SOP-C-106

Determination of Orthophosphate as Phosphorus

Revision 16

Approval:

_____ 3-12-24

Laboratory Manager Date

_____ 03-12-2024

Concurrence Date

Effective date: 3-16-24

Renewal date: _____ Initials: _____

Texas Institute for Applied Environmental Research

I. **Identification of the Method**:
   a. Standard Methods 4500P E, latest online edition, approved 2018. *Differentiations* from SM-4500P E:
      i. Narrow range (6-8) pH paper is used to check sample pH. The pH adjustment is not done using addition of phenolphthalein to the samples. The use of pH paper eliminates some contamination concerns.
      ii. Deionized water is used instead of distilled water for all reagents and standards.
      iii. The method calls for glass-stoppered bottles, but Teflon-lined lid may be used instead.
      iv. The same proportion of color reagent is added to sample aliquots, but volumes differ from SM. This is due to equipment size specific to the analytical instrument (0.64 mL CR to 4 mL sample instead of 8 mL CR to 50 mL sample). Waste and costs are also decreased.
      v. Antimony Potassium Tartrate Trihydrate is currently used rather than Antimony Potassium Tartrate Monohydrate.

II. **Applicable Matrices:**
   a. Natural waters, wastewaters, and aqueous extracts

III. **Limits of detection and quantitation:**
   a. Limit of Detection (LOD) about 0.002 mg/L determined annually; Limit of Quantitation (LOQ) at 0.005 mg/L. Low range detection may be extended with longer path cell and lower standards. LODs determined annually.

IV. **Scope and application, including parameters to be analyzed:**
   a. Orthophosphate as phosphorus; Total phosphorus may also be determined by this method upon neutralization after hydrolyzing organic and other forms to orthophosphate in accordance with SOP-C-103, “Determination of Total Kjeldahl Nitrogen and Total Phosphorus”.

V. **Summary of the method:**
   a. Spectrophotometric determination at 880 nm from ascorbic acid-molybdate-salicylate color complex

VI. Definitions:

a. Refer to QAM-Q-101, “Laboratory Quality Control”.

VII. Interferences:

a. Arsenates as low as 0.1 mg/L can react to form a blue color similar to that formed with phosphate.
b. Hexavalent chromium and NO_2- interfere to give results about 3% low at 1 mg/L and 10 to 15% low at 10 mg/L.
c. High iron concentrations can cause precipitation and subsequent loss of phosphorous.
d. The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%. Sample color that absorbs in the photometric range used for analysis will also interfere.
e. Highly colored samples may be analyzed after filtering without addition of AA & APT instead of total color reagent, after which any absorbance may be subtracted from a reacted sample aliquot of the same sample.
f. The neutral pH is crucial to proper color development.

VIII. Safety:

a. Sulfuric acid and sodium hydroxide are reactive and can damage skin and eyes. All aspects of this procedure comply with QAM-S-101, "Laboratory Safety".
b. The color reagent contains acidic, irritative, and toxic chemical species. The technician reads the MSDS for each of the species prior to performing orthophosphate analysis.
c. The analyst always wears protective eye wear, with optional gloves, and a laboratory coat or apron while performing the analysis operations.

IX. Equipment and supplies:

a. Beckman-Coulter DU-640 UV-VIS, Hach DR3900 or Perkin Elmer UV Lambda 365+ spectrophotometer. Other equivalent instruments may be used if results of LOD studies and Demonstration of Performance (DOP) requirements are met. Occasional cleaning of the systems with surfactants or low caustic solutions may be required.

b. 1.0 cm (10mm) flow cell
c. 10.0cm (100mm) sample cell
d. Autosampler (optional)
e. Auxiliary computer with software (optional)
f. Disposable glass or plastic 12x75mm culture tubes.
g. Sample vial boats compatible with autosampler and tubes.
h. Adjustable or small-volume repetitive pipetting dispensers.
i. Alarmed timer/stopwatch.
j. Micro-pipet, glass pipets or 2-5 mL adjustable pipets and tips.
k. Various sizes class A volumetric flasks.
l. Assortment of glass beakers, flasks and graduated cylinders.
m. Analytical balance capable of weighing to nearest 0.1 mg.
n. Mid-range pH indicator paper (pH 6-7 minimum).

X. Reagents and standards:

a. Reagents

i. Ascorbic acid (AA) solution (0.1 M): Add 1.76g of analytical (or better) grade ascorbic acid to a 100 mL class A volumetric flask, bring to volume and dissolve. Store at >0-≤6°C. Stable for one week.
ii. Sulfuric Acid (5.0 N): Slowly stir in 140mL of concentrated sulfuric acid into about 600mL of DI in a 1000mL beaker. Cool the solution to room temperature. Decant solution into class A 1000mL volumetric flask, dilute to volume and mix carefully. Store the solution in a glass stoppered bottle. Some acid may be also diluted for sample pH adjustment, if needed. Stable for 6 months.
iii. Antimony Potassium Tartrate Solution (APT): Add 400mL DI into 500mL class A volumetric flask. Add 1.3715g of analytical grade (or better) antimony potassium tartrate hydrate ($C_8H_4K_2O_{12}Sb_2*3H_2O$). Stir until all of the APT is dissolved. Dilute the solution to 500mL and mix. Store at >0-≤6° C in dark in a glass-stoppered bottle. Stable for 6 months.
iv. Ammonium Molybdate Solution (AM): Add about 400mL DI to a class A 500mL volumetric flask. Add 20g of analytical grade (or better) ammonium molybdate tetra-hydrate $(NH_4)_6Mo_7O_{24}*4H_2O$. Stir the mixture until all of AM is dissolved. Dilute to 500mL and mix. Store at >0-

≤6° C in dark in a glass-stoppered bottle. Stable for 6 months.

v. Color Reagent (or Combined Reagent) (CR): Ensure all solutions above have reached room temperature. Add the following and swirl after each addition in this order: 50 mL of the 5.0 N sulfuric acid solution. 5 mL of the antimony potassium tartrate solution. 15 mL of the ammonium molybdate solution. 30 mL of the 0.1 M ascorbic acid solution. Swirl to homogenize the mixture solution. This solution is good for 4 hours. Other amounts in proportion may be made. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding.

vi. Sodium hydroxide solution (5 N): Add 200g NaOH to 1 liter DI and dissolve carefully. This solution may be diluted for pH adjustment of acidified samples. If acid is needed, use the sulfuric acid above.

vii. Narrow range pH paper (6-8) is used to check sample pH. Record the lot or TIAER Chemical Inventory ID of the paper on all readings.

b. *Standards*

i. Commercially prepared stock standards are acceptable, and may be preferred, if they meet traceability requirements. Use class A glassware or calibrated pipetters for preparation and mix well.

ii. The ICV, CCV, LCS and LCSD are prepared from a source other than that of the calibration standards. The Laboratory Manager may authorize concentration variances and provides direction in the preparation and source of these check standards, but it is normally at the midrange of the calibration curve. The curve equation is listed on the instrument.

iii. Stock phosphate-P solution (1000 mg/L phosphorus): Dry about 5-10 g of potassium dihydrogen phosphate (KH_2PO_4) in an oven at 104° C. Cool in a desiccator. Add 4.390 g of KH_2PO_4 to about 800 mL DI and dissolve. Dilute the solution to 1000 mL and mix again. Store the solution at >0-≤6° C in a dark, glass stock bottle for no more than 6 months.

iv. A spiking solution (500 mg/L) is prepared in the same manner as described above, but using 2.195 g of KH_2PO_4 or equivalent preparation from a commercial standard. Store the solution at >0-≤6° C in a dark, glass stock bottle for no more than 6 months.

v. Working stock solution (WS, 100 mg/L phosphorus): Pipette 25 mL of the stock phosphate-P solution into a 250 mL DI. Store in a dark, glass stock bottle for less than 3 months.

vi. All calibration or other standard dilutions prepared from the WS that are less than 100 mg/L P have only a 48 hour shelf life when stored in refrigeration (same as environmental sample holding time). Different levels of the 6 required calibration standard dilutions may be used with approval of the Laboratory Manager. Calibration standards from the WS are generally: 1.00, 0.75, 0.50 (ICV/CCV/LCS/LCSD also at 0.50, but from a different source than calibration standards), 0.200, and 0.100 mg/L.

vii. 0.005 mg/L LOQ Calibration Standard: 1.0 mL of the 0.500 mg/L calibration standard above or CCV into 100 mL DI.

viii. Stock Laboratory Control Standard (LCS or LCSD) 100 mg/L phosphorus: 1:10 dilution of a 1000 mg/L phosphorus solution.

ix. Laboratory control standard (LCS/LCSD) 0.50 mg/L phosphorus: 0.5 mL of the stock LCS into a 100 mL DI.

x. 0.005 mg/L LOQ/LOQ Calibration Verification Standard: 1.0 mL of the LCS, LCSD or CCV into 100 mL DI.

xi. Lower level standards may be prepared as required by the project QAPP and at the direction of the Laboratory Manager. Generally these standard levels are: ICV/CCV/LCS at 0.05 mg/L, LOQ at 0.001 mg/L with calibration standards between 0.001 and 0.1 mg/L. Performance and acceptance criteria will be met prior to data reporting.

XI. Sample collection, preservation, shipment and storage:

a. TIAER Lab does not collect the samples.
b. Refrigerate sample to >0 to ≤6° C (frozen per some projects).

c. Holding Time: 48 hours. Holding times may be extended for projects as required by specific Quality Assurance Project Plans (QAPPs). Samples are not acidified normally.
d. Shipping priority will be determined by remaining holding times.

XII. Quality control:

a. QC: QAM-Q-101, "Laboratory Quality Control." Samples are filtered immediately (within 15 minutes) after collection or compositing, or identified otherwise.
b. Training: QAM-Q-107, "Laboratory Personnel Training."
c. Instrument operation, calibration and troubleshooting: QAM-I-103, "Operation and Calibration of the UV/Vis Spectrophotometer," and various instrument operations manuals
d. Record all preparation of reagents in the Reagents Logbook and standards in the Standards Logbook (or E-logs).
e. See Q-100, "Quality Assurance Manual."

XIII. Calibration and standardization:

a. Instrument Calibration

i. Label and arrange necessary test tubes in a test tube holder.
ii. Load the test tubes in the autosampler.
iii. After the ten minute color development period (see procedure below), analyze samples within 20 minutes using a 1.000 factor.
iv. Measure and record each value.
v. Transfer the data to the auxiliary computer and print it or record manually as required.
vi. Calculate the factor using the measured absorbance values versus the calculated concentration values by dividing the calculated value of each standard by its measured absorption value to obtain the factor. Each standard is within 75-125% of the expected value when the factor is applied.
vii. After calculating the factor value of each standard, calculate the mean value for the set.
viii. Enter the mean value as the factor value.
ix. All standards must be within 75-125% of the actual values. If not, reanalyze to ensure it actually fails. If it fails again, stop

and initiate corrective action (write CAR, remake the failed standard, and notify the lab manager for guidance).

x. The correlation coefficient of the curve may not be less than 0.995.

xi. This factor is maintained until next calibration. Record the reference to logbook and page number of calibration on all subsequent sample runs using this calibration factor.

xii. The instrument may also be calibrated periodically at the direction of the Laboratory Manager, or when significant alterations or maintenance have been performed on the instrument.

XIV. Procedure:

a. Water Sample Preparation

i. Membrane filter preparation and sample filtration are described in detail in QAM-Q-111, "Aliquot Preparation and Sample Preservation." Filter at least 75 mL of each sample through a 0.45 µm membrane filter for OPO_4-P analysis, possibly more for lower detection levels.

ii. Transfer 25 mL or more of the filtered sample into a 125 mL Erlenmeyer flask labeled with the sample identification number.

iii. Cover and store the samples at >0 to ≤6° C until analysis.

iv. Check the pH of the filtered samples using the multi-range indicator paper. If the pH is not between 6 and 8, adjust dropwise with 5N H_2SO_4 or 5N NaOH (or dilutions of the acid or base as needed).

v. Orthophosphate must be analyzed within 48 hours of sampling time.

b. Instrument Set-up

i. See QAM-I-103, "Operation and Calibration of the UV-Vis Spectrophotometer".

ii. Check all interfaces to ensure they are connected and turned on while allowing instrument to warm-up and stabilize for 30 minutes.

c. Sample Analysis

i. Load test tubes into a test tube rack.

ii. Label all tubes with short content designation.

iii. In each culture tube, pipet 4mL of the designated solution or sample, i.e. DI, standard, QC, sample, spike, etc.

iv. Utilizing a calibrated micropipetter, dispense 0.64 mL of freshly prepared color reagent into each culture tube.
v. Cover with Parafilm® and invert the culture tubes several times to obtain a homogenous solution. Place on the autosampler and begin run after a 10 minute reaction time.
vi. For every batch of samples the following are analyzed: Prep Batch (PB)-an MB, LCS and LCSD; Quality Control Batch (QCB)- sample, sample duplicate, matrix spike, and matrix spike duplicate, CCB and CCV. An LCS/LCSD pair are run with every group of twenty samples or less samples (PB). The LOQ Ck Std is placed at the beginning of a PB and passes acceptance criteria before continuing. If the LOQ Ck Std fails initial and rerun, reblank the instrument and try again. An ICV and IBV are analyzed at the beginning of an Analytical Run Batch (ARB).
vii. Label in the following order: ICB, ICV, IBV, LOQ Ck Std, rLOQ Ck Std, LCS, LCSD, method blank, sample, sample dup, MS, MSD, up to nine more samples not including field splits, CCV and CCB. Non-environmental samples and dilutions of high samples previously run do not count in the batch number.
viii. Field Splits are labeled with the sample number followed by a “FS” suffix. Spikes are labeled with the sample number followed by an “MS” suffix. Sample duplicates are labeled with the sample number followed by a "D" suffix.
ix. Samples that are anticipated or proven higher than 1.0 mg/L must be diluted with DI up to 4 mL in the tube. The dilution factor is recorded for data handling. Dilutions of x10 or higher should be prepared volumetrically.
x. Prepare each spike by pipetting 15 mL of the sample and spiking with 0.015 mL of 500 mg/L P Spiking Solution into a small container. Other volumes may be prepared in proportion.
xi. After the instrument has completed the analysis, transfer the data to the auxiliary computer, print results and affix to personal logbook.
xii. An approved spreadsheet uses the factor to calculate the concentration of phosphorus as orthophosphate in mg/L, if not done automatically by the program.

xiii. Perform all required quality control calculations and control charting in accordance with QAM-Q-101, "Laboratory Quality Control."
xiv. Report the value of each sample in a personal logbook as mg/L orthophosphate as phosphorus. Printouts are affixed in the logbook.
xv. Report all quality control data with the sample concentration data if requested by the client or end user.
xvi. Automated or manual data entry into ESDMS or another database may be used in conjunction with the hard copy. The QC module of ESDMS will display results for data acceptance.

d. Instrument Shut-Down

i. After completing all orthophosphate analysis, the instrument must be placed into the inactive mode.
ii. Remove the samples from the auto-sampler and decant the remaining solutions into a hazardous acidic-waste container.
iii. Final cleanout of the system is an ethanol flush followed by DI. Flush 3 times with each.
iv. Monthly washing with surfactants or cleaning solutions is performed using the above flushing steps. This may performed more frequently if needed, for instance if the analyst observes fluctuations in the baseline.
v. Turn off the lamp.

e. Low level OPO4P instrument modification

i. The Shimadzu has been modified to accept a custom made cell holder of 100 mm path length. The DU-640 may also be used.
ii. Repeat the above procedure without using the autosampler.
iii. Volumes of sample and color reagents are increased proportionally (50 mL & 8 mL, respectively) to obtain a sufficient volume to rinse and fill the longer cell.
iv. React samples in DI-soaked Erlenmeyer flasks.
v. Rinse cell with small amount of reacted sample or standard prior to filling.
vi. Tip the cell at a slight angle when filling to avoid trapping bubbles. Fill completely.
vii. Replace caps in ports at each end of cell.
viii. Wipe both ends of the cell with a lint free cloth or wipe.

ix. Hold the cell lengthwise up to light to inspect for water droplets, bubbles or smudges and remove if present.
x. Carefully place the cell into the holder and read and record the absorbance after reading stabilizes.
xi. Plot absorbance vs. concentration and obtain calculated values for samples and QC from comparing absorbance to this calibration curve. This may be done on an Excel spreadsheet. A previously determined factor may also be used if no significant instrument maintenance or changes have occurred since last calibration. Record the logbook and page number of the calibration data.
xii. Rinse cell with DI. Fill with DI & replace caps for storage.
xiii. Perform all data handling functions as described previously.

XV. Data analysis and calculations:

a. Other than dilutions and calibration, all data analysis or calculations are described in the procedure above. The QC module of ESDMS will perform final calculations on data acceptability. Calibration data source is described on every analytical run.

XVI. Method performance:

a. Refer to QAM-Q-101, "Laboratory Quality Control."

XVII. Pollution prevention:

a. Generally, waste is deposited in the acid waste barrel and neutralized for disposal.

XVIII. Data assessment and acceptance criteria for quality control measures:

a. Refer to QAM-Q-101, "Laboratory Quality Control." The TCEQ may have other data acceptance requirements not listed in the published method, but delineated in project specific QAPPs

XIX. Corrective actions for out-of-control data:

a. Refer to QAM-Q-101, "Laboratory Quality Control."

XX. Contingencies for handling out-of-control or unacceptable data:

a. Refer to QAM-Q-105, "Corrective Actions."

XXI. Waste management:

a. All waste is placed into the proper waste receptacle and disposed of in accordance with QAM-W-101, “Disposal of Laboratory Waste”.

XXII. References:

a. Standard Methods for the Examination of Water and Wastewater, Arnold E. Greenburg, et al., APHA, AWWA, Washington, D.C., Method 4500-P E (approved 2018).
b. National Environmental Laboratory Accreditation Conference TNI standard, 2016, The NELAC Institute (TNI).
c. Reduced Laboratory Detection Limits for Spectrophotometrically Measured Soluble Reactive Phosphorus: A Modified Method, Mark Murphy, TIAER publication TR0211, May 2002.
d. TIAER QAM-Q-101, “Laboratory Quality Control”
e. TIAER QAM-S-101, “Laboratory Safety”
f. TIAER QAM-W-101, “Disposal of Laboratory Waste”
g. TIAER QAM-Q-105, “Corrective Actions”
h. TIAER Quality Assurance Manual, QAM-Q-100 and all laboratory QA procedures
i. TIAER QAM-I-103, “Operation and Calibration of the UV-Vis Spectrophotometer

XXIII. Tables, diagrams, flowcharts and validation data:

a. CR prep
b. Batch setup

a. Color Reagent (CR) Prep:

*50 mL **H_2SO_4** + 5 mL **APT** + 15 mL **AM** + 30 mL **AA***

Good for 4 hours.
10 minute color development
30 minute maximum between addition and reading

c. Batch setup:

1.	ICB (Initial Calibration Blank)	Before autorun start
2.	ICV (Initial Calibration Verification)	0.45-0.55 mg/L
3.	IBV (Initial Blank Verification)	<0.005 mg/L
4.	LOQ Ck Std (Limit of Quanititation)	0.0035-0.0065 mg/L
5.	rLOQ Ck Std (rerun of LOQ Ck Std)	0.0035-0.0065 mg/L
6.	LCS (Laboratory Control Sample)	0.40-0.60 mg/L
7.	LCSD (LCD duplicate)	20% RPD of LCS
8.	MB (Method Blank)	<0.005 mg/L
9.	Sample 1	
10.	Duplicate	20% RPD of Sample 1
11.	MS (Matrix Spike)	75-125% of Sample 1 + 0.5 mg/L
12.	MSD (Matrix Spike Duplicate)	20% RPD of MS
13.	Up to 9 more samples	
14.	CCV (Continuing Calibration Verification)	0.45-0.55 mg/L
15.	CCB (Continuing Calibration Blank) ends QCB	<0.005 mg/L
16.	Repeat 9-15 to end maximum Prep Batch (PB). One PB is about all that can be run with one reaction of CR within the 30-minute maximum.	