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Protocol for colorimetric analysis of phosphate
From EPA Method 365.1

6. Apparatus

6.1Technicon AutoAnalyzer consisting of:

6.1.1 Sampler.
6.1.2 Manifold (AAI) or Analytical Cartridge (AAII).
6.1.3 Proportioning pump.
6.1.4 Heating bath, 50 degrees C.
6.1.5 Colorimeter equipped with 15 or 50 mm tubular flow cell.
6.1.6 650-660 or 880 nm filter.
6.1.7 Recorder.
6.1.8 Digital printer for AAII (optional).

6.2 Hot plate or autoclave.

6.3 Acid-washed glassware: All glassware used in the determination should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphorus and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCI and reagents is only required occasionally. Commercial detergent should never be used.

7. Reagents

7.1 Sulfuric acid solution, 5N: Slowly add 70 ml of conc. H_2SO_4 to approximately 400 ml of distilled water. Cool to room temperature and dilute to 500 ml with distilled water.

7.2 Antimony potassium tartrate solution: Weigh 0.3 g $K(SbO)C_4H_4O_6 \times 1/2H_2O$, dissolve in 50 ml distilled water in 100 ml volumetric flask, dilute to volume. Store at 4 degrees C in a dark, glass-stoppered bottle.

7.3 Ammonium molybdate solution: Dissolve 4 g $(NH_4)_6Mo_7O_{24} \times 4H_2O$ in 100 ml distilled water. Store in a plastic bottle at 4 degrees C.

7.4 Ascorbic acid, 0.1M: Dissolve 1.8 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if prepared with water containing no more than trace amounts of heavy metals and stored at 4 degrees C.

7.5 Combined reagent (AAI): Mix the above reagents in the following proportions for 100 ml of the mixed reagent: 50 ml of 5N H_2SO_4 (7.1), 5 ml of antimony potassium tartrate solution (7.2), 15 ml of ammonium molybdate solution (7.3), and 30 ml of ascorbic acid solution (7.4). Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until the turbidity disappears before processing. This volume is sufficient for 4 hours operation. Since the stability of this solution is limited, it must be freshly prepared for each run.

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NOTE 1: A stable solution can be prepared by not including the ascorbic acid in the combined reagent. If this is done, the mixed reagent (molybdate, tartrate, and acid) is pumped through the distilled water line and the ascorbic acid solution (30 ml of 7.4 diluted to 100 ml with distilled water) through the original mixed reagent line.

7.6 Sulfuric acid solution, 11 N: Slowly add 310 ml conc. H2S04 to 600 ml distilled water. When cool, dilute to 1 liter.

7.7 Ammonium persulfate.

7.8 Acid wash water: Add 40 ml of sulfuric acid solution (7.6) to 1 liter of distilled water and dilute to 2 liters. (Not to be used when only orthophosphate is being determined).

7.9 Phenolphthalein indicator solution (5 gal): Dissolve 0.5 g of phenolphthalein in a solution of 50 ml of ethyl or isopropyl alcohol and 50 ml of distilled water.

7.10 Stock phosphorus solution: Dissolve 0.4393 g of pre-dried (105 degrees C for 1 hour) KH2PO4 in distilled water and dilute to 1000 ml. 1.0 ml = 0.1 mg P.

7.11 Standard phosphorus solution: Dilute l00.0 ml of stock solution (7.10) to 1000 ml with distilled water. 1.0 ml = 0.01 mg P.

7.12 Standard phosphorus solution: Dilute 100.0 ml of standard solution (7.11) to 1000 ml with distilled water. 1.0 ml = 0.001 mg P.

7.13 Prepare a series of standards by diluting suitable volumes of standard solutions (7.11) and (7.12) to 100.0 ml with distilled water. The following dilutions are suggested:

ml of Standard Phosphorus Solution (7.12)	Conc, mg P/l
0.0	0.00
2.0	0.02
5.0	0.05
10.0	0.10

ml of Standard Phosphorus Solution (7.1.1)	mg P/l
2.0	0.20
5.0	0.50
8.0	0.80
10.0	1.00

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8. Procedure

8.1 Phosphorus

8.1.1 Add 1 ml of sulfuric acid solution (7.6) to a 50 ml sample and/or standard in a 125 ml Erlenmeyer flask.

8.1.2 Add 0.4 g of ammonium persulfate.

8.1.3 Boil gently on a preheated hot plate for approximately 30-40 minutes or until a final volume of about 10 ml is reached. Do not allow sample to go to dryness. Alternately, heat for 30 minutes in an autoclave at 121 degrees C (15-20 psi).

8.1.4 Cool and dilute the sample to 50 ml. If sample is not clear at this point, filter.

8.1.5 Determine phosphorus as outlined in (8.3.2) with acid wash water (7.8) in wash tubes.

8.2 Hydrolyzable Phosphorus

8.2.1 Add l ml of sulfuric acid solution (7.6) to a 50 ml sample and/or standard in a 125 ml Erlenmeyer flask.

8.2.2 Boil gently on a preheated hot plate for 30 10 minutes or until a final volume of about 10 ml is reached. Do not allow sample to go to dryness. Alternatively, heat for 30 minutes in an autoclave at 121 degrees C (15-20 psi).

8.2.3 Cool and dilute the sample to 50 ml. If sample is not clear at this point, filter.

8.2.4 Determine phosphorus as outlined in (8.3.2) with acid wash water (7.8) in wash tubes.

8.3 Orthophosphate

8.3.1 Add l drop of phenolphthalein indicator solution (7.9) to approximately 50 ml of sample. If a red color develops, add sulfuric acid solution (7.6) drop-wise to just discharge the color. Acid samples must be neutralized with 1 N sodium hydroxide (40 g NaOH/l).

8.3.2 Set up manifold as shown in Figure 2, AAI or Figure 3. AAII.

8.3.3 Allow both calorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through the sample line.

8.3.4 For the AAI system, sample at a rate of 20/hr, I minute sample, 2 minute wash. For the AAII system, use a 30/hr, 2:1 cam, and a common wash.

8.3.5 Place standards in Sampler in order of decreasing concentration. Complete filling of sampler tray with unknown samples.

8.3.6 Switch sample line from distilled water to Sampler and begin analysis.

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9. Calculation

9.1 Prepare a standard curve by plotting peak heights of processed standards against known concentrations. Compute concentrations of samples by comparing sample peak heights with standard curve. Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.

10. Precision and Accuracy (AAI system)

10.1 Six laboratories participating in an EPA Method Study, analyzed four natural water samples containing exact increments of orthophosphate, with the following results:

Increment as Orthophosphate mg P/liter	Precision as Standard Deviation mg P/liter	Accuracy as Bias, %	Accuracy as Bias, mg P/liter
0.04	0.019	+16.7	+0.007
0.04	0.014	- 8.3	-0.003
0.29	0.087	-15.5	-0.05
0.30	0.066	-12.8	-0.04

10.2 In a single laboratory (EMSL), using surface water samples at concentrations of 0.04, 0.19, 0.35, and 0.84 mg P/l, standard deviations were +/-0.005, +/-0.000, +/-0.003, and +/-0.000, respectively.

10.3 In a single laboratory (EMSL), using surface water samples at concentrations of 0.07 and 0.76 mg p/l, recoveries were 99% and 100%, respectively.

Bibliography

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