**Determination of Total Dissolved Phosphorus (TDP) 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 from 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 Student's t value (3.14, n=7) times the standard deviation of a minimum of 7 replicates.
1.4 The Quantitation 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.
1.7 This procedure conforms to Standard Methods #4500-P.B.5, #4500 P.E, and EPA Method 365.1 (1979).

## 2. SUMMARY

2.1 An exact amount of filtered samples are 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 Table 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. A **preparation batch** is composed of one to 300 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 10 hours. 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 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 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to 3.14 times 7 replicates that make up the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.24 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.

3.25 Laboratory Reagent Blank (LRB) – A blank matrix (i.e., DI 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.

3.26 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)

3.27 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. (ACS)

3.28 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.

3.29 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).

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

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

3.32 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.

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

3.34 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.

3.35 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)

3.36 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.

3.37 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.

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

3.39 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.

3.40 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.

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

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

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

3.44 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.

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

3.46 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 should 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 Business Manager of the incident. Contact the CBL Business Manager 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 Material Safety Data Sheets (MSDS).

| Chemical | Health | Flammability | Reactivity | Contact | Storage |
|---|---|---|---|---|---|
| Sodium Hydroxide | 3 | 0 | 2 | 4 | White Stripe |
| Sulfuric Acid | 4 | 0 | 2 | 4 | White |
| Ammonium molybdate | 2 | 0 | 1 | 2 | Orange |
| Ascorbic Acid | 1 | 1 | 0 | 1 | Orange |
| Potassium antimonyl tartrate hemihydrate | 1 | 0 | 0 | 1 | Green |
| Potassium dihydrogen phosphate | 1 | 0 | 0 | 1 | Green |
| Chloroform | 3 | 1 | 1 | 3 | Blue |
| Hydrochloric Acid | 3 | 0 | 2 | 4 | White |
| Clorox | 3 | 0 | 2 | 4 | White |
| Potassium Persulfate | 2 | 0 | 1 | 0 | Yellow |
| Boric Acid | 2 | 0 | 1 | 2 | Green |

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)
STORAGE
Red – Flammability Hazard: Store in a flammable liquid storage area.
Blue – Health Hazard: Store in a secure poison area.
Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
White – Contact Hazard: Store in a corrosion-proof area.
Green – Use general chemical storage (On older labels, this category was orange).
Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.

## 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 or XP 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 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 |
| Deionized water | up to 200 mL |

In a 200 mL volumetric flask, add approximately 120 mL deionized water. Add 54.4 mL $H_2SO_4$ to the deionized 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 |
| Deionized 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 deionized water. Bring flask to volume. Store flask in dark at room temperature. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot 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

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|---|---|
| Potassium antimonyl tartrate | 0.6 g |

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

7.7 Triple Reagent

| | |
|---|---|
| 9.8 N Sulfuric Acid | 38.2 mL |
| Deionized 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 deionized 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 2 weeks.

7.8 Orthophosphate Stock Standard, 12,000 µM –

| | |
|---|---|
| Potassium dihydrogen phosphate ($KH_2PO_4$), primary standard grade, dried at 45 C | 1.632 g |
| Deionized water | up to 1000 mL |

In a 1 L volumetric flask, dissolve 1.632 g of potassium dihydrogen phosphate in approximately 800 mL deionized water. Bring flask to volume with deionized 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 in the Analytical Reagent log book. Make fresh every 6 months.

7.9 Secondary Orthophosphate Standard –

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|---|---|
| Stock Orthophosphate Standard | 1.0 mL |
| Deionized water | up to 100 mL |

In a 100 mL volumetric flask, dilute 1.0 mL of stock orthophosphate standard to 100 mL with deionized 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 Regular Orthophosphate Standard for TDP – See Table 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 –

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|---|---|
| B-Glycerophosphoric acid, disodium salt, 5 hydrate | 0.0473 g |
| Deionized water | up to 500 mL |
| Chloroform ($CHCl_3$) | 0.5 mL |

In a 500 mL volumetric flask, dissolve 0.0473 g of glycerophosphoric acid in about 400 mL of deionized water and dilute to 500 mL with deionized water. Add 0.5 mL of chloroform as a preservative. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Standard log book.

7.12 Working Glycerophosphate Standard for TDP – See Table 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 –

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

In a 1000 mL volumetric flask, dissolve 3g of sodium hydroxide and 20.1 g of potassium persulfate in ~800mL of deionized water. Dilute to 1000 mL with deionized water. Write the name of preparer, preparation date, reagent manufacturer, manufacturer lot number in the Analytical Reagent log book. Make fresh daily. See Table 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 |
| Deionized water | up to 1000 mL |

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

7.15 Aquakem Cleaning Solution –

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|---|---|
| Clorox | 75.0 mL |

In a 100 mL volumetric flask, dilute 75.0 mL of Clorox to volume with deionized water to yield a concentration of 75% Clorox. Recent (2012) trends in commercially available Clorox, have necessitated altering this formula to 55.0 mL Clorox in 100 mL flask with the addition of 45 mL deionized water. 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 deionized 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 (TDP) – 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) – MDLs should be established for TDP using a low level ambient water sample, typically three to five times higher than the estimated MDL. To determine the MDL values, analyze seven replicate aliquots of water. Perform all calculations defined in the procedure (Sections 11.6-11.8 and Section 13) 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 MDLs should be determined annually, whenever there is a significant change in instrumental response, change of operator, or a new matrix is encountered.

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 Nanopure 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 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. Set up an accuracy chart by using percent recovery since the concentration of the QCS/SRM varies. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5 Continuing Calibration Verification (CCV) – Following every 18-23 samples, two CCV are analyzed to assess instrument performance. The CCVs are made from the different material than the calibration standards (KH2PO4), and are to be within TV ± 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 Table 1.

9.4 Assessing Analyte Recovery - % 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 for each spiked sample should fall within 80-120%. Where:
%SR = (Actual/Expected) 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.

## 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 water is used as the "zero point" in the calibration.

10.2 Working TDP Standards –Table 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 0.997.

## 11 PROCEDURE – DAILY OPERATIONS QUALITY CONTROL

11.1 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.

11.2 Discard any water remaining in the water reservoir from the previous analytical run. Fill the water reservoir with fresh deionized water.

11.3 Organize and label cups for samples that will be analyzed that day. Begin daily bench sheet documentation.

11.4 Once water reservoir is full, "perform washes" – complete five wash cycles and then initiate "start-up" at main menu.

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

11.6 Once startup is complete, check that the instrument water blank of water from the reservoir has performed within acceptance limits. If any of the instrument functions 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.

11.7 Load reagents in specified position in reagent carousel and place in refrigerated reagent compartment.

11.8 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.

11.9 Select the method to be calibrated in the software. See Table 1 for the method to be calibrated.

11.10 Begin calibration – 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.

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

11.12 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.

11.13 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.

11.14 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 or greater to ten percent of the total number of samples in the analytical batch.

11.15 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 calibration range it was run within, the samples can be automatically diluted by the instrument and reanalyzed.

11.16 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, 2005 would be named 010305. The file is converted to Microsoft Excel for data work up.

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

11.18 Aquakem Cleaning Solution is inserted into the instrument and shut down procedures are initiated. Daily files are cleared from the instrument software, the software is exited and the instrument is shut down. The computer is shut down.

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

## 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 Prepare working standards, QCS, and CCV in labeled 100 mL volumetric flasks:

12.2.1 Select concentration range for both TDN/TDP that best fits the sample batch from Table 1.

12.2.2 Fill 100 mL volumetric flasks with 80 mL deionized water.

12.2.3 Add appropriate amount of $KNO_3$ and $KH_2PO_4$ to each labeled working standard volumetric flask from Table 1.

12.2.4 Add appropriate amount of glutamic/glycerophosphate to each labeled CCV and % recovery volumetric flask from Table 1.

12.2.5 Bring up to 100 mL volume with deionized water.

12.2.6 Mix each 100 mL labeled volumetric flask thoroughly

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

12.3.1 Prepare 2, 30mL labeled test tubes for each working standard concentration.

12.3.2 Sample rinse each test tube with the appropriate working standard.

12.3.3 Add exactly 10mL of each working standard to each test tube.

12.3.4 Prepare 2 labeled test tubes with exactly 10 mL deionized water for “0” in the calibration curve.

12.3.5 Set aside 2 empty labeled test tubes to be digested with the batch with digestion reagent only.

12.3.6 Prepare 2, 30mL labeled test tubes for glutamic/glycerophosphate for % recovery by adding exactly 10mL to each test tube.

12.3.7 Prepare 2, 30mL labeled test tubes for glutamic/glycerophosphate for CCV by adding exactly 10mL of the designated CCV solution to each test tube.

12.3.8 Thaw a Quality Control Sample (CRM) sample stored in freezer and sub-sample exactly 10mL into a labeled 30mL test tube to be used for QCS.

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

12.4.1 Rinse volumetric flask with deionized water.

12.4.2 Add 20.1 g Potassium Persulfate directly to the volumetric flask.

12.4.3 Add deionized water until the meniscus is slightly below full volume.

12.4.4 Add 3 g Sodium Hydroxide to the Potassium Persulfate and water solution, cap immediately and mix thoroughly.

12.4.5 Bring to volume with deionized water.

12.4.6 Make fresh daily.

12.4.7 Digestion Reagent has a shelf life of about 4 hours.

12.5 When ready to digest, thaw frozen samples at room temperature.

12.6 Rinse dispensing vessel with deionized 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 deionized 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 1 hour by turning down the temperature setting.

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 and then to a Lotus 123 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 METHOD PERFORMANCE

14.1 On 53 separate dates from May 2009 through September 2010, 63 replicate analyses of SPEX® Corporation QC 6-42 NUT 1 were performed by TDP Alkaline Persulfate Digestion/Ascorbic Acid method. This produced a mean value of 0.2567 mg TDP as $PO_4$-P/L, SD 0.0117, Relative Percent Difference of 4.2% from the expected value of 0.25 ± 10%. This is a mean recovery of 103%.

## 15 REFERENCES


15.1 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.

15.2 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.

15.3 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 | Spike Conc. | Potassium Persulfate | CCV and % Recovery |
|---|---|---|---|---|---|---|
| | 0 | 0 | DI H2O | 2.5 ml of | | |
| TWS TDP | 1.2 | 0.0372 | 1.0 | 400 umole NO3 & | | |
| 5 ml sample | 2.4 | 0.0744 | 2.0 | 12 umole PO4 | 13.4 g/2000 mL and 2 g NaOH | 2.0 mLGlycerophosphate |
| 15 ml persulfate | 6.0 | 0.186 | 5.0 | Added to 2.5 ml | 6.7 g/L and 1g NaOH | |
| | 12.0 | 0.372 | 10.0 | sample prior to | | |
| | | | | digestion | | |
| | 0 | 0 | DI H2O | | | |
| | | | | 12 umole PO4 | | |
| Low | 0.12 | 0.0037 | 0.1 | | 20.1 g/L | 1.0mLGlycerophosphate |
| 10 ml sample | 0.3 | 0.0093 | 0.25 | | and 3g/L NaOH | |
| 5 ml persulfate | 0.6 | 0.0186 | 0.5 | | | |
| | 2.4 | 0.0744 | 2.0 | | | |
| | 4.8 | 0.1488 | 4.0 | | | |

**Table 1. Methods and Standards Used for TDP Orthophosphate**

April 10, 2015