascorbic Acid	3.6 g

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

7.7 Triple Reagent

9.8 N Sulfuric Acid	38.2 mL
Reagent Water	1.8 mL
Ammonium molybdate solution	12 mL
Potassium antimonyl tartrate solution	4.0 mL

Add 38.2 mL 9.8N sulfuric Acid and 1.8 mL reagent water 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 6 times to mix. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Reagent is stable for two weeks.

7.8 Orthophosphate Stock Standard, 12,000 µM –

Potassium dihydrogen phosphate (KH_2PO_4), primary standard grade, dried at 45 C	0.816 g
Reagent water	up to 500 mL

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 (1mL contains 12 µmoles P). Add 1 mL chloroform as a preservative. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance number in the Analytical Reagent log book. Make fresh every 6 months.

7.9 Secondary Orthophosphate Standard –

Stock Orthophosphate Standard	1.0 mL
Reagent water	up to 100 mL

In a 100 mL volumetric flask, dilute 1.0 mL of stock orthophosphate standard to 100 mL with reagent water to yield a concentration of 120 µM PO_4 –P/L (1 mL contains 1.2 µmoles P). Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 4 weeks.

7.10 Working Orthophosphate Standards for TDP – See Appendix 1 for all working orthophosphate standards for TDP. Working orthophosphate standards for TDP are made with Secondary Orthophosphate Standard. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh for every digestion batch.

7.11 Glycerophosphate Stock Standard –

B-Glycerophosphoric acid, disodium salt, 5 hydrate	0.0473 g
Reagent water	up to 500 mL

In a 500 mL volumetric flask, dissolve 0.0473 g of glycerophosphoric acid in about 400 mL of reagent water and dilute to 500 mL with reagent water. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance number in the Analytical Standard log book. Make fresh every 6 months.

7.12 Working Glycerophosphate Standard for TDP – See Appendix 1 for all working glycerophosphate standards for TDP.
Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh for every digestion batch.

7.13 Potassium Persulfate Digestion Reagent –

Sodium Hydroxide (NaOH)	3 g
Potassium Persulfate ($K_2S_2O_8$), Low N	20.1 g
Reagent water	up to 1000 mL

In an empty 1000 mL volumetric flask, add 20.1 g of potassium persulfate. Fill the flask to just under the meniscus with reagent water. Dissolve 3g of sodium hydroxide, cap immediately, and mix thoroughly. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance number in the Analytical Reagent log book. Reagent is stable for about 4 hours. See Appendix 1 for potassium persulfate digestion reagent used with Targeted Watershed Samples (TWS).

7.14 Borate Buffer Solution –

Boric Acid (H_3BO_3)	61.8 g
Sodium Hydroxide (NaOH)	8 g
Reagent water	up to 1000 mL

In a 1000 mL volumetric flask, dissolve 61.8 g of boric acid in ~ 300mL reagent water. Add 8g of sodium hydroxide and dilute to 1000mL with reagent water. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number, and balance number in the Analytical Reagent log book. Make fresh every 4 months.

7.15 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 Water collected for TDP should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.
8.2 Prior to initial use, capped 30 mL test tubes must be digested with Digestion Reagent, then rinsed thoroughly with reagent water following laboratory glassware cleaning methods.
8.3 A prescribed amount (typically 10mL) of sample should be added to each sample rinsed, capped 30mL test tube.
8.4 Water collected for TDP should be frozen at ≤-20° C.
8.5 Frozen TDP samples may be stored longer than 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.
8.6 Digested TDP samples may be stored up to three months.
8.7 TDP samples may be refrigerated at 4° C for no longer than one day.

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 Capability
- 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 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed during 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.
- 9.2.3 Method Detection Limits (MDLs) – Initial MDLs should be established for TDP using a spiked water sample, typically two to 10 times the estimated MDL and no more than ten times higher than the

estimated MDL. Process a minimum of seven spiked samples and seven blank samples through all steps of the method. The samples used for the MDL must be prepared and analyzed in at least three batches on three separate calendar dates. If multiple instruments are used for this analysis, MDLs must include data/calculations from all instruments.

9.2.3.1 Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

9.2.3.3 Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

9.2.3.3 Compute the MDLs (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,\ 1-\alpha=0.99)} S_S$$

where:
MDLs = the method detection limit based on spiked samples
$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile
t statistic and a standard deviation estimate with n-1 degrees of freedom.
S_S = sample standard deviation of the replicate spiked sample analyses.

9.2.3.4 Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDLb does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDLb equal to the highest method blank result. If more than 100 method blanks are available, set MDLb to the level that is no less than the 99^{th} percentile of the method blank results. For "n" method blanks where $n \geq 100$, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are ... 1.5,

1.7, 1.9, 5.0, and 10, then 164 x 0.99 = 162.36 which rounds to the 162nd method blank result.
Therefore, MDL_b is 1.9 for n =164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result).
Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.
If all of the method blanks for an individual analyte give numerical results, then
calculate the MDLb as:

$$MDL_b = \bar{X} + t(n-1, 1-\alpha=0.99)S_b$$

where:
MDL_b = the MDL based on method blanks
$\bar{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's t-value appropriate for the single-tailed 99th percentile
t statistic and a standard deviation estimate with n-1 degrees of freedom.
S_b = sample standard deviation of the replicate method blank sample analyses.

9.2.3.5 The verified MDL is the greater of the MDLs or MDLb. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above 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.)

9.2.3.6 MDLs should be determined annually, whenever there is a significant change in instrumental response or a significant change in instrument configuration. Data for annual MDL calculation and verification is analyzed at least quarterly, throughout the year.

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 reagent water treated the same as the samples. An amount of analyte above the MDL (TDP) 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 sample 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 samples and digested reagent blanks is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). These values are derived from stated values of the QCS/SRM. The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5 Calibration Verification, Initial and Continuing (ICV/CCV) – Immediately following calibration (ICV) and following every 18-23 samples, two calibration verifications are analyzed to assess instrument performance. The CCVs are made from the different material than the calibration standards (KH2PO4), and are to be within the expected value ± 3s. 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. Specific CCV's can be found in Appendix 1.

9.4 Assessing Analyte Recovery - Percent Recovery

9.4.1 Analyte recovery is assessed through percent recoveries of laboratory spikes. Analyte recovery is also assessed through the percent recovery of an organic standard that was digested with each batch of samples.

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

9.5 Assessing Analyte Precision – Relative Percent Difference (RPD)

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

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 (LRB) and CCV are tracked daily in the raw data file, copied to Reagent Blank (LRB) and CCV Control Charts.

Table 2

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Correlation Coefficient	≥ 0.998	If <0.998, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.	1 per batch if acceptable.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	± 10%	If QCS value is outside ± 10% of the target value reject the run, correct the problem and rerun samples.	Beginning of run and at end of run.
Initial Calibration Verification (ICV)	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Continuing Calibration Verification (CCV)	± 10%	If outside ± 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 18-23 samples.
Method Blank/Laboratory Reagent Blank (LRB)	≤ Method Quantitation Limit	If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	Following the ICV and after every 18-23 samples following the CCV.

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 problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	1/10 (spike OR duplicate)
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)

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. See Table 1 for the calibrators used for TDP analysis. All calibrators are made in replicates of two. ASTM Type I digested water (reagent water) is used as the zero point in the calibration.

10.2 Working TDP Standards –Appendix 1 defines all working TDP Standards.

10.3 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 0.998.

11 PROCEDURE – DAILY OPERATIONS QUALITY CONTROL

11.1 Organize and label cups for samples to be analyzed. Begin daily bench sheet documentation.

11.2 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.

11.3 Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh reagent water.

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

11.5 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 Washing. When complete, press "Q" to quit until you are able to bring up the Main Page.

11.6 Insert waste cuvette box, and perform Start Up operations by clicking Start Up at the bottom of the Main Page.

11.7 Gather reagents from refrigerator during startup. Assess standards and reagents. Prepare any reagent that has exceeded the time over which it is considered stable.

11.8 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.

11.9 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.

11.10 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.)

11.11 Select the method to be calibrated by clicking Calibr./QC Selection on the bottom of the main page. See Appendix 1 for the method to be calibrated. Click Calibrate at the bottom of the page. The method will now show as pending. Return to the main page.

11.12 Start instrument and calibration by clicking Page Up on the keyboard. This may also be 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

- 165 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/L P 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.

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

11.14 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.

11.15 Once calibration curves are accepted, samples are loaded into the sample segments and loaded into the instrument for analysis. After the Reagent Blank, the first sample analyzed 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.

11.16 Samples are loaded into the segments and analyzed. CCV (Continuing Calibration Verification) samples follow every 18-23 samples. Standard Reference Material (SRM) samples, as well as Laboratory Reagent Blanks (LRB) are scattered throughout the analytical batch. Throughout the analytical batch, samples are chosen 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.

11.17 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.

11.18 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 then named by the run date. The daily

report file for analytical batch of January 3, 2017 would be named 010317. The file is converted to Microsoft Excel for data work up.

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

11.20 Click on Stand By on the bottom of the main page and insert Aquakem Cleaning Solution into the instrument. This initiates shut down procedures. 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 turned off.

11.21 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 PROCEDURE – SAMPLE DIGESTION

12.1 TDN/TDP samples are digested simultaneously in the same ampule. In our procedures, this ampule is a 30 mL screw cap test tube.

12.2 Thaw frozen samples at room temperature.

12.3 Prepare working standards, QCS (CRM), and CCV in labeled 100 mL volumetric flasks:

12.3.1 Select concentration range for both TDN/TDP that best fits the sample batch from Appendix 1. TDN and TDP working standards may be combined in the same volumetric flask.

12.3.2 Fill 100 mL volumetric flasks with 80 mL reagent water.

12.3.3 Add appropriate amount of KNO_3 and KH_2PO_4 to each labeled working standard volumetric flask from Appendix 1.

12.3.4 Add appropriate amount of glutamic/glycerophosphate to each labeled CCV and percent recovery volumetric flask from Appendix 1.

12.3.5 Bring up to 100 mL volume with reagent water.

12.3.6 Mix each 100 mL labeled volumetric flask thoroughly

12.4 Sub-sample working standards into 30mL screw cap test tubes:

- 12.4.1 Prepare 2, 30mL labeled test tubes for each working standard concentration.
- 12.4.2 Sample rinse each test tube with the appropriate working standard.
- 12.4.3 Add exactly 10mL of each working standard to each test tube.
- 12.4.4 Prepare 2 labeled test tubes with exactly 10 mL reagent water for the zero point in the calibration curve.
- 12.4.5 Set aside 2 empty labeled test tubes to be digested with the batch with digestion reagent only (labeled RB).
- 12.4.6 Prepare 2, 30mL labeled test tubes for glutamic/glycerophosphate for percent recovery and CCV by adding exactly 10mL to each test tube.
- 12.4.7 Prepare a Quality Control Sample (CRM) and sub-sample exactly 10mL into a labeled 30mL test tube to be used for QCS.

12.5 Prepare Digestion Reagent by dissolving 20.1 g Potassium Persulfate and 3 g Sodium Hydroxide in a 1000 mL volumetric flask:

- 12.5.1 Rinse volumetric flask with reagent water.
- 12.5.2 Add 20.1 g Potassium Persulfate directly to the volumetric flask.
- 12.5.3 Add reagent water until the meniscus is slightly below full volume.

12.5.4 Add 3 g Sodium Hydroxide to the Potassium Persulfate and water solution, cap immediately and mix thoroughly.
12.5.5 Make fresh daily.
12.5.6 Digestion Reagent has a shelf life of about 4 hours.

12.6 Rinse dispensing vessel with reagent water and sample rinse with digestion reagent.

12.7 Add thoroughly mixed digestion reagent.

12.8 Set dispensing vessel for desired dispensing volume (Typically 5mL).

12.9 Add desired amount of digestion reagent, cap tube, shake for mixing and add test tube to pressure cooker.

12.10 Add desired amount of digestion reagent to the standards at the beginning and end of the sequence of loading the samples.

12.11 When all samples and standards have received digestion reagent and have been loaded into the pressure cooker, place pressure cooker on hot plate, add reagent water until tubes are 75% immersed, wet the gasket on the lid with a few drops of water and place lid on the pressure cooker.

12.12 Turn the hot plate on maximum temperature and have the pressure cooker come up to full steam. (This takes about 1 hour.)

12.13 When full steam is achieved, place the pressure regulator on the steam vent. Maintain heat for the cooker containing samples and standards at 3-4 psi for 55 minutes.

12.14 Turn off pressure cooker and unplug the hot plate when finished. Keep the lid on the pressure cooker.

12.15 After samples have cooled, usually the next day, remove the pressure cooker lid, add 1 mL Borate Buffer to each tube, cap, and shake.

12.16 Sample batch is now ready to analyze and is stable for 3 months.

13 DATA ANALYSIS AND CALCULATIONS

13.1 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 January 3, 2015 would be named 010315. The file is saved to a Microsoft Excel file for data work up. The instrument software has calculated final sample concentration from the designated standard curve, correcting each concentration for associated blank response and also for any user or instrument specified dilution. Dilution by the instrument is noted by software as analysis ensues and, also, 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.

14 REFERENCES

14.1 USEPA. 1979. Method No. 353.2 *in* Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of

Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

14.2 Frank, J. M., C.F. Zimmermann and C. W. Keefe (2006). Comparison of results from Konelab Aquakem 250 and existing nutrient analyzers. UMCES CBL Nutrient Analytical Services Laboratory, Dec. 2006.

14.3 Strickland, J.D.H. and T.R. Parsons. 1965. A Manual of Sea Water Analysis, 2nd ed. Fisheries Research Board of Canada, Ottawa.

Range	umoles PO4/L	mg P/L	ml 2° PO4 std/100ml	Potassium Persulfate	Sample to Persulfate Ratio	Spike Conc.	CCV and % Recovery
TWS TDP	0	0	0	13.4 g K2S2O8 and 2 g NaOH in 2000mL 6.7 g/L K2S2O8 and 1g/L NaOH	5 ml sample 15 ml persulfate	2.5 ml of top std (500 umol NO3 & 12 umol PO4) Added to 2.5 ml of sample prior to digestion	2.0 mL Glycerophosphate
	1.2	0.0372	1.0				
	2.4	0.0744	2.0				
	6.0	0.186	5.0				
	12.0	0.372	10.0				
Low	0	0	0	20.1 g/L K2S2O8 and 3g/L NaOH	10 ml sample 5 ml persulfate	12 umol PO4	1.0mLGlycerophosphate
	0.12	0.0037	0.1				
	0.3	0.0093	0.25				
	0.6	0.0186	0.5				
	2.4	0.0744	2.0				
	4.8	0.1488	4.0				

Appendix 1. Methods and Standards Used for TDP Orthophosphate