Aug 04, 2025

Ascorbic Acid Method for Ortho-P Measurement

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpzkr8lzp/v1

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Protocol Citation: Hezhou Ding, Christopher Good, Douglas F. Call 2025. Ascorbic Acid Method for Ortho-P Measurement. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpzkr8lzp/v1

Protocol status: Working
We use this protocol and it's working

Created: September 17, 2024

Last Modified: August 04, 2025

Protocol Integer ID: 107761

Keywords: ortho-phosphate, ascorbic acid method for ortho, phosphate in aqueous sample, ascorbic acid method, house measurement of ortho, soluble ortho, phosphate, ortho, aqueous sample

Abstract

This method was developed for in-house measurement of ortho-phosphate in aqueous samples. This method can cover ortho-P concentrations ranging from 0.1-90 mg-P/L. This method is intended for only soluble ortho-phosphate.

Guidelines

This method was developed for in-house measurement of ortho-phosphate in liquid samples. This method can cover ortho-P concentrations ranging from 0.1-75 mg-P/L.
Reaction: $PO_4^{3-} + 12MoO_4^{2-} + 27H^+ \rightarrow H_3PO_4(MoO^3)_{12} + 12H_2O$

Materials

1. Apparatus

- 13 mm x 100 mm digestion tubes
- If a lab has plate reader with absorbance measurement at 880 nm, this test can be done using micro-plates.
- Beakers, acid washed, used only for P measurement
- Graduated cylinder, acid washed, used only for P measurement
- P10000 pipette with pipette tips
- P1000 pipette with pipette tips

2. Reagent

1. L-(+)-Ascorbic acid, ACS, 99+%
2. Molybdate Reagent Solution, CAT# 69888-100ML (Sigma). This solution is concentrated compared to the standard method (The standard method's combined reagent has 6 g/L $(NH4)6Mo_7O_{24}\cdot4H_2O$, 0.14 g/L $K(SbO)C_4H_4O_6\cdot0.5H_2O$):
   i. (NH4)6Mo7O24.4H2O (1235.86 g/mol): 26 g/L
   ii. H2SO4: 300ml Sulfuric acid 96%
   iii. K(SbO)C4H4O6.0.5H2O: 0.7 g/L

Before start

Make sure samples do not have extreme pH (e.g., strong acid or base).
If samples contains colors, the absorbance will need to be adjusted using a sample as the blank.

Preparation

1 **Preparation**

1.1 Prepare standard solutions for the targeted measurement range.

1.2 Prepare clean glassware for the reactions. To clean the glassware, use diluted HCl (1%) to soak the glassware for several hours. Rinse well (three times at least) with DI water. If the glassware is used for the first time, wash the glassware with the combined molybdate and ascorbic acid reagent (mentioned in the Reagent Section) to react with any residual phosphorus. And rinse clean with DI water again. The reagent wash only needs to be done for one time. Acid wash is needed occasionally afterwards.

1.3 Check to see if any digestion vials have scratches that will affect the reading in the spectrophotometer.

Reagents Required

2 **Make reagents**

2.1 Make 0.1 M ascorbic acid solution: Dissolve 1.7612 g ascorbic acid in 100 mL DI water (0.8806 g/50 mL). This solution is stable under 4°C for two weeks. Wait for it to reach room temperature before use.

2.2 Dilute molybdate reagent: dilute the molybdate reagent (see the material list) solution with DI water 3 times (volume 1:2).

2.3 Make combined reagent: mix diluted molybdate reagent and ascorbic acid solution in a 7:3 ratio. Each sample/blank/QC requires 1 mL of the combined reagent. This mixture is stable for 4 h at room temperature. Light protection is not required.

For the range of 5 - 90 mg-P/L, light path: 1.3 cm

3 **Procedure for the range of 5 - 75 mg-P/L**

3.1 Add DI water to the combined reagent. The combined reagent to DI water ratio is 1:5 (for example, 10 mL combined reagent + 50 mL DI water).

3.2 Add 6 mL of reagent mixture with DI water into the digestion vial.

3.3 Pipet 0.1 mL of prefiltered sample into a digestion tube. Prepare a Blank by adding 0.1 mL of DI water into a digestion tube.

3.4 Invert the vials a few times to mix.

3.5 Let sit for 15 min at room temperature. The blue colors will develop.

3.6 Invert the vials several times until the solution is well mixed. Measure the absorbance at 880 nm in reference to the Blank.

For the range of 0.5 - 7.5 mg-P/L, light path: 1.3 cm

4 **Procedure for the range of 0.5 - 7.5 mg-P/L**

4.1 Add DI water to the combined reagent. The combined reagent to DI water ratio is 1:4 (for example, 10 mL combined reagent + 40 mL DI water).

4.2 Add 5 mL of reagent mixture into the digestion vial.

4.3 Pipet 1 mL of sample into a digestion tube. Prepare a Blank by adding 1 mL of DI water into a digestion tube.

4.4 Invert the vials a few times to mix.

4.5 Let sit for 15 min at room temperature. The blue colors will develop.

4.6 Invert the vials several times until the solution is well mixed. Measure the absorbance at 880 nm in reference to the Blank.

For the range of 0.1 - 1.5 mg-P/L, light path: 1.3 cm

5 **Procedure for the range of 0.1 - 1.5 mg-P/L**

5.1 Add DI water to the combined reagent. The combined reagent to DI water ratio is 1:4 (for example, 10 mL combined reagent + 40 mL DI water).

5.2 Add 1.5 mL of reagent mixture into the digestion vial.

5.3 Pipet 5 mL of sample into a digestion tube. Prepare a Blank by adding 5 mL of DI water into a digestion tube.
*Note: if the samples are not color-less, it may affect the result)

5.4 Invert the vials a few times to mix.

5.5 Let sit for 15 min at room temperature. The blue colors will develop.

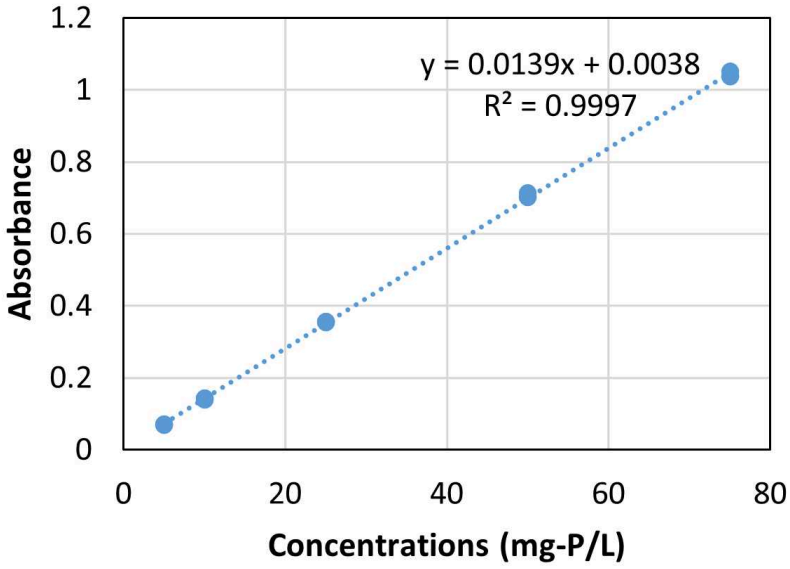
5.6 Invert the vials several times until the solution is well mixed. Measure the absorbance at 880 nm in reference to the Blank.

Standard Curves

6 To make the standard curve for each measurement range, prepare standard solutions for 5 concentrations or more using a analytical grade (such as HPLC grade) stock standard phosphate solution. For example, use a 100 mg-P/L stock solution to make 5, 10, 25, 50, 90 mg-P/L standard solutions.

Use the same reagents for the samples described in previous sections make the standard curves. Triplicate is desired.

For example:



Standard curve for a measurement range of 5-75 mg-P/L. Each concentration has triplicates.

Protocol references

Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation. 4500-p phosphorus In: Standard Methods For the Examination of Water and Wastewater. Lipps WC, Baxter TE, Braun-Howland E, editors. Washington DC: APHA Press.
DOI: 10.2105/SMWW.2882.093