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Standard Operating Procedure

AMBL-208-B

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Reactive Phosphorous by Ascorbic Acid Reduction

METHOD SUMMARY

This SOP describes a procedure for determining total and dissolved reactive phosphorous in water using ascorbic acid to reduce phosphomolybdic acid forming a molybdenum blue that is proportional to orthophosphate concentration and analyzed colorimetrically. This method is adapted from Method 4500-P E (Standard Methods, 2017) and Method 8048 (Hach). A procedure to correct for interference of arsenate (As-V), silica, germanium and ferrous iron is adapted from van Schouwenburg and Walinga (1967).

ENVIRONMENTAL HEALTH AND SAFETY

Hazards Assessment: This method uses small quantities of reagents in pre-prepared packets that minimizes hazards from handling reagents and uses a small volume of hydrochloric acid rinsing solution (1:1) that must be handled with caution, as well as a small volume of concentrated sulfuric acid (2.8 mL) to prepare a sodium metabisulfite-sodium thiosulfate reducing solution when eliminating known interferences. . Potential health hazards associated with using the extracting solution are as follows.

1. Hydrochloric acid is a clear, colorless and corrosive liquid that is very hazardous when in contact with skin, eyes or ingested. Potential health hazards associated with using hydrochloric acid are as follows.

   Skin Contact: Will permeate skin causing severe burns, with the destruction of skin tissue within 1 hour (GHS Category 1B).

   Eye Contact: Will cause severe burns and severe eye damage that may not reverse after 21 days (GHS Category 1). Permanent blindness can result if the exposure is severe enough.

Ingestion: Although, unlikely to occur during the performance of this method, ingestion can cause severe burns to the lips, tongue, throat and stomach. Nausea, stomach cramps, diarrhea and vomiting are all systems of an exposure by ingestion.

Inhalation: Exposure by inhalation is not expected unless the acid is heated or misted, or used outside of the fume hood. This exposure pathway is very hazardous and will cause irritation to the nose and throat, and can cause significant damage to respiratory system tissue leading to production and accumulation of fluid in the lungs. Coughing, shortness of breath, breathing difficulty and tightness in the chest are all symptoms of acid vapor or mist exposure by inhalation.

2. Sulfuric acid is a clear, colorless and corrosive liquid that can burn skin, eyes and clothing. Potential health hazards associated with using sulfuric acid are as follows.

   Skin Contact: Will permeate skin causing severe burns, with the destruction of skin tissue within 3 minutes (GHS Category 1A).

   Eye Contact: Will cause severe burns and severe eye damage that may not reverse after 21 days (GHS Category 1). Permanent blindness can result if the exposure is severe enough.

   Ingestion: Although, unlikely to occur during the performance of this method, ingestion can cause burns to the lips, tongue, throat and stomach. Nausea, stomach cramps, diarrhea and vomiting are all systems of an exposure by ingestion.

   Inhalation: Exposure by inhalation is not expected unless the acid is heated or misted. This exposure pathway is very hazardous and will cause irritation to the nose and throat, and cause significant damage to respiratory system tissue leading to production and accumulation of fluid in the lungs. Coughing, shortness of breath, breathing difficulty and tightness in the chest are all symptoms of acid vapor or mist exposure by inhalation. Severe exposures can lead to death.

3. Sodium metabisulfite is a white crystalline solid (powder). Potential health hazards associated with using sodium metabisulfite are as follows.

   Skin Contact: May cause irritation.

   Eye Contact: Can cause serious eye damage with effects that may not be reversible within 21 days (GHS Category 1).

   Ingestion: Although, unlikely to occur during the performance of this method, ingestion will irritate mucous membranes and requires immediate medical attention. Considered harmful if swallowed (GHS Category 4).

   Inhalation: Direct inhalation of this chemical is unlikely when performing this method, but should inhalation occur, it is considered toxic. Of particular significance is the noticeable liberation of toxic sulfur dioxide fumes when mixed with water or acid.

4. Sodium thiosulfate is a white crystalline solid (powder). Although not considered a hazardous substance by GHS, use precautions to avoid contact with skin or eyes, and do not handle in a manner that will result in ingestion or inhalation.

Safety Equipment and Engineering Controls: This method requires the preparation and use of the hydrochloric acid rinse solution in a fume hood, with a glass dish to contain possible spillage. Preparation of the sodium thiosulfate reduction solution must also be done in the fume hood. An eye wash station must be located nearby.

Personal Protective Equipment (PPE): This method requires the use of the following PPE.

Gloves (nitrile)

Safety goggles or glasses

Laboratory coat

Analysis-derived Wastes and Disposal:

Waste Generated	Hazardous (Y / N)	Disposal
This procedure generates small volumes (10 mL) of expended sample containing reagents used in the test	N	May be disposed in a sink.
This procedure may result in a small volume (≤80 mL) of residual hydrochloric acid rinse solution.	Y	Slowly neutralize the remaining volume of acid with sodium bicarbonate or sodium carbonate until no further carbon dioxide gas bubble evolve. May be disposed in sink.

METHOD DESCRIPTION

1.0 Introduction and Applicability

Phosphorous in natural waters and wastewaters predominantly occurs as phosphates. Orthophosphate is a salt or ester of orthophosphoric acid and is measured as the phosphate ion, PO_4^{3-}. Orthophosphate responds to colorimetric determination without having to be digested and is thus simply referred to as “reactive phosphorous.” Orthophosphate may be measured as total reactive phosphorous or dissolved reactive phosphorous, with suspended reactive phosphorous being determined by difference calculation.

This method is used to determine reactive phosphorous in the range of 0.0065 to 1.0 mg/L PO_4^{3-} as P for samples that do not present a color interference and is applicable to natural surface water and groundwater, agricultural runoff water, municipal wastewater, industrial wastewater and

seawater. This method also presents modifications that are applicable for determining reactive phosphorous in the Olsen extracting solution obtained from a soil sample. Reactive phosphorous greater than 1.0 mg P/L is determined by diluting and then analyzing the diluted sample.

All glassware or plastic containers used in transferring, diluting, collecting or otherwise holding samples, as well as rubber and glass stoppers, must be rinsed with a hydrochloric acid solution followed by a reagent water before used. If possible, dedicate all glassware and plastic containers used for use only with this method to minimize error introduced from cross- or residual-contamination.

2.0 Interferences

The presence of arsenate, silica, germanium and ferrous iron at high enough concentrations can have a positive interference (on color development) when using this method for determining reactive phosphorous. Eliminating these interferences is possible by treating the sample with a sodium metabisulfite - sodium thiosulfate (SMB-ST) reducing solution. When adding reducing solution to eliminate these interferences, the manual instrument mode and a separate calibration curve to account for the volume of reducing solution added must be used. The addition of this reducing solution extends the reaction time for stable color development and the measurement must be done after a 15 minute reaction time, rather than after 2 minutes. The color is known to remain stable for 45 minutes.

3.0 Apparatus

a. Membrane filter, 47 mm diameter, 0.45 µm pore size

b. Glass-fiber filter, with a 47 mm diameter, nominal pore size ≤ 2.0 µm and ≥ 1.0 µm

c. Filtration funnel assembly for a 47 mm size diameter filter.

d. Vacuum suction flask, 1000 mL capacity.

e. Glass test tube, 50-mL, to collect filtered sample. If possible, have one filtrate collection tube for each sample filtered.

f 50-mL, 20-mL, 10 mL and 5-mL volumetric pipets, Class A

g. 1000-mL and 500 mL volumetric flasks, Class A

h. 100- or 125-mL Erlenmeyer flasks

i. Magnetic stirrer and stir bar.

j. 10 mL sample cells, matching pair of 1-cm square cuvettes, (Hach 2495402). Inspect sample cells before use to verify that there are no abrasions, scratches or other marks on the glass walls. Replace the matching pair if any one of the cell is damaged.

k. DR 3900 or DR 2010 spectrophotometer (Hach), or an equivalent spectrophotometer capable of being set at a single wavelength.

l. Rubber stoppers for 10-mL sample cells (size 1)

4.0 Reagents

a. Stock phosphate solution: Dissolve 219.5 mg anhydrous KH_2PO_4 in distilled water and dilute to 1000 mL (1.00 mL = 50.0 µg PO_4^{3-} as P). Store in amber bottle.

b. Standard phosphate solution. Prepare fresh by diluting 50.0 mL of stock phosphate solution to 1000 mL of reagent water (1.00 mL = 2.5 µg PO_4^{3-} as P).

c. PhosVer 3 Phosphate reagent packet for 10-mL sample (Hach, 2106069)

d. SMB-ST reducing solution: Prepare this solution fresh daily and immediately prior to use by mixing together in the following sequence; prepare 20 mL of 5.0 N sulfuric acid (2.8 mL concentrated H_2SO_4 + 17.2 mL reagent water), then add 40 mL of 10% sodium metabisulfite (4 g of $Na_2S_2O_5$ in 40 mL reagent water), and then add 40 mL of 1% sodium thiosulfate (0.4 g of $Na_2S_2O_3$ in 40 mL of reagent water).

e. Reagent water, distilled water.

f. Hydrochloric acid rinse solution (1:1), 50 mL concentrated HCl + 50 mL reagent water. All glassware or other surfaces

5.0 Filtration of Samples

a. Determine dissolved reactive phosphorous or remove color from the sample by first filtering through a 0.45 µm membrane filter. Pre-filter samples through a pre-washed glass fiber filter (see 3.0 b) if filtration time is greater than 10 minutes. Do not filter sample portions on which you intend to determine total or suspended reactive phosphorous.

b. Similarly, filtering to remove color from a sample only allows dissolved reactive phosphorous to be determined.

c. Wash filters before use to remove trace amounts of phosphorous. Prepare only the number of filters (membrane and glass fiber) that you plan to use.

   1. Presoak filters overnight in 2 L of reagent water.

   2. Decant reagent water and soak again for another 1 hour in 2 L of reagent water.

   3. Decant reagent water, remove filters and store them in a smaller holding container (a glass petri dish works just fine). Add fresh reagent water.

d. Carefully remove a filter from the holding container, place it on the filtration apparatus support screen and draw a slight vacuum to remove all excess water from the filter to near dryness. Secure the filtration funnel over the filter and turn off the vacuum.

e. Remove the vacuum apparatus from the vacuum flask and carefully place the 50-mL tube in the flask. Replace the vacuum apparatus so that the filtrate tube on the bottom of the filtration apparatus is inside the test tube.

f. Turn on the vacuum just enough to filter the sample without causing sample to be splash out of the filtrate collection tube. Collect approximately 40 mL of filtered sample.

g. Remove the vacuum apparatus from the vacuum flask and carefully remove the 50-mL tube from the flask. Analyze as soon as possible or cover the tube with a wax film or transfer this sample to a cleaned and rinsed 60-mL plastic sample container and secure the cap.

h. Before filtering the next sample, thoroughly rinse the vacuum apparatus and the 50-mL filtrate collection tube with reagent water. Remove excess water from the vacuum apparatus by placing it on the flask and apply a vacuum until dry. Remove excess water from the collection tube by inverting and shaking the water out, and then leave the tube inverted (in a tube rack) to finish drying.

6.0 Procedure for Analysis of Phosphorous

6.1 Select the Instrument Mode

The instruments used to measure reactive phosphorous can be set to perform this measurement in either a stored program mode or a manual mode. The manual mode is preferred over the stored program mode.

a. Turn on the DR3900 or DR 2010 spectrophotometer allowing it to initialize and warm up according to manufacturer's instructions. The instrument is ready to use after initialization has completed.

b. Once the instrument is ready, select to measure phosphorous either by using a stored program, or by using a manual mode.

c. Stored Program Mode Measurements. The stored program mode uses a preprogrammed calibration that is recalled from memory by entering the appropriate stored program code.

    1. DR 3900. On the main menu screen, select Stored Programs to display the alphabetical list of stored programs and program code numbers. Scroll through the list to find **P React. PV** (reactive phosphorous (orthophosphate), PhosVer 3 ascorbic acid method) and touch the screen to highlight this method and press START to

run the program. The preprogramed wavelength used by this instrument's program is 880 nm.

Alternately, after selecting Stored Programs from the main menu screen, you may press the "Select by Number" option from the bottom, enter program code 490 and then press OK. Press START to run the program.

2. DR 2010. Enter program code 490 (press keys 4-9-0-Enter) and dial the wavelength setting to 890 nm.
3. When using the stored program mode the default instrument readout it typically set to display units of mg/L for the PO_4^{3-} chemical form. Change the display to read "P" so that chemical form is expressed "as mg/L P." The chemical form display units may be changed as follows:
   a) DR 3900. While the program is running, select "Options" from the bottom menu bar, then select "more," then select "Chemical Form:" to bring up the chemical form display options window. You may then select PO_4^{3-}, P or P_2O_5 to change the display to the desired chemical form.
   b) DR 2010. While the program is running, use the scroll down or scroll up arrow keys to change the displayed to the desired chemical form; PO_4^{3-}, P or P_2O_5 may be selected.

d. Manual Measurement Mode (preferred). The manual mode is used obtain sample absorbance readings that are then compared to a concentration-absorbance calibration curve to determine the concentration of the unknown sample.

1. DR 3900. On the main menu screen select Single Wavelength to display the options menu for single wavelength mode. Check that the reading option displayed is set to absorbance (Abs), otherwise press Abs/%Trans./Ext to switch between % transmittance, concentration and absorbance until Abs is displayed. Press the wavelength icon (λ) and, using the numeric keypad, enter 880. NOTE: You can also display absorbance readings when using the stored program mode by pressing the Abs, % Trans, Conc Option key from the Options menu until "Abs" is displayed.
2. DR 2010. Press the SHIFT key and then the ABS (8) key, and then dial the wavelength setting to 890 nm. NOTE: Pressing the SHIFT and ABS keys will also display the absorbance reading when running a stored program.

6.2 Prepare the Sample for Measurement

a. When using the DR 2010 a 10-mL cell riser must be inserted into the cell compartment. Check if a cell riser is already in the cell compartment and if it is not, insert one.

b. If sample concentration is known to be greater than the upper limit of the calibration curve, dilute the sample with reagent water to bring the diluted sample concentration to a value within the calibration range. Use the dilution factors in the following table as guidance when diluting samples.

Expected Final Concentration Range, P mg/L	Dilution Factor	Diluted Concentration Range, mg/L
1.0 to 10.0	1/10	0.10 to 1.0
10 to 20	1/20	0.5 to 1.0
20 to 50	1/50	0.4 to 1.0

c. All samples known to contain interferences associated with arsenate, silicon, germanium or ferrous iron and samples derived from soils using the Olsen extraction method that either contain or do not contain these interferences, must be pre-treated prior analysis.

1. Samples known to contain arsenate, silicon, germanium or ferrous iron are pre-treated with reducing solution by adding 5 mL of the reducing solution to 30 mL of sample. Mix this solution thoroughly and allow to react for at least 15 minutes but no more than 30 minutes.
2. Samples derived from using the Olsen extraction solution in soils and known to contain arsenate, silicon, germanium or ferrous iron are pre-treated as follows.
    a) Add 0.4 mL of concentrated sulfuric acid to 30 mL of sample. This neutralizes most of the carbonate in the Olsen solution and adjusts the samples pH to about 6.6. Perform this neutralization step in a 100- or 125-mL Erlenmeyer flask that is moderately stirred to promote the formation and release of carbon dioxide gas from the solution.
    b) Continue stirring the 30 mL sample in this flask and add 5 mL of the reducing solution. Additional carbon dioxide gas forms, further adjusting the pH to about 2.2. Allow the reducing solution to react for at least 15 minutes but no more than 30 minutes.
    c) Turn off the stirrer and remove the stir bar when done.

3. Samples derived from using the Olsen extraction solution in soils without interference from arsenate, silicon, germanium or ferrous iron are pre-treated as follows.

    a) Add 0.1 mL of concentrated sulfuric acid to 10 mL of sample. This neutralizes most of the carbonate in the Olsen solution and adjusts the samples pH to about 6.8. . Perform this neutralization step in a 100- or 125-mL Erlenmeyer flask that is moderately stirred to promote the formation and release of carbon dioxide gas from the solution.

    b) Turn off the stirrer and remove the stir bar when done.

4. These pre-treated samples must be analyzed using a calibration curve that has been generated using calibration standards that have been prepared and analyzed identical to the samples.

d. Quantitatively transfer two 10.0 mL aliquots of the sample or use two pre-treated 10 mL samples already available (from 6.2.c.3 above) into each of two optically matched 10-mL sample cells. One of these sample cells will be the sample blank used to zero the instrument.

    1. When preparing multiple samples, quantitatively transfer the two 10 mL aliquots into two 15-mL centrifuge tubes (polypropylene). One of these tubes will be the sample blank used to zero the instrument.

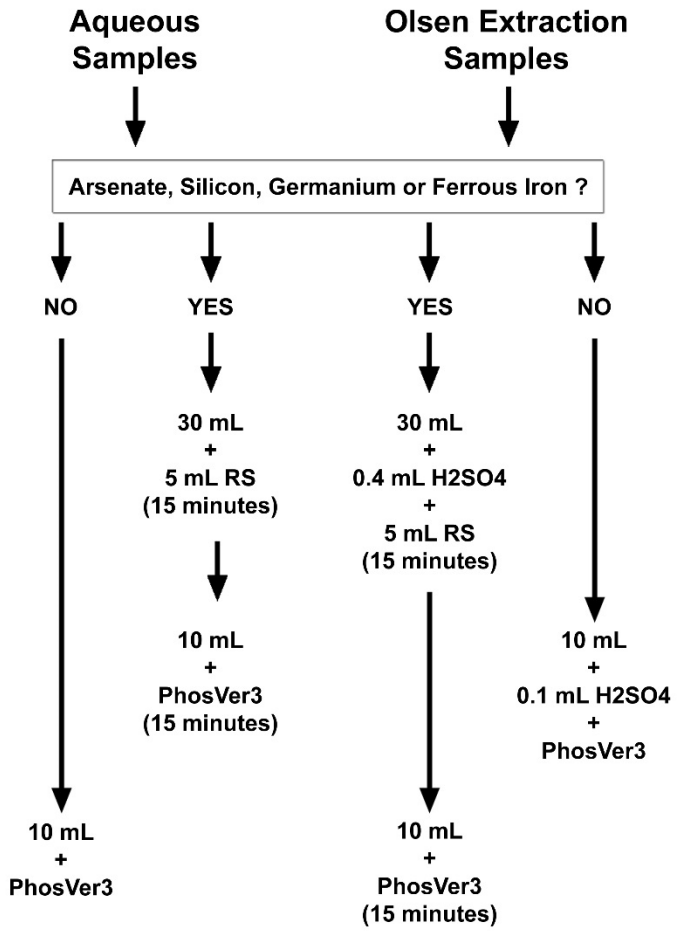
    2. When preparing multiple samples of the Olsen extracting solution, quantitatively transfer the two 10 mL aliquots into two new 50-mL centrifuge tubes (polypropylene). One of these tubes will be the sample blank used to zero the instrument.

e. Add the entire contents of one PhosVer 3 Phosphate reagent packet to one of the sample cells – this is the measured sample. Immediately place the rubber stopper in the sample cell and mix vigorously for 30 seconds.

    1. When preparing multiple samples with reducing solution that have been transferred to the 15-mL tubes, add the entire contents of the PhosVer 3 Phosphate reagent packet to one of the 15-mL tubes – this is the measured sample. Immediately place the caps on all tubes and if possible, using a tube rack, mix all tubes vigorously at the same time for 30 seconds.

    2. When preparing multiple samples of the Olsen extracting solution that have been transferred to two 50-mL centrifuge tubes (polypropylene), add the entire contents of the PhosVer 3 Phosphate reagent packet to one of the 50-mL tubes – this is the measured sample. Immediately place the caps on all tubes and if possible, using a tube rack, mix all tubes at the same time for 30 to 60 seconds. Avoid splashing sample up into the cap. Release any carbon dioxide gas that forms.

3. The following diagram summarizes all the steps used for the different samples applicable to this method.



f. Immediately after the PhosVer 3 Phosphate reagent and sample have been mixed, start the instrument timer, which will provide a 2-minute reaction time (Note: A reaction time of at least 15 minutes must be provided when reducing solution has been added.).

   1. DR 3900. On the Toolbar display, located on the right side of the screen, press Timer to select and activate the timer function. If using a Stored Program, the timer is pre-programmed. Press OK to begin the 2-minute reaction period. If using the Single Wavelength (manual mode) the numeric keypad is displayed. Enter 2-0-0 (1-5-0-0 when reducing solution added) and press OK to begin the 2-minute reaction period. The timer function display will show the time remaining. A sound will be emitted when the reaction period is finished.
   2. DR 2010. When using a Stored Program mode, the timer is pre-programmed. Press the SHIFT key and then the Timer (5) key. This

begins the 2-minute reaction period and the display will show the time remaining. If the stored program does not have a pre-programmed time or if using Manual measurement mode, press the SHIFT key and then the TIMER key to display "Timer=MM:SS" and then press 2-0-0 (1-5-0-0 when reducing solution added). Press the ENTER key to begin the 2-minute reaction period. Three series of fours beeps will sound when the reaction period is finished.

3. When samples are prepared in either the 15-mL or 50-mL centrifuge tubes, transfer (pour) the entire 10-mL contents of both tubes into the two matched samples cells after the reaction time has completed.
4. When using sample cells that have just been rinsed, either completely dry the inside of the sample cells (preferred) or conduct a final rinse using left over sample and remove all drops of water to the extent possible.

g. During the reaction time, use a soft cloth to clean the outside glass of both sample cells.

6.3 Conduct the Measurement

a. When the reaction period has completed (the instrument will emit a sound or series of beeps), the instrument must be zeroed before the sample measurement reading can be obtained.

b. Insert the blank sample cell into the cell compartment and close the cover. Zero the instrument.

1. DR 3900. The 10-mL mark on the sample cell must face towards the front of the instrument. Press Zero. This also activates the Read key. Repeat if the instrument does not display zero.
2. DR 2010. The 10-mL mark on the sample cell must face towards the left of the instrument. Press the Zero key. Repeat if the instrument does not display 0.00 mg/L or 0 absorbance.

Open the cover and remove the blank sample cell.

c. Remove the rubber stopper from the sample cell, and then insert the sample cell into the cell compartment with the 10-mL mark on the sample cell facing the same direction as the blank sample cell (see 6.3b above). (Note: when conducting measurements on samples with reducing solution and using the centrifuge tubes during preparation, there will be no rubber stopper.)

1. DR 3900. Press Read to display the results.
2. DR 2010. The display will show the results or you may press the Read key to refresh the display.

Open the cover and remove the sample cell.

d. Immediately after finishing with each pair of blank and sample cell measurements, the contents of each cell may be disposed in the sink. Thoroughly rinse the sample cells and the rubber stopper with tap water followed by a reagent water rinse. **NEVER allow sample or water to remain in a sample cell.** Place the sample cells upside down on a clean adsorbing pad to dry. To avoid scratching the glass walls, **NEVER place the sample cells on the drying rack or with other glassware on the drain shelf or in the Contrad solution bath.** To avoid etching the sample cell matching numbers from the glass, **NEVER use the Contrad solution bath to clean the cells.**

7.0 Preparing Calibration Curves

7.1 Calibration Curve without Reducing Solution

a. Preparation and use of an external calibration curve is preferred and recommended to ensure greater control of the measurement process. However, the pre-programmed calibration provided with a stored program is acceptable as long as it is verified using a known calibration check sample and adjusted as necessary.

b. Prepare calibration standard solutions as follows.

To obtain a calibration solution having this concentration of PO_4^{3-} as P	Dilute this volume of standard phosphate solution to 500 mL.
1.0 mg/L	200 mL
0.80 mg/L	160 mL
0.50 mg/L	100 mL
0.25 mg/L	50 mL
0.10 mg/L	20 mL
0.05 mg/L	10 mL
0.01 mg/L	a
0.0065 mg/L	b
Blank	c

a Prepare the 0.01 mg/L standard by diluting 50 mL of the 0.10 mg/L standard to a total volume of 500 mL.
b Prepare the 0.0065 mg/L standard by diluting 65 mL of the 0.05 mg/L standard to a total volume of 500 mL.
c A calibration blank, consisting of reagent water and reagents and also known as a reagent water blank, is included.

c. Allow prepared calibration standards to equilibrate to the same temperature as the samples, equilibrating both to the temperature of the room.

d. Using reagent water as the blank, conduct the measurement of each calibration standard in the manual mode as described above in 6.1.d, 6.2 and 6.3.

e. The most recent calibration curve data for both the DR 2010 and DR 3900 are available in an Excel spreadsheet file that may be downloaded from https://www.cefns.nau.edu/~teb/ambl/ambl_SOPs.html. When updating the calibration data, enter the standard concentration values, if different than provided, in the "Concentration" column, and enter the absorbance readings for each concentration in the "Abs" column. The Excel file automatically calculates the slope, intercept and r-squared values from a linear regression analysis of the calibration data. The resulting calibration curve and a basic calculator that uses the linear calibration curve equation for calculating a sample's concentration are also provided in this file.

7.2 Calibration Curve with Reducing Solution

a. Color intensity is influenced by the solution concentration of phosphorous, which will change when reducing solution is added to the sample.

b. When using reducing solution, prepare a separate calibration curve by treating standard solutions exactly as samples are treated.

c. Prepare standards as described above in 7.1, and add 5 mL of reducing solution to 30 mL of each standard solution and allow the same 15 to 30-minute reaction time that is used with samples having reducing solution added.

d. Using reagent water as the blank, conduct the measurement of each calibration standard in the manual mode as described above in 6.1.d, 6.2 and 6.3.

e. The most recent calibration curve data for both the DR 2010 and DR 3900 are available in an Excel spreadsheet file that may be downloaded from https://www.cefns.nau.edu/~teb/ambl/ambl_SOPs.html. When updating the calibration data, enter the standard concentration values, if different than provided, in the "Concentration" column, and enter the absorbance readings for each concentration in the "Abs" column. The Excel file automatically calculates the slope, intercept and r-squared values from a linear regression analysis of the calibration data. The resulting calibration curve and a basic calculator that uses the linear calibration curve equation for calculating a sample's concentration are also provided in this file.

7.3 Calibration Curve with Olsen Extracting Solutions

a. Color development and its intensity is also influenced by pH of the solution, which is buffered by the carbonate in the Olsen extracting solution.

b. When using Olsen extracting solution is used, prepare a separate calibration curve by treating standard solutions exactly as samples are treated.

c. Prepare standards as described above in 7.1, using Olsen extracting solution for dilutions instead of reagent water.

d. Neutralize calibration standards and blank prepared with Olsen extracting solution identically as the samples, and conduct the measurement of each calibration standard and blank in the manual mode as described above in 6.1.d, 6.2 and 6.3.

e. The most recent calibration curve data for both the DR 2010 and DR 3900 are available in an Excel spreadsheet file that may be downloaded from https://www.cefns.nau.edu/~teb/ambl/ambl_SOPs.html. When updating the calibration data, enter the standard concentration values, if different than provided, in the "Concentration" column, and enter the absorbance readings for each concentration in the "Abs" column. The Excel file automatically calculates the slope, intercept and r-squared values from a linear regression analysis of the calibration data. The resulting calibration curve and a basic calculator that uses the linear calibration curve equation for calculating a sample's concentration are also provided in this file.

8.0 Calculation and Reporting

a. Direct Readout. When using the direct readout mode, the phosphorous concentration is taken directly from the display and no calculation is necessary.

b. Using the calibration curve: When using the manual mode with a concentration-absorbance calibration curve, the absorbance reading is taken from the display and the phosphorous concentration is calculated as follows:

$$\text{Reactive } PO_4^{3-}\text{-P}, mg/L = \frac{A - b}{m \times D}$$

where A = absorbance reading

b = intercept from regression analysis of calibration standards

m = slope from regression analysis of calibration standards

D = the dilution factor

This calculation is the same one performed in the calibration curve spreadsheet described in 7.1.e, 7.2.e and 7.3.e above.

c. Report the concentration of reactive phosphorous according to the following table.

Range of Concentration, mg/L	Report to the Nearest mg/L
0.01 to <1.00	0.01
1.0 to <10.0	0.1
10 to <50	1
≥50	5

d. Identify results reported according to the form of reactive phosphorous measured; either as “total reactive phosphorous” or “dissolve reactive phosphorous.”

9.0 Minimum Required Quality Control

a. Continuing Calibration Verification (CCV) sample. When using the manual mode and before analyzing any samples, analyze a CCV sample. Thereafter, analyze a CCV sample after each batch of 20 or fewer samples. Select a concentration that is in the mid-range of the calibration curve (typically either the 0.5, 1.0 or 2.0 mg/L standard). Prepare a new calibration curve if the calibration check sample differs from the most recent calibration by more than 10%.

b. Reagent water blank (or Olsen Extraction solution blank). Analyze one reagent water blank, as any other sample, for each batch of 20 or fewer samples. The result of the reagent water blank must be less than the most recently determined method detection limit (MDL). If not, the source of the error must be determined and corrected until an acceptable reagent water blank is analyzed, and all samples since the previous acceptable reagent water blank must be reanalyzed.

c. Duplicate sample or Laboratory Fortified Matrix (LFM) and Laboratory Fortified Matrix Duplicate (LFMD). Analyze at least one sample in duplicate for each batch of 20 or fewer samples. If a single sample is being analyzed, this sample must be analyzed in duplicate. When reactive phosphorous is not measured in a matrix type, prepare and analyze a LFM and LFMD. Acceptance criteria (precision) for duplicate analysis using this method has not yet been established.

10.0 Bibliography

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