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Method 365.1, Revision 2.0: Determination of Phosphorus by Semi-Automated Colorimetry

METHOD 365.1

DETERMINATION OF PHOSPHORUS BY SEMI-AUTOMATED COLORIMETRY

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METHOD 365.1

DETERMINATION OF PHOSPHORUS BY AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of specified forms of phosphorus in drinking, ground, and surface waters, and domestic and industrial wastes.

1.2 The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pretreatment of the sample, the various forms of phosphorus that may be determined are defined in Section 3.0 and given in Figure 1.

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

1.3 The applicable range is 0.01-1.0 mg P/L. Approximately 20-30 samples per hour can be analyzed.

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 manual sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by manual persulfate digestion.[2] The developed color is measured automatically.

2.3 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Section 9.0, Quality Control.

3.0 DEFINITIONS

3.1 **Calibration Blank (CB)** -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 **Calibration Standard (CAL)** -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 **Instrument Performance Check Solution (IPC)** -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4 **Laboratory Fortified Blank (LFB)** -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 **Laboratory Fortified Sample Matrix (LFM)** -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6 **Laboratory Reagent Blank (LRB)** -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7 **Linear Calibration Range (LCR)** -- The concentration range over which the instrument response is linear.

3.8 **Material Safety Data Sheet (MSDS)** -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 **Method Detection Limit (MDL)** -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 **Quality Control Sample (QCS)** -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The

QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11 **Stock Standard Solution (SSS)** -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source

3.12 **Total Phosphorus (P)** -- All of the phosphorus present in the sample regardless of forms, as measured by the persulfate digestion procedure.

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

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

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

3.13 **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.

3.13.1 Dissolved Orthophosphate (P-D ortho) -- As measured by he direct colorimetric analysis procedure.

3.13.2 Dissolved Hydrolyzable Phosphorus (P-D, hydro) -- As measured by the sulfuric acid hydrolysis procedure and minus predetermined dissolved orthophosphates.

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

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

3.14.1 Insoluble Phosphorus (P-I) = (P) - (P-D).

3.14.1.1 Insoluble Orthophosphate (P-I, ortho) = (P, ortho) - (P-D, ortho).

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

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

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

4.0 INTERFERENCES

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

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

4.3 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.

4.4 Sample turbidity must be removed by filtration prior to analysis for orthophosphate. Samples for total or total hydrolyzable phosphorus should be filtered only after digestion. Sample color that absorbs in the photometric range used for analysis will also interfere.

4.5 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sulfuric acid (Sections 7.2 and 7.7)

6.0 EQUIPMENT AND SUPPLIES

6.1 Balance -- Analytical, capable of accurately weighing to the nearest 0.000l g.

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Hot plate or autoclave.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

6.5 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 HCl and reagents is only required occasionally. Commercial detergent should never be used.

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Sulfuric acid solution, 5N: Slowly add 70 mL of conc. H_2SO_4 (CASRN 7664-93-9) to approximately 400 mL of reagent water. Cool to room temperature and dilute to 500 mL with reagent water.

7.3 Antimony potassium tartrate solution: Weigh 0.3 g $K(SbO)C_4H_4O_6 \bullet 1/2H_2O$ (CASRN 28300-74-5) and dissolve in 50 mL reagent water in 100 mL volumetric flask, dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

7.4 Ammonium molybdate solution: Dissolve 4 g $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ (CASRN 12027-67-7) in 100 mL reagent water. Store in a plastic bottle at 4°C.

7.5 Ascorbic acid, 0.1M: Dissolve 1.8 g of ascorbic acid (CASRN 50-81-7) in 100 mL of reagent 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°C.

7.6 Combined reagent: Mix the above reagents in the following proportions for 100 mL of the mixed reagent: 50 mL of 5N H_2SO_4 (Section 7.2), 5 mL of antimony potassium tartrate solution (Section 7.3), 15 mL of ammonium molybdate solution (Section 7.4), and 30 mL of ascorbic acid solution (Section 7.5). 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 a four hour operation. Since the stability of this solution is limited, it must be freshly prepared for each run.

Note: 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 7.5 diluted to 100 mL with reagent water) through the original mixed reagent line.

7.7 Sulfuric acid solution, 11 N: Slowly add 155 mL conc. H_2SO_4 to 600 mL reagent water. When cool, dilute to 500 mL.

7.8 Ammonium persulfate (CASRN 7727-54-0).

7.9 Acid wash water: Add 40 mL of sulfuric acid solution (Section 7.7) to 1 L of reagent water and dilute to 2 L. (Not to be used when only orthophosphate is being determined).

7.10 Phenolphthalein indicator solution (5 g/L): Dissolve 0.5 g of phenolphthalein (CASRN 77-09-8) in a solution of 50 mL of isopropyl alcohol (CASRN 67-63-0) and 50 mL of reagent water.

7.11 Stock phosphorus solution: Dissolve 0.4393 g of predried (105°C for one hour) Potassium phosphate monobasic KH_2PO_4 (CASRN 7778-77-0) in reagent water and dilute to 1000 mL. 1.0 mL = 0.1 mg P.

7.12 Standard phosphorus solution: Dilute 10.0 mL of stock solution (Section 7.11) to 100 mL with reagent water. 1.0 mL = 0.01 mg P.

7.13 Standard phosphorus solution: Dilute 10.0 mL of standard solution (Section 7.12) to 100 mL with reagent water. 1.0 mL = 0.001 mg P.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH <2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9.0 QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2 Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by ±10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ±10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going

analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[5] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]

S = standard deviation of the replicate analyses

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within ±10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where, R = percent recovery
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample

9.4.3 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare a series of at least three standards, covering the desired range, and a blank by pipetting and diluting suitable volumes of working standard solutions (Section 7.12 or 7.13) into 100 mL volumetric flasks. Suggested ranges include 0.00-0.10 mg/L and 0.20-1.00 mg/L.

10.2 Process standards and blanks as described in Section 11.0, Procedure.

10.3 Set up manifold as shown in Figure 2.

10.4 Prepare flow system as described in Section 11.0, Procedure.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

10.6 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based regression curve fitting techniques. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.7 After the calibration has been established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed ±10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis.

Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

11.1 Phosphorus

11.1.1 Add 1 mL of sulfuric acid solution (Section 7.7) to a 50 mL sample and/or standard in a 125 mL Erlenmeyer flask.

11.1.2 Add 0.4 g of ammonium persulfate (Section 7.8).

11.1.3 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. Alternately, heat for 30 minutes in an autoclave at 121°C (15-20 psi).

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

11.1.5 Determine phosphorus as outlined (Section 11.3.2) with acid wash water (Section 7.9) in wash tubes.

11.2 Hydrolyzable Phosphorus

11.2.1. Add 1 mL of sulfuric acid solution (Section 7.7) to a 50 mL sample and/or standard in a 125 mL Erlenmeyer flask.

11.2.2 Boil gently on a pre-heated hot plate for 30-40 minutes 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).

11.2.3 Determine phosphorus as outlined (Section 11.3.2) with acid wash water (Section 7.9) in wash tubes.

11.3 Orthophosphate

11.3.1 Add 1 drop of phenolphthalein indicator solution (Section 7.10) to approximately 50 mL of sample. If a red color develops, add sulfuric acid solution (Section 7.7) drop-wise to just discharge the color. Acid samples must be neutralized with 1 N sodium hydroxide (40 g NaOH/L).

11.3.2 Set up manifold as shown in Figure 1.

11.3.3 Allow system to equilibrate as required. Obtain a stable baseline with all reagents, feeding reagent water through the sample line.

11.3.4 Place standards in sampler in order of decreasing concentration. and complete filling of sampler tray.

11.3.5 Switch sample line from reagent water to Sampler and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.l Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.

l2.3 Report results in mg P/L.

13.0 METHOD PERFORMANCE

13.1 Six laboratories (using Technicon AAI equipment) 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/L	Precision as Standard Deviation mg P/L	Accuracy As	
		Bias %	Bias mg P/L
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

13.2 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.

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

13.4 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg PO_4-P/L.

l4.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous

opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Section 14.3.

16.0 REFERENCES

1. Murphy, J. and Riley, J., "A Modified Single Solution for the Determination of Phosphate in Natural Waters". Anal. Chim. Acta., 27, 31 (1962).

2. Gales, M., Jr., Julian, E., and Kroner, R., "Method for Quantitative Determination of Total Phosphorus in Water". Jour. AWWA, 58, No. 10, 1363 (1966).

3. Lobring, L.B. and Booth, R.L., "Evaluation of the AutoAnalyzer II; A Progress Report", Technicon International Symposium, June, 1972, New York, N.Y.

4. Standard Methods for the Examination of Water and Wastewater, 18th Edition, p. 4-116, Method 4500-P F (1992).

5. Code of Federal Regulations 40, Ch. 1, Pt. 136, Appendix B.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

Number of Values Reported	True Value (T)	Mean (X)	Residual for X	Standard Deviation (S)	Residual for S
54	0.150	0.1530	-0.0017	0.0128	-0.0010
69	0.351	0.3670	0.0140	0.0368	0.0084
88	0.625	0.6090	-0.0141	0.0413	-0.0069
87	1.80	1.7374	-0.0444	0.1259	-0.0072
57	2.50	2.4867	0.0146	0.1637	-0.0200
69	2.75	2.8344	0.1158	0.2019	0.0002
53	3.50	3.5619	0.1038	0.2854	0.0295
87	3.60	3.4957	-0.0610	0.2137	-0.0495
64	4.00	3.8523	-0.0989	0.3158	0.0237
57	7.01	6.9576	0.0383	0.5728	0.0632
88	8.20	8.0995	0.0068	0.5428	-0.0528
63	9.00	8.6717	-0.2099	0.6770	0.0236

REGRESSIONS: $X = 0.986T + 0.007$, $S = 0.072T + 0.003$

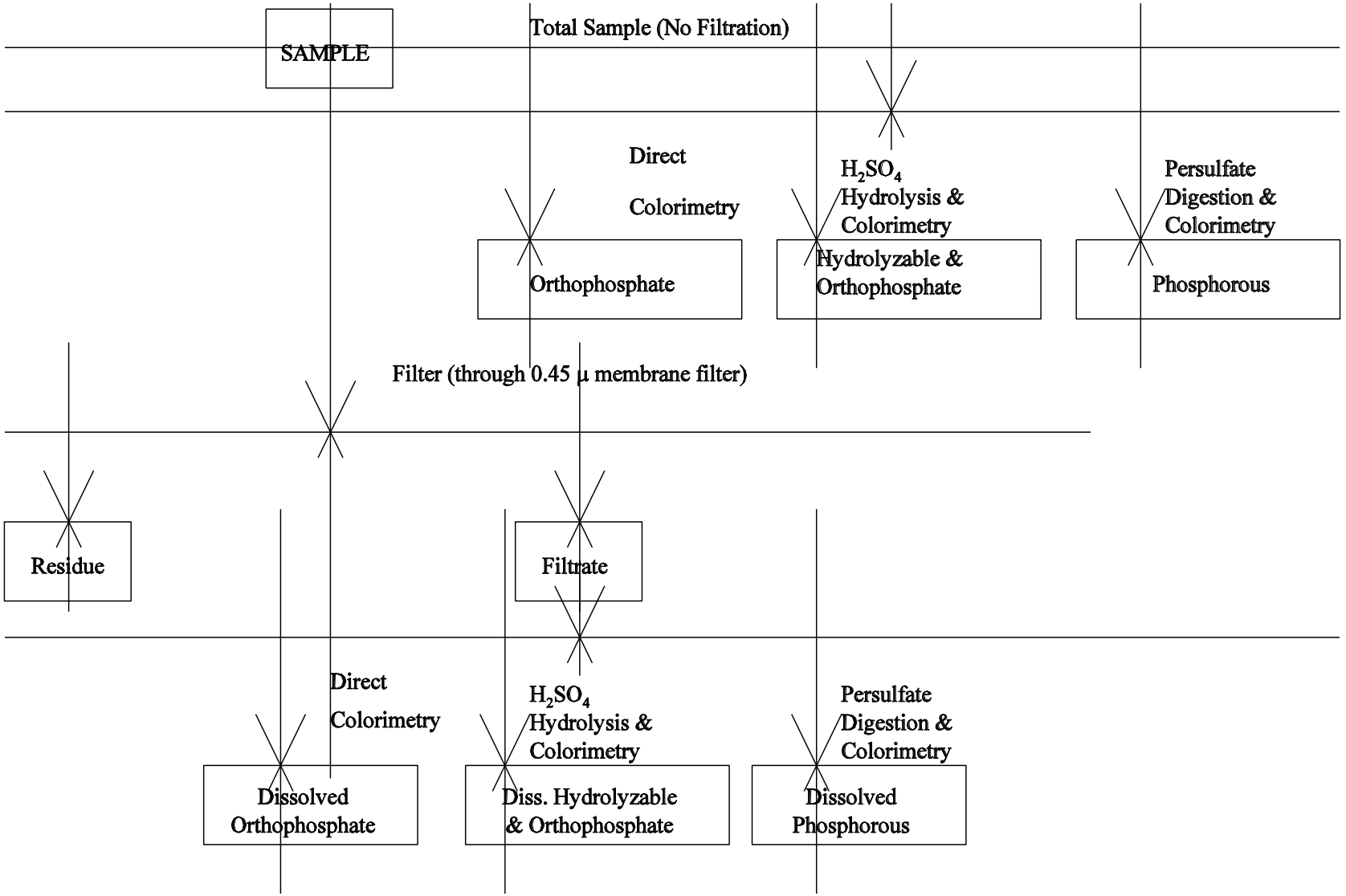

Figure 1. Analytical Scheme for Differentiation of Phosphorous Forms

Figure 2
Phosphorous Manifold