

Standard Operating Procedure for Total Phosphorus and Orthophosphate Analysis By AQ300 Discrete Analyzer

Laboratory Services and Applied Sciences Division
US EPA, Region 1 New England
11 Technology Drive
North Chelmsford, MA 01863

Prepared by Holly Westbrook, Chemist, Laboratory Services Branch, LSAD

Reviewed by _____
Paul Toompas, Chemist
Technical Reviewer, Laboratory Services Branch, LSASD

Reviewed by _____
 Maureen Freedman, Environmental Engineer
 Quality Assurance Officer, Laboratory Quality Assurance Branch, LSASD

Approved by _____
 Robert Reinhart, Manager Effective Date _____
 Laboratory Services Branch, LSASD

Note: The effective date is considered to be the last approval date.

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1.0 Scope and Application

The SOP covers the determination of dissolved reactive phosphorus (mostly a measure of orthophosphate) and total phosphorus in drinking, surface, waste and saline waters as well as soils.

2.0 Summary of Method

Ammonium molybdate and antimony potassium tartrate react at acidic pH with orthophosphate ion (PO_4^{3-}) to form an antimony-phospho-molybdate complex. Ascorbic acid reduces this complex to a complex with an intensely blue color. The intensity of the color is proportional to the concentration of orthophosphate in the sample. Orthophosphate is the only form of phosphate that will form the blue complex. All organic forms of phosphorus present in the sample may be converted to orthophosphate form by persulfate digestion.

3.0 Acronyms/ Definitions

3.1 Calibration Blank (CCB) -- A volume of reagent water, the same matrix as the calibration standards but without the analytes.

3.2 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.3 Laboratory Fortified Sample Matrix (LFM or MS/MSD) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.4 Reagent Blank (BLK) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents, that are used with other samples. The BLK is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus. Analyzed after CCV (every 10 injections)

3.5 Continuing Calibration Blank (CCV)-- An individual CAL solution which is analyzed after every tenth injection which verifies the previously established calibration curves and confirms accurate analyte quantitation for the previous ten injections analyzed.

3.6 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.

3.7 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. 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.

3.8 Instrument Calibration Verification (ICV) -- A solution prepared from a second source to test the performance of the calibration with respect to a defined set of criteria.

4.0 Health and Safety Warnings

Sulfuric acid used in this method can cause severe burns and should be handled by an analyst trained to work with this chemical. Gloves and protective clothing must be worn, and chemicals should be kept under a fume hood. The reagents used are toxic and similar precautions should be taken when handling them. Safety information is available in the form of SDS sheets and can be obtained on the internet. Links are available on the Region 1 Intranet site.

5.0 Interferences

- Silica forms a pale blue complex that absorbs at 880 nm. This interference is generally insignificant at silicate concentration of approximately 50 mg SiO₂/L would be required to produce a 0.0008 mg P/L positive error in orthophosphate.
- High iron concentrations can cause phosphorus to precipitate out of solution.
- Phosphorus contamination is a common problem. For that reason, commercial detergents should never be used to clean glassware. All glassware is cleaned with 10% HCl and rinsed with distilled deionized water. The glassware must be dedicated to the phosphorus methods and be rinsed with DI water after use.
- Sample turbidity must be removed by filtration prior to analysis for orthophosphate. Samples for total phosphorus should be filtered only after digestion if needed.

6.0 Personnel qualifications

The analyst should have at least 4-year degree in physical science. The analyst must have a satisfactory IDC/MDL in place before analyzing samples. All personnel shall be responsible for complying with all QA/QC requirements that pertain to their organizational/technical function.

7.0 Equipment and Supplies

- Balance - analytical, capable of accurately weighing to the nearest 0.0001 g.
- Glassware - Class A volumetric flask and pipettes or plastic containers as required.
- DigiTubes - Acid-washed
- Discrete Analyzer – SEAL Analytical AQ300

8.0 Procedures

8.1 Sample collection and preservation

- Sample containers may be plastic or Pyrex glass.
- For total phosphorus: If the analysis cannot be performed the day of collection, the sample should be preserved by addition of 1 mL concentrated H_2SO_4 per liter, preferably in the field, and refrigerated at 4°C . Sample analysis must be performed within 28 days of sample collection.
- For orthophosphate: Samples need to be filtered upon collection, refrigerated at 4°C , and analyzed within 48 hours. Samples do not require preservation.

8.2 Reagents and Standards

All reagents and standards should be stored in the appropriate bottles and labeled with the following information:

Manufacturer, Lot number, Date of preparation, Date of expiration,
Concentration, Initials of Preparer

Use DI water for all reagents when analyzing drinking, surface, waste waters and soils where salinity is not an interferent.

NOTE: For samples with high salinity (25 ppt or above), all solutions should be prepared in ASTM D1141 certified synthetic sea water. Synthetic sea water should be used as diluent for calibration standards, QC standards, blank (orthophosphate), and used to prepare Reagent 3 when analyzing total phosphorus samples. *Historical data has shown Reagents 1*

and 2 do not need to be prepared in sea water diluent for matrix matching for high salinity samples.

- **Reagent 1. Molybdate Color Reagent**

- Total phosphorus

- To a 100 mL volumetric flask, add about 50 mL of DI water and then add 0.020 g potassium antimonyl tartrate and swirl to dissolve. Then dissolve 0.80 g ammonium molybdate. Add 5.6 mL concentrated sulfuric acid and swirl gently to mix. CAUTION: The solution will get hot during preparation. Once cool, dilute to the mark and invert to mix. Store at 4°C in a plastic bottle. Stable for 3 weeks. Discard if the reagent turns blue or becomes turbid.

- Orthophosphate

- To a 100 mL volumetric flask, add about 50 mL of DI water and then add 7 mL of concentrated sulfuric acid CAUTION: The solution will get hot during preparation. Once cool, add 0.3 g of ascorbic acid. Swirl to dissolve, then add 0.0137 g of potassium antimonyl tartrate. Swirl to dissolve, then add 0.6 g ammonium molybdate. **Ammonium molybdate must be added last.** Swirl to dissolve then fill to 100 mL with DI. Make fresh daily and keep refrigerated when not in use. Discard if reagent turns blue or becomes turbid.

- **Reagent 2. Ascorbic Acid Reducing Solution, 15 g/L, TP Only (with phosphate spike)**

- To a 100 mL volumetric flask, add about 80 mL of DI water and dissolve 1.5 g ascorbic acid. Spike with 1.0 mL of Working Standard 1 (10 ppm $(\text{PO}_4)^{3-}\text{-P}$). Swirl to mix and dilute to the mark with DI water. Store at 4°C. Stable for 1 week. Discard if the solution becomes yellow.

- **Reagent 3. Sulfuric Acid, 0.036 M (blank and diluent for total phosphorus only)**

- To a 1L volumetric flask, add 500 mL water and 2 mL concentrated sulfuric acid. Once cool, dilute to the mark and shake to mix. For high salinity samples, this reagent must be made in sea water. Store up to 6 months.

- **Reagent 4. Sulfuric Acid, 5.6M (For persulfate digestion)**

- To a 1 L volumetric flask, add about 500 mL DI water and 310 mL conc. sulfuric acid. Dilute to the mark and shake to mix. Store at room temperature for up to 6 months.

- **Reagent 5. DI Water:** Blank and diluent for orthophosphate only.
- **Reagent 6. ASTM D1141 certified synthetic sea water:** Blank and diluent for high salinity orthophosphate samples. Used to make Reagent 3 in high salinity samples.
- **Reagent 7. Ammonium persulfate ($(\text{NH}_4)_2\text{S}_2\text{O}_8$):** Use in digesting total phosphorus samples.
- **Reagent 8. Special Cleaning solution:**
1.0g of EDTA (FW 372.2) plus 4.0 g of NaOH (FW 40) to 500 mL with DI water. Store at room temperature for up to 2 months.
- **Reagent 9. SEAL Cleaning Solution:** Provided by the vendor.

8.3 Calibration and Standardization

Three different stock standards (lot or vendor) solutions (1000 ppm orthophosphate as P solution), commercially prepared and purchased. Certificate of analysis required. One is used for calibration standards, second for ICV, and the third one for LFB/MS/MSD.

8.3.1 **Working standard 1:** 10 ppm $(\text{PO}_4)^{3-}$ as P: To a 100 mL volumetric flask, add 1.0 mL of stock standard 1 and dilute to the mark with DI water. One autocalibration standard needs to be prepared for the calibration curve, as well as one CCV. Prepare fresh before every run.

Auto-calibration standard: 0.2 ppm $(\text{PO}_4)^{3-}$ as P: To a 25 mL volumetric flask add 0.5 mL of working standard 1 and dilute to the mark with diluent.

CCV: 0.08 ppm $(\text{PO}_4)^{3-}$ as P: To a 25 mL volumetric flask add 0.20 mL of working standard 1 and dilute to the mark with diluent.

Calibration Curve:

Calibration Point	1	2	3	4	5	6	7	8
Concentration as $\mu\text{g (PO}_4\text{)}^{3-}$ as P/L	0	8*	16	20	30	60	120	200
		12**						
Percent of auto calibration standard used	-	4.0*	8	10	15	30	60	100
		6.5**						

* For Orthophosphate

** For Total Phosphorus

8.3.2 **Working standard 2:** 10 ppm $(\text{PO}_4)^{3-}$ as P: To a 100 mL volumetric flask, add 1.0 mL of stock standard 2 and dilute to the mark with DI water. Used for ICV quality control sample. Prepare fresh before every run.

ICV: 80 ppb $(\text{PO}_4)^{3-}$ as P: To a 25 mL volumetric flask add 0.20 mL of working standard 2 and dilute to the mark with diluent.

8.3.3 **Working standard 3:** 2 ppm $(\text{PO}_4)^{3-}$ as P: To a 100 mL volumetric flask, add 0.2 mL of stock standard 3 and dilute to the mark with DI water. Used for LFB and MS/MSD quality control samples. Prepare fresh before every run.

LFB Low:

OPHOS: 8.0 ppb $(\text{PO}_4)^{3-}$ as P: To a 25 mL volumetric flask, add 0.1 mL of working standard 3 and dilute to the mark with diluent.

TP: 12 ppb $(\text{PO}_4)^{3-}$ as P: To a 25 mL volumetric flask, add 0.15 mL of working standard 3 and dilute to the mark with diluent.

LFB: 80 ppb $(\text{PO}_4)^{3-}$ as P: To a 25 mL volumetric flask add 1 mL of working standard 3 and dilute to the mark with diluent.

MS/MSD: 80 ppb $(\text{PO}_4)^{3-}$ as P:

Waters: To a 25 mL volumetric flask add 1 mL of working standard 3 and dilute to the mark with sample.

Soils: To a 25 mL volumetric flask add 1 mL of working standard

3, about 0.25 grams of sample, and dilute to the mark with Reagent 3.

8.4 Sample Preparation

8.4.1 Total Phosphorus (aqueous):

- 8.4.1.1 Begin heating autoclave prior to sample preparation.
- 8.4.1.2 Prepare samples and standards as described in section 8.2.
- 8.4.1.3 Take an acid-washed DigiTube and measure out 25 mL of sample or standard using the marks.
- 8.4.1.4 Add 0.5 mL of (Reagent 4) 5.6 M sulfuric acid
- 8.4.1.5 Add 0.2 g ammonium persulfate (Reagent 7).
- 8.4.1.6 Remove the plastic liner from the caps and place them slightly ajar on all samples to release any pressure.
- 8.4.1.7 Place samples in the autoclave, seal the chamber and start the preloaded method that is set to digest samples for at least 30 min at 121°C at constant pressure.
- 8.4.1.8 After cycle is finished, release pressure to open doors and remove samples.
- 8.4.1.9 Allow to cool to room temperature and analyze samples. No adjusting of volume is required in this method.

8.4.2 For orthophosphate, samples do not need any preparation.

8.4.3 Total Phosphorus (soils):

- 8.4.3.1 Take an acid washed DigiTube and add approximately 0.25 g of sample and bring it up to 25 mL volume mark with Reagent 3.
- 8.4.3.2 Add 0.5 mL of (Reagent 4) 5.6 M sulfuric acid.
- 8.4.3.3 Add 0.2 g ammonium persulfate (Reagent 7).
- 8.4.3.4 Remove the plastic liner from the caps and place them slightly ajar on all sample to release any pressure.
- 8.4.3.5 Place samples in the autoclave, seal the chamber and start the preloaded method that is set to digest samples for at least 30 min at 121°C at constant pressure.
- 8.4.3.6 After cycle is finished, release pressure to open doors and remove samples.
- 8.4.3.7 Allow to cool to room temperature and analyze samples.

8.5 Analysis

- 8.5.1 Allow at least 40 minutes for the lamp to warm up.

- 8.5.2 For orthophosphate, samples should be brought to the room temperature prior to analysis.
- 8.5.3 Perform the daily AQ300 daily startup.
- 8.5.4 Place samples and standards in the carousel.
- 8.5.5 Fill reagent wedges and place in the correct positions and start the analysis.
- 8.5.6 AQ300 Analytical Sequence:
 - Auto calibration standard
 - ICV
 - CCV
 - BLK
 - LFB
 - Samples
- 8.5.7 At the end of the run, fill a reagent wedge with SEAL Cuvette Cleaning Solution (Reagent 9) and place it in position 1 of the reagent rack. Run an extra wash within the SEAL software.
- 8.5.8 If a large volume of orthophosphate or total phosphorus has been run, run the extra wash with the Special Cleaning Solution (Reagent 8).

9.0 Data and Record Management

9.1 Data Analysis and Calculations

Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample's concentration response with standard curve. Sample concentration is calculated from regression equation automatically. The computer yields results directly in $\mu\text{g/L}$ as $(\text{PO}_4)^{3-}$ as P.

9.2 Reporting Limits

For aqueous samples, the RL is equal to the lowest calibration standard.

For soil samples: using 25 mL reflux tube:

$$\text{RL (mg/Kg)} = \frac{0.025 \text{ L} \times \text{low cal. std. (ug/L)}}{\text{wt. (g)}}$$

9.3 Data Package

All standards must be traceable back to the original vendor stock and that standard must be identified in detail (vendor, lot number, and certificate). Each data package must include a copy of the following:

- Sample preparation sheet
- Standards preparation sheet
- Reagent preparation sheet

- Raw instrument data and analytical sequence (reports from SEAL instrument).

Prep sheets are available in Y:\LSB\Wetchem\Forms

9.4 Project review

Upon completion of a project a project review form should be filled out and accompany the final report in the report folder. The first section (requested analysis and data folder completeness check) should be completed by the analyst. The last two sections (data evaluation and final report) should be completed by two different chemists that have knowledge of the method. Project Review forms are available in Y:\LSB\Wetchem\Forms

10.0 Quality Control and Quality Assurance

10.1 Demonstration of capability

The analyst must make an initial demonstration of capability (IDC) to generate acceptable accuracy and precision with this method. Continuing displays of proficiency (C-DOP) are repeated annually and each time a method modification is made.

10.2 Lab Reagent Blank (or BLK):

One per each batch of 20 samples. Use 25 mL of distilled deionized water or other blank matrices treated in the same manner as sample. Because silica forms complexes that absorb at the same 880 nm as phosphate, DI water is used for both water and soil blanks to avoid false positive results from using silica-based sand as a soil blank.

10.3 Lab Fortified Blank (80 ppb orthophosphate):

One per 20 samples. Used for waters and soils.

10.4 Matrix Spike and Matrix Spike Duplicate (80 ppb orthophosphate):

One of each per 10 samples or less.

10.5 Initial Calibration:

An initial calibration is performed by analysis of a blank and at least five standards. The correlation coefficient must be less than or equal to 0.990 for the calibration to be valid. Investigate and apply corrective action if criterion is not met.

10.6 Initial Calibration Verification Standard (80 ppb orthophosphate):

Middle level of initial calibration standards. Recoveries must be within

10% of the true value, otherwise re-calibration of the instrument is necessary.

10.7 Continuing Calibration Verification Standards (80 ppb orthophosphate):

Analyze CCV after the ICV, after every ten samples, and at completion of analysis. Recoveries must be within 10% of the true value. If recoveries fall outside of this range the cause of the failure needs to be determined, corrected, and the instrument may need to be recalibrated.

10.8 Duplicate sample analysis (DUP):

One per ten samples or less.

10.9 Method Detection Limit:

A low LFB of a concentration two to three times the last calculated MDL is run with each analysis. At least once every thirteen months the MDL_s is calculated from low LFBs and the MDL_b is calculated from the blanks as described in 40 CFR 136 Appendix B. The verified MDL is the greater of the two values and should be less than half the reporting limit. Acceptance criteria are given in the Appendix 1 Table. If acceptance criteria cannot be met, corrective actions must be taken and if possible, the samples must be redistilled and re-analyzed within holding time. The laboratory maintains performance records to document the quality of data that is generated.

11.0 Pollution Prevention and Waste Management

LSASD encourages all chemist and biologists to investigate micro analytical techniques, innovative technologies, and chemical substitution in laboratory processes to reduce waste and prevent pollution. As analytical SOPs are reviewed, on an annual basis, the responsible chemist or biologist will incorporate waste minimization practices where practicable and where these practices have been demonstrated to return data of equivalent quality.

Chemists and biologists must refer to the Waste Management Program SOP for proper disposal of laboratory waste. Personnel should contact the Environmental, Safety and Health Department if changes in the analytical SOP will generate new waste streams. Questions regarding the proper disposal of laboratory waste and purchase of new reagents should be directed to the Environmental, Safety and Health Department in advance of actually initiating a change in the analytical method.

Effluent from the channels as well as the sample effluent are acidic. They need to be disposed of in a labeled waste satellite container.

12.0 Preventive maintenance & Troubleshooting

Daily maintenance includes emptying and replacing the rinse water, replacing used reaction wells, and running the “Daily Startup” function in the software to rinse the cuvette and perform the daily water baseline check. Verify smooth movement and good aspiration.

Weekly maintenance includes cleaning the sampler and aspiration wash baths, inspecting the probes to make sure they are straight/clean/not dripping, and verifying that the baseline voltages don’t indicate that the lamp needs changing.

Monthly maintenance includes replacing and lubricating the pump tubes, bleaching the wash container with a 2% bleach solution, and removing and washing the fan air filter.

Six month maintenance includes replacing the syringe assembly/o-ring, cleaning and lubricating the syringe screw drive, replacing the lamp, replacing the reagent wedges, replacing the probe wash assembly, and inspecting the cuvette tubing and probes.

The molybdate to acid ratio is very important for the phosphate chemistry. If this ratio is off, then the curve will be quadratic. If curve is quadratic, check age of the acid and molybdate solutions. Remake color reagent and stocks.

If running a lot of phosphate on the AQ300, it is recommended to run the Special Cleaning Solution (section 8.4) to clean the cuvette. To do this, fill a reagent wedge with Special Cleaning Solution and place it in Position 1 of the reagent rack. Within SEAL AQ software, go to Maintenance—Cuvette Functions and select Extra Wash.

13.0 Method References

- 1) Method 365.1, Determination of Phosphorus by Semi-Automated Colorimetry, Revision 2.0 August 1993.
- 2) AQ300 Method EPA-156-D Rev. 1 o-Phosphate-P in Water.
- 3) AQ300 Method EPA-119-D Rev. 3A Phosphorus-P, Total, in Surface and Saline Waters, Domestic and Industrial Wastes
- 4) Soils digestion - method developed in LSASD

Appendix I: Acceptance Criteria

QA/QC Sample	Frequency	Acceptance Criteria	Corrective Action	
			Orthophosphate (OP)	Total Phosphorus (TP)
Blanks: Digested for T.P. Not digested for OP	1 per batch (up to 20 samples) after calibration, continuing calibration, every 10 injections and at the end of the run	< RL	-	If results for samples are below RL, report all data. If results for samples are less than 10 times of the blank value, re digest entire batch. If not enough sample, qualify all data with B and write an explanation.
Initial Cal. Verification (ICV)	After calibration	$\pm 10\%$ of true value	Prepare new calibration curve, prepare a new ICV if failed, terminate analysis, correct problems, re analyze all samples	Re prep entire batch
Continuing Calibration Verification (CCV)	After ICV, every 10 injections and at the end of the run	$\pm 10\%$ of true value	Prepare new calibration curve. Prepare new CCV if failed, terminate analysis, correct problems, re analyze all samples	Re prep entire batch
Laboratory Fortified Blank (LFB)	1 per batch (up to 20 samples)	$\pm 10\%$ of true value	Prepare new calibration curve and LFB. If failed, terminate analysis; correct problems, re analyze all samples	Re prep entire batch. If not enough sample is available, qualify all data.
MS/MSD	1 per 10 samples	$\pm 10\%$ of true value for drinking water control limits for soils and wastewater	Qualify data for the sample if LFB is within spec.	

Laboratory Duplicate	1 per 10 samples	$\pm 10\%$ RPD for drinking water $\pm 20\%$ RPD or control charts for soils and waste water ¹	Qualify data (J) for sample with explanation	
Samples Samples Holding Time	Section 8.1	Samples must be analyzed within holding times	If re-sampling is not available, results are estimated (J) for all samples and project notice submitted with the report.	
IDC/LOQ	One a year per analyst	4 replicates at concentration 2 to 5 times reporting limit 80–120% recovery <20% RSD ¹	Correct problems; reanalyze all samples.	Correct problems; re-prep and reanalyze all samples
MDL	Analyze a low LFB with each run and calculate at least once every thirteen months.	Half RL	Correct problems and repeat.	Correct problems and repeat.
QCS	Run as PT studies.	-	-	

¹= Acceptance criteria calculated from in-house historical data (control charts)

Appendix 2: Suggested timing parameters for total phosphorus and orthophosphate:

Orthophosphate

PARAMETER	AQ SETTING
Test name	o-PHOS_low
Units	mg P/L
Decimals	3
Test type	End Point
Sample volume (μL)	500
Water volume (μL)	0
Number of mixes	2
Cuvette primes	4
Cuvette washes	2
Baseline on Wash	Ticked
Reaction time (seconds)	540
Wavelength (nm)	880
Polynomial order	1
Number of reagents	1
1. PO4 color (μL)	40
Advanced Test Parameters	<input checked="" type="checkbox"/> Extra Debubbling Action

Total Phosphorus Test Parameters

PARAMETER	AQ SETTING
Test name	T-PHOS 1
Units	mg P/L
Decimals	4
Test type	End Point
Sample volume (μL)	300
Water volume (μL)	0
Number of mixes	2
Cuvette primes	4
Cuvette washes	2
Baseline on Wash	Ticked
Reaction time (seconds)	480
Wavelength (nm)	880 (or 660)
Polynomial order	1
Number of reagents	2
1. TP Working Color (μL)	140
2. TP Ascorbic Acid (μL)	65
Advanced Test Parameters	<input checked="" type="checkbox"/> Extra Debubbling Action

Appendix 3: Method Comparison

Method	EPA Series Method 365.1	LSBSOP-OPHOS-TP13
Parameter		
Applicability	Drinking, surface, domestic and industrial wastes.	Drinking, surface, domestic and industrial wastes
Number of Analytes	Phosphorus	Total Phosphorus and Orthophosphate
Method Validation	<p>Initial demonstration of performance:</p> <ol style="list-style-type: none"> 1. The Linear Calibration Range (LCR) must be determined initially and verified every 6 months. The verification of linearity must use a minimum of a blank and 3 standards. 2. A Quality Control sample (QCS), an independent standard, is prepared and analyzed at least quarterly to verify the calibration standards and instrument performance. If not within $\pm 10\%$ of stated value, determine source of problem and correct before continuing with analyses. 3. Determine MDLs by analyzing seven replicates of Laboratory fortified blanks at concentration of 2 to 3 times the estimated detection limit. MDLs must be determined every six months. 	<p>Initial demonstration of performance:</p> <ol style="list-style-type: none"> 1. The Calibration Range (CR) must be determined initially and verified every run. A blank and 6 standards used for calibration 2. An independent standard (ICV) analyzed with every batch to verify the calibration standards and instrument performance. Acceptance criteria $\pm 10\%$ of the true value. 3. Determine MDLs by analyzing seven replicates of Laboratory fortified blanks at concentration of 2 to 4 times the reporting limit. MDLs need to be run one per new instrument or if major changes were done to the method.
QC Check Standards/ Samples	After calibration is completed, verify by analyzing QCS. If not within $\pm 10\%$ of stated value, terminate analysis and re calibrate instrument.	After calibration is completed, verify by analyzing ICV. If not within $\pm 10\%$ of stated value, terminate analysis and re calibrate instrument.

Method	EPA Series Method 365.1	LSBSOP-OPHOS-TP13
Parameter		
QC Check Standards/ Samples	Prepare and analyze a Laboratory Fortified Blank (LFB) with each batch of samples by fortifying laboratory reagent water with the QCS. If the recovery of the analyte is not within 90–110%, the analyte is judged out of control. Determine source of problem and correct before continuing with analyses.	Prepare and analyze a Laboratory Fortified Blank (LFB) with each batch of samples by fortifying laboratory reagent water with the standard. If the recovery of the analyte is not within 90–110%, the analyte is judged out of control. Determine source of problem and correct before continuing with analyses.
Standard Solution Expiration	Stock standard: Not specified.	Stock standard used by expiration date. Working standard solution: Prepare before running
Initial Calibration	Minimum of 3 levels and a blank. Range: 0.01–1.0 mg P/L	Up to 7 levels and a blank. Range: 8–200ug P/L
Continuing Calibration	<p>Analyze instrument performance check (IPC) solution (mid-range check standard) immediately following calibration, after every 10 samples and at the end of the run.</p> <ol style="list-style-type: none"> 1. If not within $\pm 10\%$ of stated value, reanalyze IPC. 2. If second analysis of IPC is not within $\pm 10\%$ of stated value, discontinue analysis, determine the cause and/or in the case of drift recalibrate instrument. Reanalyze all samples since last compliant IPC. 	<ol style="list-style-type: none"> 1. Analyze CCV solution (mid-range check standard) immediately following calibration, after every 10 injection and at the end of the run. 3. If not within $\pm 10\%$ of stated value, reanalyze CCV 4. If second analysis of CCV is not within $\pm 10\%$ of stated value, discontinue analysis, determine the cause and/or in the case of drift recalibrate instrument. Reanalyze all samples since last compliant CCV

Method	EPA Series Method 365.1	LSBSOP-OPHOS-TP13
Parameter		
Accuracy/ Precision	<p>Spike and analyze one sample out of every 10 (Laboratory Fortified Matrix: LFM).</p> <p>The added analyte concentration should be the same as that used in the LFB. %R = 90–110</p>	<p>Spike and analyze one sample out of every 10 (Laboratory Fortified Matrix: LFM).</p> <p>The added analyte concentration should be the same as that used in the LFB. %R = 90–110 for drinking water and %R = 85–115 for soil and wastewater</p>
Blanks	<p>A Laboratory reagent blank (LRB) is carried through the entire sample preparation and analysis scheme with each batch of samples.</p> <p>Values that exceed the MDL indicate contamination should be suspected and corrective actions must be taken before continuing analysis.</p> <p>A Calibration blank (CCB) is to be analyzed after each IPC solution.</p>	<p>A Laboratory reagent blank (LRB) is carried through the entire sample preparation and analysis with each batch of samples.</p> <p>Values that exceed the RL indicate contamination should be suspected and corrective actions must be taken before continuing analysis.</p> <p>Lab blank (CCB) is to be analyzed after each CCV solution.</p>
Preservation/ Storage Conditions	pH <2 with H ₂ SO ₄ for TP and not preserved for OP 4°C storage required	pH <2 with H ₂ SO ₄ for TP not preserved for OP 4°C storage required
Holding Time	28 days for TP. As soon as possible for OP	28 days for TP. As soon as possible for OP and not exceed 48 hours
Field Sample Amount Required	Glass or plastic container.	Glass or plastic container.
Amount for Digestion	50 mL for TP	25 mL for TP

Method	EPA Series Method 365.1	LSBSOP-OPHOS-TP13
Parameter		
Reagent Preparation	Combine color reagent: mix 50 mL of 5 N H ₂ SO ₄ , 5 mL of antimony potassium tartrate solution, 15 mL of ammonium molybdate solution and 30 mL of ascorbic acid solution in 100 mL volume. Prepare freshly for each run	<p>Reagent 1-orthophosphate: 7 mL concentrated sulfuric, 0.3 g ascorbic acid, 0.0137 g of potassium antimonyl tartrate, and 0.6 g ammonium molybdate brought to 100 mL in DI. Prepare fresh daily.</p> <p>Reagent 1-total phosphorus: 0.020 g Antimony potassium tartrate, 0.80 g Ammonium molybdate, 100 mL of water and 5.6 mL of concentrated H₂SO₄. Stable for 3 weeks.</p>