Determination of Total Dissolved Phosphorus (TDP) in Fresh/Estuarine/Coastal Waters Using Alkaline Persulfate Digestion of Phosphorus to Orthophosphate (PO4)

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 all ranges of salinity.
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-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 Colorimeter – Detector found in Bran & Luebbe Single-Channel Industrial Colorimeter. Color is quantitatively detected with 199-B021-04 phototubes using 880 nm monochromatic filters and 50 mm long flow cell with 1.5 mm internal diameter. Comparisons are made between signals from the colored solution in the flow cell to the signal of air in the reference cell. Signals from the Colorimeter are transmitted to a Recorder.

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

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

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

3.18 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.

3.19 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)

3.20 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.

3.21 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.

3.22 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.

3.23 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.

3.24 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to 3.14 times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.25 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.

3.26 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.

3.27 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)

3.28 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)

3.29 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.

3.30 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).

3.31 Manifold – The module whose configuration of glass connectors, fittings, mixing coils, tubing and 37º C heating bath precisely reduces

the antimony-phospho-molybdate complex to an intensely blue-colored complex by ascorbic acid to orthophosphate.

3.32 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.

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

3.34 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.

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

3.36 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)

3.37 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.

3.38 Proportioning Pump – A peristaltic pump that mixes and advances samples and reagents through proscribed precision pump tubes proportionately for the reactions to take place and for the concentration to be measured.

3.39 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.

3.40 Recorder – A graphic recorder used to record electronic output from the colorimeter.

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

3.42 Sampler – An automated rotational device that moves sample cups sequentially to aspirate an aliquot into the proscribed analytical stream. As the loaded sample tray rotates, a metal probe dips into the sample cup and aspirates sample for a preset time, rises from the sample cup and aspirates air for approximately one second and goes into a deionized water-filled wash receptacle, where deionized water is aspirated. After another preset interval, the probe rises from the wash receptacle, aspirates air and moves into the next sample cup. The sampler moves at a rate of 40 samples per hour with a sample to wash solution ratio of 9:1.

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

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

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

3.46 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.

4 INTERFERENCES

4.1 Suspended matter in the sample will restrict flow through the apparatus. All samples must be filtered. See Section 8.

4.2 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
Acetone	1	4	2	1	Red
Clorox	3	0	2	4	White
Potassium Persulfate	2	0	1	0	Yellow
Boric Acid	2	0	1	2	Green
Sodium dodecyl sulfate (SDS)					

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 Technicon Bran & Luebbe AutoAnalyzer II (now owned by Seal Analytical) sampler, proportioning pump, manifold and colorimeter capable of analyzing for TDP as orthophosphate are used in this laboratory. A PMC Industries Flat Bed Linear recorder is used to record electronic output from the colorimeter.

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 4.9 N Sulfuric Acid

Sulfuric Acid (concentrated H_2SO_4)	136mL
Deionized water	up to 1000 mL

In a 1000 mL volumetric flask, add approximately 700 mL deionized water. Add 136mL 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 200 mL

In a 200 mL plastic volumetric flask, dissolve, with immediate inversion, 8 g of ammonium molybdate, in approximately 180 mL deionized water. Bring flask to volume. Store flask in the 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

Potassium antimonyl tartrate	0.6 g

In a 200 mL plastic volumetric flask dissolve 0.6g potassium antimonyl tartrate hemihydrate, in approximately 180 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.

7.7 Ascorbic acid solution

Ascorbic Acid	1.8 g

In a 100 mL plastic volumetric flask dissolve 1.8 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.

7.8 Triple Reagent

4.9 N Sulfuric Acid	75 mL
Ammonium molybdate solution	22.5 mL
Potassium antimonyl tartrate solution	7.5 mL
SDS	0.15 g

Add 0.15 g SDS to a 200 mL Erlenmeyer glass flask. Add 75 mL sulfuric acid to the flask. Carefully add 22.5 mL ammonium molybdate solution to the flask. Carefully add 7.5 mL potassium antimonyl tartrate solution to the flask. Carefully swirl the flask to mix the reagent together. Triple reagent is made and used on the day of analysis only.

7.9 Working Ascorbic acid solution

Ascorbic Acid	50 mL
SDS	0.1 g

In a plastic 50 mL beaker, add 0.1 g SDS. Pour in approximately 30 mL Ascorbic acid solution and swirl. When SDS is dissolved, fill beaker up to 50 mL with ascorbic acid solution. When analysis is complete, cover remaining working ascorbic acid solution with parafilm and store in refrigerator. Reagent is stable for one month.

7.10 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.

7.11 Secondary Orthophosphate Standard –

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.

7.12 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.

7.13 Glycerophosphate Stock Standard –

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.

7.14 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.

7.15 Potassium Persulfate Digestion Reagent –

Sodium Hydroxide (NaOH)	3 g
Potassium Persulfate ($K_2S_2O_8$), Low N	20.1 g
Deinoized 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.

7.16 Borate Buffer Solution –

Boric Acid (H_3BO_3)	61.8 g
Sodium Hydroxide (NaOH)	8 g
Deinozed 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 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 and process through the entire analytical procedure. 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:

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

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 (KH_2PO_4), 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. Four point calibrations are used with the Technicon Bran & Luebbe AutoAnalyzer II in replicates of three. 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 Prepare standard curve by plotting response on recorder of standards processed through the manifold against TDP as PO_4 –P/L concentration in standards.

10.4 Compute sample mg TDP/L concentration by comparing sample response on recorder with standard curve.

11 PROCEDURE – DAILY OPERATION

11.1 Attach pump tubes to end rails of Proportioning Pump. Put platen on Proportioning Pump. Allow deionized water to run through the sample line, deionized water mixed with SDS to run through the diluent line and deionized water mixed with SDS to run through both the Ascorbic Acid and Triple Reagent lines. Check for good flow characteristics (good bubble pattern).

11.2 Turn on Colorimeter and Recorder. Set Colorimeter Standard Calibration setting to 1.00. Let liquid pump through the Manifold and Colorimeter for 15 minutes.

11.3 At the conclusion of the 15 minutes, turn Baseline Knob on Colorimeter to obtain 5 chart units deflection on Recorder.

11.4 Insert the Ascorbic Acid line into the Ascorbic Acid solution and the Triple Reagent line into the Triple Reagent solution. At a Colorimeter Standard Calibration setting of 1.00, note deflection on the Recorder. Reject Triple Reagent if deflection is more than 8 out of total 100 chart units. Turn Baseline Knob on the Colorimeter to obtain 0 deflection on Recorder.

11.5 At desired Standard Calibration (See Table 1), analyze inorganic linearity check also listed in Table 1. Repeat the top standard to check for good replication. If replicates are not within ± 10%, repeat. If repeating fails a second time, remake Triple Reagent components and Triple Reagent solution.

11.6 Analyze Working TDP Standards using the NAP Software Program. (For NAP Software Program procedures, see Appendix A.) The NAP Software Program will prepare standard curve by plotting response on recorder of standards processed through the manifold against TDP as PO_4 –P/L concentration in standards.

11.7 Analyze samples. The NAP Software Program will compute sample TDP as PO_4 –mg P/L concentration by comparing sample response on Recorder with standard curve.

11.8 Change the Standard Calibration setting if a sample peak is larger than 100%. Standard Calibration setting of 8.0 can be turned down to 5.0, then 2.0 and finally 1.0. Calculate the Change in Gain by multiplying the peak height

times 100/55.5 for correcting to a Standard Calibration of 5.0, 100/55.5x100/47.6 for correcting to a Standard Calibration of 2.0, and 100/55.5x100/47.6x100/66.6 for correcting to a Standard Calibration of 1.0. This will give a corrected peak height. Use the corrected peak height with the daily regression in order to calculate the sample concentration in mg/L.

11.9 Allow deionized water to flow through the sample line for 10 minutes. Allow deionized water to flow through the sample, Ascorbic Acid and Triple Reagent lines for one minute. Turn Proportioning Pump switch to fast pump for its allotted time.

11.10 Turn off Sampler, Colorimeter and Recorder. Release and remove Proportioning Pump platen. Release pump tubes from end rails.

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 3, 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 3 labeled test tubes with exactly 10 mL deionized water for "0" in the calibration curve.

12.3.5 Set aside 3 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, middle 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.
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 Lotus 123 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 010315tdnp. The

instrument software has calculated final sample concentration from the designated standard curve in a program called New Analyzer Program (NAP) Software. Dilution by the analyst is noted and recalculated by multiplying the original peak height times the dilution factor to calculate a corrected peak height. Use the corrected peak height with the daily regression to calculate the sample concentration in mg/L. The analyst examines each peak height and peak marker within the NAP Software and compares it to the peak height from the chart recorder, correcting the placement of the peak marker if necessary. Results are eliminated that are outside the limits of the calibration range.

14 METHOD PERFORMANCE

14.1 On 27 separate dates from February through July 2008, 27 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 0.2635 mg TDP as PO_4-P/L, SD 0.0076, Relative Percent Difference of 3.3% from the expected value of 0.255 ± 10%. This is a mean recovery of 103%.

15 REFERENCES

15.1 Technicon Industrial Method No. 158-71 W/A Tentative. 1977. Technicon Industrial Systems. Tarrytown, New York, 10591.

15.2 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.

Range	Pump Tubes	umoles PO4/L	mg P/L	ml 2° PO4 std/100ml	Spike Conc.	Inorganic Check For Linearity	Glycerphosphate for % Recovery	NAP File Created	CCV	Correction Coefficient
		0	0	DI H2O						
TWS TDP 5 ml sample 15 ml persulfate	Red/Red sample Orn/Orn DI w/SDS Std Cal 8.0	2.4 6.0 12.0	0.0744 0.186 0.372	2.0 5.0 10.0	2.5 ml of 400 umole NO3 & 12 umole PO4 Added to 2.5 ml sample prior to digestion	1.8 PO4 1.2 PO4 0.6 PO4 0.3 PO4 0.12 PO4	2 ml Glycerophos.	TWS TDP (group) TWS TDP (samp. table)	1.0 mLGlycerophosphate	2.4 umoles PO4
		0	0	DI H2O						
Low 10 ml sample 5 ml persulfate	Red/Red sample Orn/Orn DI w/SDS Std Cal 8.0	0.6 2.4 4.8	0.0186 0.0744 0.1488	0.5 2.0 4.0	12 umole PO4	1.8 PO4 1.2 PO4 0.6 PO4 0.3 PO4 0.12 PO4	1ml Glycerophos.	Low TDP (group) lowp (samp. table)	0.5 mLGlycerophosphate	2.4 umoles PO4
		0	0	DI H2O						
Low and Salty (Above 30 ppt) 10 ml sample 5 ml persulfate	Wht/Wht sample Wht/Wht DI w/SDS Std. Cal 8.0	0.6 2.4 4.8	0.0186 0.0744 0.1488	0.5 2.0 4.0	12 umole PO4	3.6 PO4 1.8 PO4 1.2 PO4 0.6 PO4 0.3 PO4	1ml Glycerophos.	Low TDP (group) lowp (samp. table)	0.5 mLGlycerophosphate	3.6 umoles PO4

Table 1. Methods and Standards Used for TDP Orthophosphate

April 10, 2015