

b. *Extraction procedure:*

$$\text{mg P/L} = \frac{\text{mg P (in 50 mL, final volume)} \times 1000}{\text{mL sample}}$$

4500-P E. Ascorbic Acid Method

1. General Discussion

a. *Principle:* Ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

b. *Interference:* Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_3^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. *Minimum detectable concentration:* Approximately 10 μg P/L. P ranges are as follows:

Approximate P Range mg/L	Light Path cm
0.30–2.0	0.5
0.15–1.30	1.0
0.01–0.25	2.0

d. *Quality control (QC):* The QC practices considered to be an integral part of each method are summarized in Table 4020-1.

2. Apparatus

a. *Colorimetric equipment:* One of the following is required:

1) *Spectrophotometer,* with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer,* equipped with a red color filter and a light path of 0.5 cm or longer.

b. *Acid-washed glassware:* See 4500-P.C.2b.

3. Reagents

a. *Sulfuric acid (H_2SO_4), 5N:* Dilute 70 mL conc H_2SO_4 to 500 mL with distilled water.

b. *Antimony potassium tartrate solution:* Dissolve 1.3715 g $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

c. *Ammonium molybdate solution:* Dissolve 20 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 500 mL distilled water. Store in a glass-stoppered bottle.

d. *Ascorbic acid, 0.1M:* Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

5. Precision and Bias

See Table 4500-P-I.

e. *Combined reagent:* Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5N H_2SO_4 , 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.

f. *Stock phosphate solution:* See 4500-P.C.3e.

g. *Standard phosphate solution:* Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50 μg P.

4. Procedure

a. *Treatment of sample:* Pipet 50.0 mL sample into a clean, dry test tube or 125-mL Erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5N H_2SO_4 solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

b. *Correction for turbidity or interfering color:* Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a sample blank by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. Subtract the sample blank absorbance from the absorbance of the sample.

c. *Preparation of calibration curve:* Prepare individual calibration curves from a series of four up to six standards, including a calibration blank, within the phosphate ranges indicated in 4500-P.E.1c. The calibration blank consists of reagent water with the combined reagent. Plot absorbance vs. phosphate concentration. Test at least one phosphate standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in approximately 50 mL, final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

The precision and bias values given in Table 4500-P-I are for a single-solution procedure given in the 13th Edition. The present procedure differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and bias to the previous technique in the analysis of both distilled water and river water at the 228- μg P/L level (Table 4500-P-II).

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