

Orthophosphate in Water Samples

Reagent A: Ammonium paramolybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$

1. Dissolve 12 g of ammonium paramolybdate in 250 mL of distilled water.
2. Dissolve 0.2908 g of potassium antimony tartarate ($\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2\cdot 3\text{H}_2\text{O}$) in 100 mL of distilled water.
3. Prepare 1000mL of 2.5M sulfuric acid (142 mL concentrated H_2SO_4)
4. Transfer the 2.5M H_2SO_4 to a 2000 mL volumetric flask. Then add the other two solutions. Dilute to 2000 mL volume, mix well, and then transfer to amber bottle and store in a dark place.

Reagent B:

1. NOTE: The amount of Reagent B needed depends on the number of samples to be analyzed and the type of container used to prepare the samples. The samples can be prepared in 25-mL volumetric flasks or in the test tubes used by the automatic sampler.
2. Determine how much reagent B will be needed for the day by multiplying the number of samples plus the number of standards. Multiply by 4 if using 25-mL volumetric flasks or by 1.6 if using spectrophotometer test tubes
3. Add 0.528 g of Ascorbic Acid per 100 mL of Reagent A (Ex. 0.792 g Ascorbic Acid per 150 mL Reagent A).

Standard Curve: (*Follow this method when using 25-mL volumetric flasks*)

1. Pipette 0, 1, 3, 5, 10, and 15 mL of a $2\ \mu\text{g P mL}^{-1}$ standard into separate 25 mL volumetric flasks.
2. Bring the volume in each standard to approximately 20 mL with deionized water. Add 4 mL of reagent B to each standard. Mix well. Dilute to 25 mL volume. Allow the samples to set for 30 minutes.
3. Read absorbance at 882 nm.

Standard Curve: (*Follow this method when using Shimadzu automatic sampler test tubes*)

1. Pipette 0, 1, 2, 3, and 5 mL of a $2\ \mu\text{g P mL}^{-1}$ standard into separate test tubes.
2. Use an adjustable pipette to bring the total volume in each tube to 10 mL. For example, the 3 mL standard needs 7 mL of water to bring the total volume to 10 mL.
3. Set the 5-mL adjustable pipette to 1.6 mL. Add 1.6 mL of Reagent B to each of the standards. Allow samples to set for at least 30 minutes.
4. Read absorbance at 882 nm.

Sample Procedure: (*This procedure is for the Shimadzu automatic sampler test tubes.*)

1. Pipette a volume of sample containing between 1 and 10 μg of ortho-P into the test tube. For runoff samples, you will probably need to use between 5 and 10 mL of sample. Record the volume of sample used.
2. Use the adjustable pipette to bring the total volume of solution in each test tube to 10 mL.
3. Add 1.6 mL of Reagent B to each sample test tube and mix well. Allow samples to set for at least 30 minutes.
4. Read absorbance at 882 nm.

Reference:

Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. In Page, A.L. et al. (eds.) Methods of Soil Analysis, Part 2 – Chemical and Microbiological Properties. 2nd ed. Agronomy Mono. No. 9. p. 413-414, 421-422.