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EPA 365.1 – Total Phosphorus in Water

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1 Scope and Application

1.1 This method covers the determination of total phosphorus in drinking, ground, and surface waters, and domestic and industrial wastes. This method determines total phosphorus, or if the sample is filtered through a 0.45micron pore size filter, the result is termed total dissolved phosphorus. The difference between the result of a sample determined directly and filtered is termed total insoluble phosphorus. The method is based on reactions that are specific for the orthophosphate ion. The orthophosphate ion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample. Polyphosphates may be converted to orthophosphate by sulfuric acid digestion and organic phosphorus may be converted to orthophosphate by persulfate digestion. This method is modified for use with the Lachat Quikchem Flow Injection Analysis (FIA) System (Quikchem Method 10-115-01-1-F).

1.2 Restricted Procedure
This procedure is restricted to use by an analyst experienced in the operation of a Lachat Quikchem (FIA) System and an Autoclave. Additionally, the analyst must complete the requirements of the GAEPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GAEPD Chemical Hygiene Plan for additional information regarding chemicals required by this method.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Plan (see SOP reference 13.3) for Quality Control Definitions.

2.2. Primary Source (PS) – A standard that is used to make up the calibration points of a curve.

2.3. Second Source (SS) – A standard made from a manufacturer other than that of the primary source.

2.4. Initial Calibration Verification (ICV) – An ICV is a second source standard that is used to verify the correctness of the primary source calibration curve. The ICV is run at a level equal to that of a Laboratory Control Sample (LCS) or the midpoint on the calibration curve.

2.5. Continuing Calibration Check (CCC) or Continuing Calibration Verification (CCV) – A standard used to verify that the response of the instrument has not changed since initial calibration. The CCC is run at a level equal to that of a Laboratory Control Sample (LCS) or the midpoint on the calibration curve.

2.6. Calibration Blank (CB), Initial Calibration Verification Blank (ICB), Method Blank (MBLK), Method Detection Limit Blank (MDLB) or Continuing Calibration Blank (CCB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.

2.7 MDLS (Method Detection Limit Spike) – MDLB spiked with analytes at the lowest calibration level to be used for the determination of MDL.

2.8 LCS (Laboratory Control Sample) and LCSD (Laboratory Control Sample Duplicate) are prepared by spiking laboratory reagent water, Ottawa sand or air sampling device with the target analyte or compound. They are used to validate the analytical batch with respect to accuracy and precision.

3 Interferences

3.1 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant, as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

3.2 Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to precipitation, and subsequent loss, of orthophosphate. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

3.3 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with reagent water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

4 Safety

4.1 Refer to the EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision (See Section 13.8).

5 Apparatus and Equipment

5.1 Analytical Balance, capable of accurately weighing to the nearest 0.0001 g.
5.2 Glassware – Class A volumetric flasks, graduated cylinders and pipettes.
5.3 Lachat Quikchem Flow Injection Analysis (FIA) System designed to deliver and react sample and reagents in the required order and ratios.
5.3.1 Lachat Autosampler
5.3.2 Reagent pump
5.3.3 Reaction unit or manifold
5.3.4 Colorimetric detector with 880 nm interference filter
5.4 Sonicator
5.5 Computer with Microsoft Windows operating system and Lachat Omnion software or equivalent.
5.6 8.0 ml plastic culture tubes, 13mm OD x 100 mm, Fisher Scientific Part # 14-956-8E or equivalent or glass culture tubes 12 mm x 75 mm VWR part # 60825-502 or equivalent for loading samples on auto-sampler.
5.7 Autoclave, with slow exhaust cycle option.
5.8 Glass test tubes for digestion of standards and samples
5.8.1 Standards – VWR 25 x 150 mm Borosilicate glass disposable culture tubes, PN# 47729-586
5.8.2 Samples – VWR 25 x 150 mm glass disposable culture tubes, PN# 89000-510
5.9 Autoclave datalogger and USB datalogger interface – Madgetech RMA-HiTemp 140 or equivalent with NIST certification. Recertification required yearly.
5.10 Air displacement pipettes of various volumes, auto-pipettor, pipette tips in various sizes. Air displacement pipettes and auto-pipettors may also be described as mechanical pipettes.
5.10.1 Each day of use, the volume dispensed by each mechanical pipette must be verified for the specific volume for which the pipette is being used.
5.10.1.1 Mechanical pipette volumes are verified by measuring the weight of a volume of water dispensed by the unit. At room temperature, 1 ml of water is equal to 1g. Mechanical pipettes must be verified to be within ± 2.5 percent of the nominal volume.
5.10.1.2 Mechanical pipettes must be professionally calibrated every 6 months.
5.10.2 Auto-pipettors may be verified by measuring the volume dispensed with a class A graduated cylinder. The volume dispensed must be within ± 2.5 percent of the nominal volume.
5.11 Autosampler racks, 90-position
5.12 Disposable pipette tips, 5-10 ml –VWR PN# 89087-532 or equivalent.
5.13 Test tube racks, autoclavable
5.14 Vacuum source (for degassing)
5.15 Rubber tubing, Serological pipet and rubber stopper to assemble degassing wand.
5.16 HDPE bottles, containing 2.5 ml of 10% sulfuric acid (preservative), for sampling.

5.17 HDPE bottles, various sizes, for storage of standards.
5.18 Glass bottles, dark amber in color, for storage of reagents.
5.19 50 ml Centrifuge Tubes: For standards. VWR Part Number 21008-240, or equivalent.
5.20 Disposable transfer pipettes:
5.20.1 Plastic - VWR® Disposable Transfer Pipets PN# 16001-190 or Fisherbrand™ Standard Disposable Transfer Pipettes PN# 13-711-7M
5.21 Magnetic stir plate
5.22 Magnetic stir bars of various sizes

6 Reagents

6.1 Reagent Water
6.1.1 Purified water which does not contain any measurable quantities of target analytes or interfering compounds for each compound of interest (Deionized, HPLC, Milli-Q water, or equivalent. Milli-Q water has a resistivity of 18.2[MΩ.cm] @ 25°C and a TOC of 50 ug/L or less).
6.2 Stock Ammonium Molybdate Solution:
6.2.1 In a 1L volumetric flask, dissolve 40.0 g of ACS grade ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in approximately 800 ml DI water. Dilute to volume and stir for four hours. Store in plastic bottle and refrigerate. Prepare fresh every two months.
6.2.2 Ammonium Molybdate Tetrahydrate is stable until expiration date on bottle or within 2 years of opening date, whichever is sooner.
6.3 Stock Antimony Potassium Tartrate Solution:
6.3.1 In a 500 ml volumetric flask, dissolve 1.5 g of ACS grade Antimony potassium tartrate (Potassium antimony tartrate hemihydrate $K(SbO)C_4H_4O_6\bullet\frac{1}{2}H_2O$) or dissolve 1.61 g of ACS grade Antimony potassium tartrate (Potassium antimony tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\ 3H_2O$) in approximately 400 ml of reagent water. Dilute to the mark and invert to mix. Store in a dark glass bottle and refrigerate. Prepare fresh every two months.
6.3.2 Antimony potassium tartrate (Potassium antimony tartrate hemihydrate $K(SbO)C_4H_4O_6\bullet\frac{1}{2}H_2O$) and Antimony potassium tartrate (Potassium antimony tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\ 3H_2O$) are stable until expiration date on bottle or within 2 years of opening date, whichever is sooner.
6.4 Molybdate Color Reagent:
6.4.1 To a 500 ml volumetric flask, add about 200 ml Reagent water, followed by 10.5 ml concentrated sulfuric acid (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add 36 ml Stock Antimony Potassium Tartrate Solution and 106.5 ml Stock Ammonium Molybdate Solution. Dilute to the mark with reagent water and invert to mix. Store in a dark glass bottle. Prepare fresh weekly. Degas prior to running analysis.
6.5 Sulfuric Acid

6.5.1 This ACS grade purchased chemical is stable until expiration date on bottle or within 2 years of opening date, whichever is sooner. Store at room temperature.

6.6 Sulfuric Acid Solution(for digestion):
Add 155 ml of concentrated sulfuric acid(H_2SO_4) to approximately 300 ml of Reagent water in a 1000 ml beaker (CAUTION: Solution becomes very hot). Stir well. Once solution has cooled enough for safe handling, transfer to a 500 ml volumetric flask. Dilute to volume. Store in dark glass bottle. Prepare fresh every 28 days.

6.7 Ammonium Persulfate Solution:

6.7.1 Add 10.0 grams of ACS grade ammonium persulfate to approximately 10 ml Reagent water in a 25 ml volumetric flask. Dilute to volume with Reagent water. Prepare fresh daily.

6.7.2 Ammonium persulfate is stable until expiration date on bottle or within 2 years of opening date, whichever is sooner.

6.8 Ascorbic Acid Reducing Solution:
In a 500 ml volumetric flask dissolve 30.0g of ACS grade ascorbic acid in about 350 ml of Reagent water. Dilute to the mark with reagent water and invert to mix. Add 0.5 g of ACS grade sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$). Store in a dark glass bottle. Prepare fresh weekly. Degas prior to running analysis. Note: If analyst notices excessive buildup of blue deposits in instrument lines, the mass of sodium dodecyl sulfate added to the solution may be doubled. Discard if the solution becomes yellow.

6.8.1 Ascorbic acid and Sodium dodecyl sulfate are purchased chemicals that are stable until expiration date on bottle or within 2 years of opening date, whichever is sooner. Store at room temperature.

6.9 Carrier: Sulfuric Acid, 0.13 M:

6.9.1 In a 1L volumetric flask, add 7.2 ml of concentrated sulfuric acid (H_2SO_4) to 500 ml Reagent water. Dilute to mark with reagent water and invert to mix. Prepare fresh weekly. Degas prior to running analysis.

6.10 NaOH-EDTA Solution:

6.10.1 Add 65.00 grams of sodium hydroxide and 6 grams of tetrasodium EDTA to approximately 700 ml Reagent water in a 1000 ml beaker. (CAUTION: Solution becomes very hot). Stir well. Once solution has cooled enough for safe handling, transfer to a 1000 ml volumetric flask. Dilute to volume. Store in a dark glass bottle. Prepare fresh every 3 months. Note: Disodium EDTA may be substituted since this is used for cleaning purposes only.

6.11 1:1 Hydrochloric Acid Solution:

6.11.1 Add 250 ml of reagent water to a clean 1L beaker. Add 250 ml hydrochloric acid. Stir well using a stir bar and allow the solution to cool. Transfer to appropriate acid wash bottle. Note: Use this solution to clean glassware for analysis.

6.11.2 Hydrochloric Acid is commercially purchased and stable until expiration date on bottle or within 2 years of opening date, whichever is sooner. Store at room temperature.

6.12 10% Sulfuric Acid:

6.12.1 Purchased from VWR, Part # BDH3358-4 or equivalent.

6.12.2 This solution is used for preservation of standards and blanks.

6.12.3 This purchased chemical is stable until expiration date on bottle or within 2 years of opening date, whichever is sooner. Store at room temperature.

6.13 Primary Source (PS) Phosphorus Stock Standard A (1000 mg P/L):

6.13.1 Dissolve 4.3962 g of ACS grade anhydrous potassium phosphate monobasic (KH_2PO_4) which has been dried for one hour at 105ºC, in a 1L volumetric flask in approximately 800 ml of Reagent water. Dilute to mark with Reagent water and invert to mix. Refrigerate. Prepare fresh every six months.

6.13.2 Anhydrous Potassium Phosphate Monobasic (KH2PO4 is stable until expiration date on bottle or within 2 years of opening date, whichever is sooner. Store at room temperaturein .

6.14 PS Phosphorus Intermediate Stock Standard Solution B (10 mg P/L):
In a 1L volumetric flask, add 10 ml of Stock Standard A (1000 mg P/L). Dilute to the mark with Reagent water. Invert to mix. Refrigerate. Prepare fresh every three months. Store in 1L HDPE bottle.

6.15 Phosphorus Spiking Solution (100 mg/L):

6.15.1 In a 100 ml volumetric flask, add 10 ml of Primary Source (PS) Phosphorus Stock Standard A (1000 mg P/L) and dilute to volume with Reagent water. Invert to mix. Store in a small dark glass bottle and refrigerate. Prepare fresh every three months.

6.16 Calibration standards:

6.16.1 Prepare working standards from PS Phosphorus Intermediate Stock Standard Solution B (10 mg P/L) as shown in chart below. The calibration standards range from 0.00 mg P/L – 2.00 mg P/L. After the standards are brought to volume with reagent water, 2.5 ml of 10% sulfuric acid per 250 ml of standard is added. Refrigerate. Prepare fresh every three months. Store in 1L HDPE bottles.

Table 6.16.1.1- Working Standards

Stock Standard B (ml)	Final volume in ml	Concentration (mg P/L)
2 ml	1000 ml	0.02
5 ml	1000 ml	0.05
20 ml	1000 ml	0.20
50 ml	1000 ml	0.50
100 ml	1000 ml	1.00
150 ml	1000 ml	1.50
200 ml	1000 ml	2.00

6.17 0.00 mg/L Standard, ICB, CCB, MBLK, MDLB and Dilution water):

Fill a 1000 ml volumetric flask to volume with reagent water. Add 10 ml of 10% sulfuric acid. Cap and mix well. Store in 1L HDPE bottle.

6.17.1 Prepare fresh every 28 days.

6.18 Volumes and amounts of reagents, chemicals and standards may be altered as long as final concentrations remain the same. Sample volumes and injection amounts may be altered as long as required detection limits can be met and sample/reagent ratios remain the same.

6.19 ICV Phosphorus Stock Solution or Second Source (SS):

6.19.1 ICV Phosphorus Stock Solution (1000 P/L)

6.19.2 The ICV stock standard is typically intended as a "QC Sample" but used as a second source standard instead.

6.19.3 This stock standard must be from a different source than the stock standard used to make the calibration standards.

6.19.4 This standard is purchased from a commercially available source. The purchased standard is stable until expiration date on bottle or within 6 months of opening date, whichever is sooner.

6.20 ICV Phosphorus Solution (1.00 mg P/L)

6.20.1 A 1 ml aliquot of the ICV Phosphorus Stock Solution (see 6.19) is pipetted into a 1L volumetric flask and diluted to volume with reagent water. After the ICV is brought to volume, add 10 ml of 10% Sulfuric Acid (See 6.12).

6.20.2 The ICV solution must be prepared fresh every 3 months. Store in 1L HDPE bottle.

7 Sample Collection

7.1 Samples are collected in 250 ml plastic bottles.

7.2 Total Phosphorus sample bottles are pre-preserved with 2.5ml of 10% Sulfuric Acid to a pH of < 2 in the field.

7.3 Sample preservation is checked in the receiving lab at time of receipt.

7.4 Samples are cooled and stored at 0-6° C (not frozen).

7.5 Sample holding time is 28 days.

8 Calibration

8.1 Calibration Standards

The calibration curve consists of the calibration standards at the following concentrations: 0.00 mg P/L, 0.02 mg P/L, 0.05 mg P/L, 0.20 mg P/L, 0.50 mg P/L, 1.00 mg P/L, 1.50 mg P/L , and 2.00 mg P/L.

8.2 Calibration Curve

The Lachat Quikchem is calibrated prior to the analysis of each analytical batch, at the calibration levels listed in section 8.1. Minimum acceptable correlation coefficient is 0.995.

8.3 Calibration Verification

An Initial Calibration Verification standard (ICV), Continuing Calibration Check standard (CCC), and an Initial Calibration Blank (ICB) must be

analyzed immediately after the calibration standards. The ICV standard must be prepared with a stock from a different source than the standards used in the calibration of the instrument. The ICV value must be within 10% of its true value and the ICB value must be less than the method RL or the run will have to be repeated. A Continuing Calibration Check (CCC) and a Continuing Calibration Blank (CCB) must be analyzed every 10 samples, and at the end of the sample run. The CCC must be within 10% of its expected value and the CCB must be less than the RL. The CCC may come from the same source as the calibration standards. If the CCC or CCB does not meet acceptance criteria, then all samples affected by the out of control CCC or CCB are to be rerun.

8.4 A MDLS (low level mdl spike) at the concentration of 0.02 mg P/L must be analyzed with each batch to perform an ongoing MDL study. All batch QC must be valid to report this result.

8.5 A MDLB (MDL blank) must be analyzed once per analytical batch to perform an ongoing MDL study. All batch QC must be valid to report this result.

9 Quality Control

9.1 Refer to Table 14.1 for Reporting Limits (RLs), Appendix A, Table A.1 for Quality Control Acceptance Criteria, and Table 14.2 for Quality Control Procedures associated with this method.

9.2 See SOP reference 13.5. for control charting procedures.

9.3 See SOP reference 13.4. for training and certification procedures.

9.3.1 For Initial Demonstrations of Capability (IDC), Method 365.1 requires a recovery range of 90% - 110% (see SOP calculation 11.6.).

9.3.1.1 The EPD Laboratory sets a 15% RSD requirement for IDC replicates (see SOP calculation 11.4.).

9.3.2 The EPD Laboratory uses the most current accuracy and precision control ranges in use for samples for Continuing Demonstrations of Capability (CDC). If 4 replicates are performed (as opposed to two LCS/LCSD pairs) a 15% RSD is required (see SOP calculation 11.4.).

9.4. Control Limits:

9.4.1 Method 365.1 recommends control limits to be adjusted through the use of control charts. For DW samples, control charting performed every six months will be used to adjust the acceptance limits within the default limits. For all other samples, control charting will be performed every six months for trending monitoring purposes only.

9.4.2 Default control limits for recovery for LCS/LCSD pairs are based on Section 9.3.3 of EPA Method 365.1(SOP reference 13.1.) as noted in Table 9.4.1. The default limits are 90% - 110% recovery.

9.4.3 The EPD laboratory sets default LCS/LCSD precision control limits to 0-15% RPD.

9.4.4 Default control limits for recovery for MS/MSD pairs are based on Section 9.4.2 of EPA Method 365.1. The default limits are 90% - 110% recovery.

9.4.4.1 Method 365.1 section 9.4.1 requires that 10% of all routine samples must be spiked.

9.4.5 MS/MSD default precision limit are set by the EPD lab as 0 – 15% RPD.

9.4.6 In-house limits based on control charts may never exceed the default limits.

9.4.7 See Administrative SOP for Control Charting and Control Limits, SOP reference 13.5 for further details.

9.5 *MDL* (method detection limit) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.

9.5.1 The actual MDL varies depending on instrument and matrix.

9.5.2 The MDL must be determined annually for each instrument prior to results being reported for that instrument. The MDL determined for each compound must be less than the reporting limit for that compound.

9.5.3 The Method Detection Limit Study for all analytes must be performed initially on a new instrument and performed after major instrument repairs or changes to procedures. There are two ways to perform the MDL. The first is with 7 samples and 7 blanks over 3 separate days. The second preferred way the MDL is run as a continuous format.

9.5.4 The 7 MDL samples study is performed by preparing 7 spiked vials, MDLSpike, spiked at the lowest calibration point of the curve, and preparing 7 clean blank vials filled with DI water, MDLBlank. These 7 sets of spiked and blank vial "pairs" are analyzed over 3 separate days, there may or may not be a non-analysis day between each of the 3 days. A total of 14 vials are prepared, 7 spiked and 7 blank.

9.5.5 A continuous format MDL study is performed where one vial is spiked as an MDLSpike, at the lowest point of the calibration curve and analyzed with every batch of samples along with the method blank vial as an MDLBlank.

9.5.6 The results of the MDLBlank will be entered into Labworks using the Method Blank test code, B_TPHOS. The MDLSpike result will be entered using the MLTPHOS. The MDL Spiked Amount will be entered into the test code MATPHOS. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-TPHOS.

9.5.7 MDL study must be performed twice yearly and before the MDL for the instrument expires.

Note: The default control limits are presented to assist in defining control limits established with control chart. Batch acceptance control limits are noted in SOP Table 14.2.

Table 9.4.1 Default Quality Assurance Criteria for Method EPA 365.1

QC Type	Analyte	Accuracy(%R) LCL	Accuracy(%R) UCL	Precision (%RPD)
LCS/LCSD	Total Phosphorus	90	110	15
MS/MSD	Total Phosphorus	90	110	15

9.6 Batching:

9.6.1 Batch samples in groups of 20. For each batch, analyze a Matrix Spike (MS) and a Matrix Spike Duplicate (MSD) for a minimum of 10% of routine samples.

9.6.2 For batches of 1 to 10 routine samples, one MS/MSD pair must be spiked. For batches of 11 to 20 routine samples, two MS/MSD pairs must be spiked using different samples for each pair.

9.6.3 Each batch must have an LCS, LCSD, Method Blank, MDLB, and MDLS.

9.7 MDL Studies:

9.7.1 MDL studies are performed on a continuing basis with each batch of samples analyzed. A report of this MDL data is generated every six months. Alternatively, an MDL study must be performed every 6 months (twice annually) or if instrument maintenance warrants a new MDL study. See SOP reference 13.7. for further details.

10 Procedure

10.1 Sample Preparation

10.1.1 Remove sample bottles, standards, and reagents from cold storage and allow them to equilibrate to room temperature prior to sample preparation and/or analysis.

10.1.2 Air displacement and other mechanical pipettors must have the delivery volume verified each day of use for each specific volume for which the device is used on that day.

10.1.3 Pull a backlog of pending TPHOS samples. Batch in groups of 20. Select QC samples for MS/MSD. QC samples are to be analyzed at a frequency of 10% of samples.

10.1.4 The ICB/CCB/MBLK/MDLB must be poured into a 250 ml sample collection bottle before it is pipetted into the appropriate tubes. Record lot # of bottle used.

10.1.5 The MDLS/0.02 mg P/L standard must be poured into a 250 ml sample collection bottle before it is pipetted into the appropriate tube. Record lot # of bottle used.

10.1.6 Prepare the LCS and LCSD at the 1.00 mg/L level by pipetting 0.25 ml of spiking solution (See SOP section 6.15) into a 25 ml volumetric flask. Dilute to volume with the blank that was stored in a 250 ml sample collection bottle. Volume of LCS and LCSD prepared may be scaled up/down as long as the final concentration remains the same. Record lot # of bottle used.

10.1.7 Prepare the MS and MSD at the 1.00 mg/L level by pipetting 0.25 ml of spiking solution (See SOP section 6.15) into a 25 ml volumetric flask. Dilute to volume with the QC sample. Volume of MS/MSD prepared may be scaled up/down as long as the final concentration remains the same.

10.1.8 Pipette 10 ml of sample and 20 ml of calibration curve standards into the respective assigned test tube and place in rack.
10.1.9 Add 0.2 ml of Sulfuric Acid Solution (See SOP section 6.6) to each 10 ml sample and 0.4 ml to each 20 ml standard.
10.1.10 Add 0.2 ml of Ammonium Persulfate Solution (See SOP section 6.7) to each 10 ml sample and 0.4 ml to each 20 ml standard.
10.1.11 Cover samples and standards with aluminum foil and start up Madgetech Data Logger.

10.2 Procedure for starting Madgetech Data Logger
10.2.1 On the Windows desktop, click the Madgetech icon to launch the Madgetech software.
10.2.2 Insert the data logger into USB Data Logger Interface.
10.2.3 Select the corresponding device on the software (see number on data logger) and click Custom Start.
10.2.4 In the pop up window, select "Now" as the Start Method.
10.2.5 Select "Manual" as the Stop Method.
10.2.6 Make sure the reading interval is set as 5 minutes and then click Start.
10.2.7 The status of the data logger should now say "Running."
10.2.8 Remove the data logger from the USB Data Logger Interface and close the Madgetech software.
10.2.9 Place the data logger in autoclave with the samples for digestion.

10.3 Procedure for use of 3-AUTOCLAVE03, 3-AUTOCLAVE04 and 3-AUTOCLAVE05.
10.3.1 Remove black cap and add Reagent water to tank until it reaches the Max line on the tube. Then replace cap.
10.3.2 Close door and lock it.
10.3.3 Turn the power switch to "ON."
10.3.4 Record Autoclave start time and data logger number on Total Phosphorus Digestion Log.
10.3.5 Press the flask button and then press Start.
10.3.6 Monitor autoclave pressure during cycle. Record pressure on Total Phosphorus Digestion Log.
10.3.7 After the cycle and slow exhaust have completed, the buzzer will sound. Turn power to "OFF."
10.3.8 Record Autoclave end time on Total Phosphorus Digestion Log.
10.3.9 Once autoclave pressure is at 0 psi, slowly crack open autoclave door, just enough to allow steam to escape. Caution: Do not open the autoclave door too quickly, as this may result in an injury from steam.
10.3.10 Once the autoclave has cooled down, remove the sample rack from autoclave using gloves. Caution: Sample tubes and sample rack are extremely hot.
10.3.11 Once samples have cooled to room temperature, they are ready for analysis. Samples can also be stored in the refrigerator, if running them the next day.

10.3.12 Remove data logger from autoclave and refer to Section 10.4 to stop data logger and download temperature readings.

10.4 Procedure for downloading Madgetech Data Logger Readings

10.4.1 On the Windows desktop, click the Madgetech icon to launch the Madgetech software.

10.4.2 Insert the High Temperature Data Logger into the USB Data Logger Interface.

10.4.3 Select the corresponding device on the software (see number on data logger) and click Stop.

10.4.4 Click Download after the device has stopped.

10.4.5 Enter the batch number in the "Choose a report title" pop up window, then click OK.

10.4.6 Click Generate Data Table

10.4.7 Enter the batch number in the "Choose a report title" pop up window, then click OK.

10.4.8 Under the Report Options tab, click Export to Excel.

10.4.9 When prompted "Would you like a chart to be displayed," click NO.

10.4.10 In Excel, save the file as the batch number under My Computer >Inorganic on 'IC PC server(dnr-labnt2)' (I:) > Madgetech datalogger for Tphos Autoclave.

10.4.11 Print the Excel spreadsheet.

10.4.12 Remove the data logger from the USB Data Logger Interface.

10.4.13 Close the Madgetech software.

10.4.14 On the Confirm save pop up window, Click OK.

10.4.15 Place the Excel sheet in your data packet.

10.4.16 Make sure to record the highest temperature reading from spreadsheet on the Total Phosphorus Digestion Log. The autoclave must reach a temperature of at least 121°C for proper sample digestion.

10.5 Instrument Setup

10.5.1 Check pump tubes for wear. Replace if necessary. Snap pump tube cartridges down into cartridge holders and adjust tension levers to tensioned position. Place instrument waste lines in labeled waste container.

10.5.2 Place lines in Reagent water and turn pump on. Turn autosampler power to "ON." Next, turn reagent pump power to "ON." Make sure speed is set to 35 and press Manual Run. Turn Lachat QuikChem power to ON. Pump Reagent water through all reagent lines and check for leaks and smooth flow. If backpressure is noticed, identify the source and perform appropriate maintenance.

10.5.3 If lines need cleaning, place the color reagent and ascorbic acid lines into the NaOH-EDTA solution and the other lines in Reagent water for 5 minutes, and then place all lines in Reagent water for 5 minutes.

10.5.4 Switch lines from Reagent water to their respective degassed reagents and allow system to equilibrate.

10.5.5 Load samples/standards in autosampler. Standards are loaded in order from high to low.

10.5.6 If a sample must be filtered, do so after digestion. If any sample in a batch requires filtration, the LCS, LCSD and Method Blank must also be filtered.

10.6 Instrument/Computer Procedure

10.6.1 On the Windows desktop, click the Omnion icon to launch the Omnion software.

10.6.2 Click the open button to open the folder containing all methods. Open the methods folder. Select the Tphos folder and click on it.

10.6.3 Open the file "TPHOS Template." If prompted to change the heater setpoint, select "Yes." Input sample batch information into the template, and save the file under the total phosphorus folder as the batch number.

10.6.4 Allow 15 minutes for heater warm-up and reagent equilibration. Use the Preview button to monitor the baseline for air spikes, flow problems, instability, etc.

10.6.5 After allowing the system to equilibrate, verify heater temperature is 37°C. Click the start button located above the run worksheet to start the run.

10.7 Printing Custom Reports and Exporting Worksheets

10.7.1 Select the Tools pull down menu and click on custom report. Click on the custom report format (yellow icon) and select layout. Change the value in the "author" field to the analyst's initials. Make sure "show current view" under the charts tab is selected.

10.7.2 Click Apply when all layout modifications have been made. Then close the custom report format menu.

10.7.3 Click on the printer button to print the report. The report should contain the calibration curve.

10.7.4 From the main menu, select the Run pull down menu and click on export worksheet to print the run log. Print two copies of the run log.

10.7.5 After the run, place the color reagent and ascorbic acid lines into the NaOH-EDTA solution and the other lines in Reagent water for 5 minutes and then place all lines in Reagent water for 5 minutes. After Reagent water rinse, allow the lines to pump dry. Remove waste lines from waste container and seal waste container.

10.7.6 To shut down the system, exit the Omnion program and shut down the computer, auto sampler, reagent pump and instrument.

10.7.7 If the response of any sample or QC sample is greater than the high standard 2.0 mg/L, the samples must be diluted, re-digested and rebatched. Dilution ratios should be determined, as nearly as possible, so that the response is near the mid-point of the calibration range. Note: Sample dilution will alter your RL by a proportion equivalent to that of the dilution.

10.7.8 To prepare dilutions, use the Dilution water (SOP Section 6.17) to dilute samples. Note: All diluted samples will have to be re-digested and re-batched.

10.7.9 If both tphos and ophos analyses are required for a sample, and the total phosphorus result is less than the ophos result for that sample, re-analyze the sample for tphos to confirm the results. Tphos results should always be greater than or equal to the ophos result for a given sample.

11 Calculations

11.1 The weighted external standard calibration is calculated by the instrument software and is documented in the instrument software.

11.2 Mean ($\overline{X}$):

$$\overline{X} = \frac{X_1 + X_2 + \cdots X_n}{n}$$

11.2.1 Where:

$X_1 + X_2 + \cdots X_n$ = The sum of a set of values Xi, i = 1 to n
n = The number of values in the set

11.3 Standard Deviation (n – 1) (σn - 1):

$$\sigma_{n-1} = \sqrt{\sum_{i=1}^{n} \frac{(X_i - \overline{X})^2}{n-1}}$$

11.3.1 Where:

$\overline{X}$ = Mean of the values
X_i = Individual values 1 through i
n = Number of values

11.4 Percent Relative Standard Deviation (%RSD):

$$\%RSD = \frac{\sigma_{n-1}}{\overline{X}} * 100$$

11.4.1 Where:

σn – 1 = Sample Standard Deviation
$\overline{X}$ = Mean of the values

11.5 Relative Percent Difference (%RPD or RPD):

$$\%RPD = \frac{|X_1 - X_2|}{\frac{(X_1 + X_2)}{2}} * 100$$

11.5.1 Where:

$|X_1 - X_2|$ = Absolute difference between two values

$\frac{(X_1 + X_2)}{2}$ = Average of two values

11.6 Percent Drift, %Drift:

$$\%\text{Drift} = \frac{(\text{Concentration}_{\text{Calculated}} - \text{Concentration}_{\text{Expected}})}{\text{Concentration}_{\text{Expected}}} * 100$$

11.6.1 Where:

$\text{Concentration}_{\text{Calculated}}$ = Concentration calculated from result
$\text{Concentration}_{\text{Expected}}$ = Theoretical concentration of the standard

11.7 Extract Concentration:

11.7.1 The extract concentration is calculated relative to the calibration curve by the instrument software.

11.8 Percent Recovery:

11.8.1 LCS/LCSD:

$$\%\text{Recovery} = \frac{\text{Conc}_{\text{spiked}}}{\text{Conc}_{\text{expected}}} * 100$$

11.8.1.1 Where:

$\text{Conc}_{\text{spiked}}$ = Concentration found in the spiked sample
$\text{Conc}_{\text{expected}}$ = Expected concentration

11.8.2 MS/MSD:

$$\%\text{Recovery} = \frac{\text{Conc}_{\text{spiked}} - \text{Conc}_{\text{unspiked}}}{\text{Conc}_{\text{expected}}} * 100$$

11.8.2.1 Where:

$\text{Conc}_{\text{spiked}}$ = Concentration found in the spiked sample
$\text{Conc}_{\text{unspiked}}$ = Concentration found in unspiked sample
$\text{Conc}_{\text{expected}}$ = Expected concentration

11.9 Calculation of Dilution Factors

$$C \times D = F$$

11.9.1 Where:

C = concentration from instrument in mg/L
D = dilution factor, if any
F = final concentration in mg/L

12 Waste Management

12.1 Refer to the EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

13 References

13.1 Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio. Revision 2.0, August 1993. Method 365.1.
13.2 Lachat Instruments, QuikChem Method 10.115-01-1-F. Revision 5, December 2007.
13.3 EPD Laboratory Quality Assurance Plan, online revision
13.4 GA EPD Laboratory SOP's – Initial Demonstration of Capability SOP 6-001, online revision and Continuing Demonstration of Capability SOP 6-002, online revision.
13.5 GA EPD Laboratory SOP-EPD Laboratory Procedures for Control Charts and Control Limits SOP, SOP 6-025, online revision.
13.6 GA EPD Laboratory SOP-EPD Laboratory Waste Management SOP, SOP 6-015, online revision.
13.7 GA EPD Laboratory SOP – Determination of Method Detection Limit, Method Detection Limit SOP MDL 6-007, online revision.
13.8 GA EPD Laboratory Safety Plan – EPD Laboratory Safety / Chemical Hygiene Plan & Fire Safety Plan, online revision.

14 Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

Table 14.1 Reporting Limits for EPA 365.1 Total Phosphorus

Parameter/Method	Analyte	Matrix (aqueous)	
		RL	Unit
EPA 365.1	Total Phosphorus	0.02	mg/L

Table 14.2 Acceptance Criteria for Method EPA 365.1 Total Phosphorus

Method	Analyte	Accuracy Water (%R)	Precision Water (RPD)
EPA 365.1	Total Phosphorus	90-110	15

Table 14.3 Summary of Calibration and QC Procedures for Method EPA 365.1

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
EPA 365.1	Total Phosphorus	Initial Calibration for all analytes	Initial calibration prior to sample analysis	Correlation coefficient ≥ 0.995 linear regression	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once per initial calibration or quarterly, whichever is sooner.	Phosphorus concentration within 10% of expected value	Correct problem then repeat initial calibration	
		Initial calibration blank (ICB)	Once per calibration	Phosphorus value must be <RL	Correct problem and repeat initial calibration.	
		Initial Demonstration: Demonstrate ability to generate acceptable accuracy and precision using four analysis of a QC check sample, a method blank and a blind sample. In addition, the analyst must prepare one standard.	Once per analyst	QC Acceptance Criteria Table, SOP 3-014 Appendix A and Initial Demonstration SOP(Reference 13.4)	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	

Table 14.3 Summary of Calibration and QC Procedures for Method EPA 365.1

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
EPA 365.1	Total Phosphorus	Continuing Demonstration: Demonstrate ability to generate acceptable accuracy and precision using a variety of analysis options of a QC sample(s)	Every 6 months	QC Acceptance Criteria Table, SOP 3-014 Appendix A and Continuing Demonstration of Capability SOP(Reference 13.4)	Recalculate results: locate and fix problem with system and then rerun demonstration for those analytes that did not meet criteria	
		Method Blank (MBLK)	One per analytical batch	Phosphorus value must be < RL.	Correct problem then analyze method blank and all samples processed with the contaminated blank	If unable to reanalyze, flag with a "B"
		Laboratory Control Sample (LCS/ LCSD)	One LCS/LCSD pair per analytical batch	QC Acceptance Criteria Table, SOP 3-014 Appendix A	Correct problem then reanalyze the LCS/LCSD and all samples in the affected batch	If unable to reanalyze, flag with a "J"
		Matrix Spike (MS/MSD)	MS/MSD at 10% of all samples over time	QC Acceptance Criteria Table, SOP 3-014 Appendix A	Evaluate out of control event, reanalyze or flag data	
		Continuing Calibration Check	Prior to analysis, after every 10 samples, and at the end of the sample run.	Phosphorus concentration within 10% of expected value	Correct problem then reanalyze CCC and all samples in affected batch	
		Continuing Calibration Blank (CCB)	After every 10 samples and at the end of the sample run.	Phosphorus concentration must be < RL	Correct problem then reanalyze CCB and all samples in affected batch	
		MDL Low level Spike (MDLS) 0.02 mg/L	Once per analytical batch	All batch QC must be valid	Correct problem then reanalyze the affected batch	MDL Low level Spike (MDLS) 0.02 mg/L
		MDL Blank (MDLB)	Once per analytical batch	All batch QC must be valid	Correct problem then reanalyze the affected batch	MDL Blank (MDLB)
		MDL study	Every six months or after major maintenance of the instrument	All Spiked MDLs must have a value greater than 0. Minimum Detection Limits established shall be < the RLs in Table 14.1	Re-do MDL Study	None

Table 14.3 Summary of Calibration and QC Procedures for Method EPA 365.1

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance criteria	Corrective Action	Flagging Criteria
		MDL analysis	Once per batch or as needed to acquire data points per SOP 6-007, online revision	All Spiked MDLs must have a value greater than 0. All other QC in the MDL blank and MDL sample (i.e. Surrogate Spike or Internal Standard, etc. if included) must meet established criteria	Correct problem and re-run the MDL sample or MDL blank once and initiate a corrective action. If the re-run fails a second time, do not use MDL data. Update corrective action, and use associated sample data	None

Appendix A – Quality Assurance Criteria for Method EPA 365.1

Table A.1 Quality Assurance Criteria for Method EPA 365.1

QC Type	Analyte	Accuracy(%R) LCL		UCL	Precision (%RPD)
LCS/LCSD	Total Phosphorus	90	-	110	15
MS/MSD	Total Phosphorus	90*	-	110*	15*

*MS/MSD Control limits are static by EPA Method/EPD Lab default.
Control Charts are generated twice annually for trend monitoring purposes only.
Control Chart data generated from 01/01/2019 - 01/01/2021

Updates to Previous Version:

Section 2
Section 4
Section 6
Section 9
Table 14.3
Appendix A