METHOD #: 365.2 Approved for NPDES (Issued 1971)

TITLE: Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Single Reagent)

ANALYTE: CAS # P Phosphorus 7723-14-0

INSTRUMENTATION: Spectrophotometer

STORET No. See Section 4

1.0 Scope and Application

1.1 These methods cover the determination of specified forms of phosphorus in drinking, surface and saline waters, domestic and industrial wastes.

1.2 The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pre-treatment of the sample, the various forms of phosphorus given in Figure 1 may be determined. These forms are defined in Section 4.

1.2.1 Except for in-depth and detailed studies, the most commonly measured forms are phosphorus and dissolved phosphorus, and orthophosphate and dissolved orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples and insoluble forms of phosphorus are determined by calculation.

1.3 The methods are usable in the 0.01 to 0.5 mg P/L range.

2.0 Summary of Method

2.1 Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

2.2 Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion[(2)].

3.0 Sample Handling and Preservation

3.1 If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.

3.2 Sample containers may be of plastic material, such as cubitainers, or of Pyrex glass.

3.3 If the analysis cannot be performed the day of collection, the sample should be preserved by the addition of 2 mL conc. H_2SO_4 per liter and refrigeration at 4°C.

4.0 Definitions and Storet Numbers

4.1 Total Phosphorus (P)--all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure. (00665)

4.1.1 Total Orthophosphate (P, ortho)--inorganic phosphorus [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure.

(70507)

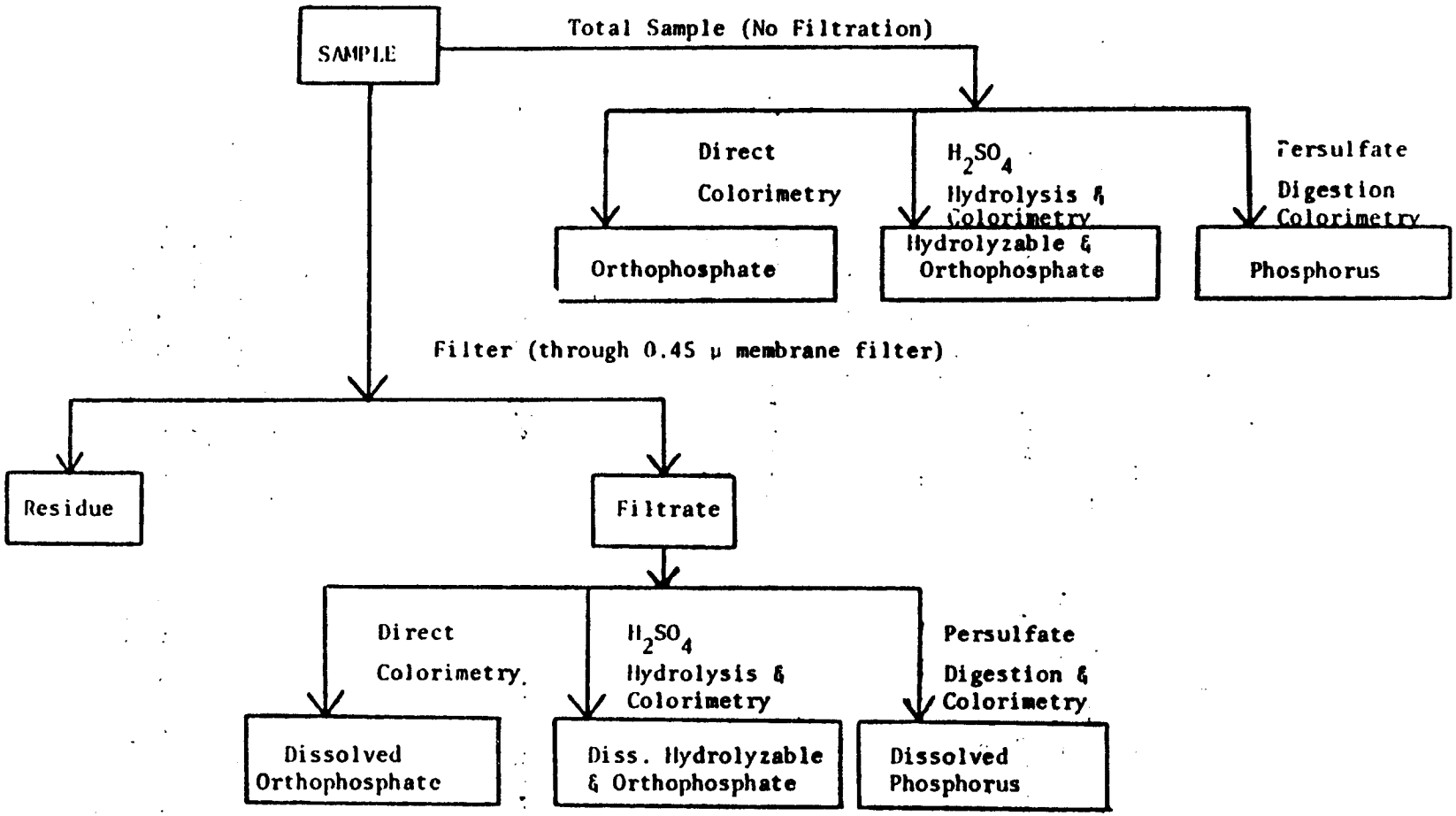

FIGURE 1. ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

4.1.2 Total Hydrolyzable Phosphorus (P, hydro) - phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus pre-determined orthophosphates. This hydrolyzable phosphorus includes polyphosphorus. [$(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$, etc.] plus some organic phosphorus. (00669)

4.1.3 Total Organic Phosphorus (P, org)--phosphorus (inorganic plus oxidizable organic) in the sample measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate. (00670)

4.2 Dissolved Phosphorus (P-D)--all of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure. (00666)

4.2.1 Dissolved Orthophosphate (P-D, ortho)--as measured by the direct colorimetric analysis procedure. (00671)

4.2.2 Dissolved Hydrolyzable Phosphorus (P-D, hydro)--as measured by the sulfuric acid hydrolysis procedure and minus pre-determined dissolved orthophosphates. (00672)

4.2.3 Dissolved Organic Phosphorus (P-D, org)--as measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate. (00673)

4.3 The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:

4.3.1 Insoluble Phosphorus (P-I) = (P)-(P-D). (00667)

4.3.1.1 Insoluble orthophosphate (P-I, ortho)=(P, ortho)-(P-D, ortho). (00674)

4.3.1.2 Insoluble Hydrolyzable Phosphorus (P-I, hydro)=(P, hydro)-(P-D, hydro). (00675)

4.3.1.3 Insoluble Organic Phosphorus (P-I, org)=(P, org) - (P-D, org). (00676)

4.4 All phosphorus forms shall be reported as P, mg/L, to the third place.

5.0 Interferences

5.1 No interference is caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in sea water. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

5.2 The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.

5.3 Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in sea water, it does not interfere.

6.0 Apparatus

6.1 Photometer - A spectrophotometer or filter photometer suitable for measurements at 650 or 880 nm with a light path of 1 cm or longer.

6.2 Acid-washed glassware: All glassware used 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 HCl and reagents is only required occasionally. Commercial detergents should never be used.

7.0 Reagents

7.1 Sulfuric acid solution, 5N: Dilute 70 mL of conc H_2SO_4 with distilled water to 500 mL.

7.2 Antimony potassium tartrate solution: Weigh 1.3715 g $K(SbO)C_4H_4O•1/2H_2O$ dissolve in 400 mL distilled water in 500 mL volumetric flask, dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

7.3 Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_70_{24}•4H_2O$ in 500 mL of distilled water. Store in a plastic bottle at 4°C.

7.4 Ascorbic acid, 0.1 M: Dissolve 1.76 g of ascorbic acid in 100 mL of distilled water. The solution is stable for about a week if stored at 4°C.

7.5 Combined reagent: 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 proceeding. Since the stability of this solution is limited, it must be freshly prepared for each run.

7.6 Sulfuric acid solution, 11 N: Slowly add 310 mL conc. H_2SO_4 to 600 mL distilled water. When cool, dilute to 1 liter.

7.7 Ammonium persulfate.

7.8 Stock phosphorus solution: Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml; 1.0 mL = 0.05 mg P.

7.9 Standard phosphorus solution: Dilute 10.0 mL of stock phosphorus solution (7.8) to 1000 mL with distilled water; 1.0 mL = 0.5 μg P.

7.9.1 Using standard solution, prepare the following standards in 50.0 mL volumetric flasks:

mL of Standard Phosphorus Solution (7.9)	Conc., mg/L
0	0.00
1.0	0.01
3.0	0.03
5.0	0.05
10.0	0.10
20.0	0.20
30.0	0.30
40.0	0.40
50.0	0.50

7.10 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in 600 mL distilled water. Cool and dilute to 1 liter.

8.0 Procedure

8.1 Phosphorus

8.1.1 Add 1 mL of H_2SO_4 solution (7.6) to a 50 mL sample in a 125 mL Erlenmeyer flask.

8.1.2 Add 0.4 g of ammonium persulfate.

8.1.3 Boil gently on a pre-heated 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. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi).

8.1.4 Cool and dilute the sample to about 30 mL and adjust the pH of the sample to 7.0 ±0.2 with 1 N NaOH (7.10) using a pH meter. If sample is not clear at this point, add 2-3 drops of acid (7.6) and filter. Dilute to 50 mL. Alternatively, if autoclaved see NOTE 1.

8.1.5 Determine phosphorus as outlined in 8.3.2 Orthophosphate.

8.2 Hydrolyzable Phosphorus

8.2.1 Add 1 mL of H_2SO_4 solution (7.6) to a 50 mL sample in a 125 mL Erlenmeyer flask.

8.2.2 Boil gently on a pre-heated hot plate for 30-40 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°C (15-20 psi).

8.2.3 Cool and dilute the sample to about 30 mL and adjust the pH of the sample to 7.0 ±0.2 with NaOH (7.10) using a pH meter. If sample is not clear at this point, add 2-3 drops of acid (7.6) and filter. Dilute to 50 mL. Alternatively, if autoclaved see NOTE 1.

8.2.4 The sample is now ready for determination of phosphorus as outlined in 8.3.2 Orthophosphate.

8.3 Orthophosphate

8.3.1 The pH of the sample must be adjusted to 7 ± 0.2 using a pH meter.

8.3.2 Add 8.0 mL of combined reagent (7.5) to sample and mix thoroughly. After a minimum of ten minutes, but no longer than thirty minutes, measure the color absorbance of each sample at 650 or 880 nm with a spectrophotometer, using the reagent blank as the reference solution. NOTE 1: If the same volume of sodium hydroxide solution is not used to adjust the pH of the standards and samples, a volume correction has to be employed.

9.0 Calculation

9.1 Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations.

9.1.1 Process standards and blank exactly as the samples. Run at least a blank and two standards with each series of samples. If the standards do not agree within ±2% of the true value, prepare a new calibration curve.

9.2 Obtain concentration value of sample directly from prepared standard curve. Report results as P, mg/L. SEE NOTE 1.

10.0 Precision and Accuracy

10.1 Thirty-three analysts in nineteen laboratories analyzed natural water samples containing exact increments of organic phosphate, with the following results:

Increment as Total Phosphorus mg P/liter	Precision as Standard Deviation mg P/liter	Accuracy as Bias, %	Accuracy as Bias, mg P/liter
0.110	0.033	+3.09	+0.003
0.132	0.051	+11.99	+0.016
0.772	0.130	+2.96	+0.023
0.882	0.128	-0.92	-0.008

(FWPCA Method Study 2, Nutrient Analyses)

10.2 Twenty-six analysts in sixteen laboratories analyzed natural water samples containing exact increments of orthophosphate, with the following results:

Increment as Orthophosphorus mg P/liter	Precision as Standard Deviation mg P/liter	Accuracy as Bias, %	Accuracy as Bias mg P/liter
0.029	0.010	-4.95	-0.001
0.038	0.008	-6.00	-0.002
0.335	0.018	-2.75	-0.009
0.383	0.023	-1.76	-0.007

(FWPCA Method Study 2, Nutrient Analyses)

Bibliography

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4. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 476 and 481, (1975).