Standard Operating Procedure for:

Total Phosphorus
(3010R02 Total P.doc)

Missouri State University

and

Ozarks Environmental and Water
Resources Institute (OEWRI)

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1   Identification of the test method
        Total phosphorus by spectrophotometer (EPA 365.2).

2   Applicable matrix or matrices
        This method is suitable for the analysis of water samples collected from rivers,
streams, lakes, and wastewater.

3   Detection Limit
        The detection limit is ≤ 0.005 mg TP/L.

4   Scope of the test method
        This Standard Operating Procedure provides Missouri State University (MSU)
laboratory personnel with guidance on the procedure for determining total phosphorus
(TP) in surface water samples. This method is not applicable to samples preserved with
$HgCl_2$.

5   Summary of test method
        5.1   All forms of phosphorus, including organic phosphorus, are converted to
                orthophosphate by an acid-persulfate digestion. The persulfate digestion
                procedure and phosphate determination follow EPA 365.2, with the size of the
                sample reduced.

        5.1   A 10-mL volume of a well-mixed water sample is combined with sulfuric acid and
                ammonium or potassium persulfate and heated to approximately 120ºC in an
                autoclave or pressure cooker. This quantitatively converts phosphorus
                compounds to orthophosphate.

        5.2   The digested sample is then analyzed for orthophosphate based on its reaction
                with a combined reagent containing ammonium molybdate, antimony potassium
                tartrate, and ascorbic acid to form intensely-colored molybdenum blue.

        5.3   The desired performance criteria for this measurement are:
                a.   Detection limit: 0.005 mgTP/L
                b.   Precision: ≤ 20%
                c.   Accuracy: ≤ 20%
                d.   Minimum Quantification Interval: 0.001 mg TP/L

        5.5   According to EPA 365.2. the applicable range for the method is 0.01 mg TP/L to
                0.5 mg TP/L and may be extended by dilution. EPA 365.2 describes
                performance for undigested orthophosphate samples but not for digested
                samples.

6   Definitions
        6.1   The definitions and purposes below are specific to this method, but have been
                conformed to common usage as much as possible.

        6.2   AMP standard: The AMP standard is used to confirm effective digestion of
                organic phosphorus.

6.3   Analytical batch: The set of samples processed at the same time to a maximum of 10 samples.

6.4   Calibration standard: A solution prepared from the primary dilution standard solution or stock standard solutions. The calibration standards are used to calibrate the instrument response with respect to analyte concentration.

6.5   De-ionized water blank (DIB): This blank should not contain any concentration of analyte and is not processed or digested like the standards, checks, or samples. The de-ionized water blank should be less than the laboratory reagent blank (LRB).

6.6   Field blank (FB): An aliquot of deionized water treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

6.7   Field duplicate (FD): Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory prcedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

6.8   Laboratory control check (LCC): A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).

6.9   Laboratory duplicate (LD): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. The division of one sample into two samples for separate analysis. A laboratory duplicate is made primarily to assess precision associated with analytical procedures.

6.10   Laboratory matrix spike (LS): An aliquot of a sample to which a known amount of analyte is added before sample preparation. The LFM is used to evaluate analyte recovery in a sample matrix.

6.11   Laboratory reagent blank (LRB): An aliquot of deionized water treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

6.12   Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.
        a.   To calculate the MDL:

      b.   Prepare a solution with the concentration of TP near the estimated MDL

      c.   Analyze seven portions of this solution over a period of at least three days

      d.   Include all sample processing steps in the determination

      e.   Calculate the standard deviation (s).

      f.   From a table of the one-sided t distribution select the value of t for $7 - 1 = 6$ degrees of freedom at the 99% level. This value is 3.14

      g.   The product 3.14 times s is the desired MDL.

6.12   Relative Percent Difference (RPD): calculated as the difference between a sample and duplicate results, divided by the average of the sample and duplicate results, multiplied by 100%.

6.13   Quality control check sample (QCC): A sample containing analytes of interest at known concentrations (true values). The quality control check sample is obtained from a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

7   Interferences

7.1   No interferences are normally observed for copper, iron, or silicate. However, high concentrations of iron can cause precipitation of, and subsequent loss, of phosphorus.

7.2   Arsenate may interfere when present at concentrations higher than phosphorus.

7.3   Sample turbidity and natural color may interfere. Turbidity may be removed by centrifugation or filtration after digestion.

7.4   Phosphate adsorbed on glass surfaces may affect measurements at low phosphate levels. Use of acid-washed glassware dedicated to this analysis prevents this interference.

7.5   A number of sources suggest that there is a problem with deposition of reaction products on cell windows. Some methods incorporate a surfactant to minimize this effect.

8   Health and safety

8.1   This analysis involves handling freshwater samples that may contain live microorganisms and therefore pose some threat of infection. Laboratory personnel who are routinely exposed to such water samples are encouraged to protect themselves from water borne illnesses by wearing clean disposable gloves and washing their hands frequently.

8.2   The calibration standards, samples, and most reagents used in this method pose no unusual hazard to an analyst employing standard safety measures including protective clothing and safety goggles. Care must be taken when handling

concentrated sulfuric acid and sodium hydroxide. Analysts should review the MSDSs for all chemicals used in this analysis.

8.3   This procedure requires use of an autoclave or pressure cooker capable of heating samples to 120ºC. All safety directions for using these devices should be followed carefully.

9   Personnel qualifications
        Laboratory and field personnel shall have a working knowledge of this analytical procedure and will have received training from an MSU employee knowledgeable of the proper sample analysis procedures. Prior to the first batch of sample analyses, the analyst will complete a demonstration of capability exercise as described below in the Quality control section.

10   Equipment and supplies
        10.1   Balance: analytical, capable of accurately weighing to the nearest 0.0001 g.

        10.2   Glassware: Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

        10.3   Glass culture tubes: with linerless polypropylene caps, 20 mm OD and 150 mm long. Clean before first use by heating to 120ºC with digestion reagent. Rinse with 6*M* HCl and deionized water between uses.

        10.4   Spectrophotometer: A spectrophotometer capable of measurements at 650 or 880 nm with a path length of 5.0 cm or longer is required. Instruments currently available that meet these requirements are Spectronic Unicam 20 Genesys, Hitachi UV-2001 or Shimadzu UV-1600 or equivalent.

        10.5   Spectrophotometer cells: Cells, including flow cells, with path lengths of 1.0 cm or longer, can be used. This procedure will normally employ a cell with 5.0 cm path length.

        10.6   Heating unit: Use either an autoclave or pressure cooker capable of heating samples to 121°C (15 – 20 PSI).

11   Reagents and standards
        11.1   Deionized water (DI): Use deionized water that has been purified with a Barnstead/Thermolyne purification system that includes ion exchange and organic purification cartridges. Use this water for all procedures.

        11.2   Sulfuric acid, 5.4*M* (11*N*): *Cautiously* add 310 mL concentrated sulfuric acid to an equal volume of water. CAUTION: This mixture will become very hot. Dilute to 1 L.

        11.3   Antimonyl potassium tartrate solution: Dissolve 0.3 g $K(SbO)C_4H_4O_6$ - ½H_2O (antimony potassium tartrate hemihydrate) in about 50 mL water and dilute to 100 mL. Store at 4°C in a dark bottle.

11.4   Ammonium molybdate reagent: Dissolve 4 g $(NH_4)_6Mo_7O_{24}$ - $4H_2O$ in 100 mL deionized water. Store at 4°C in a plastic bottle. Note: This solution (4% (w/v) ammonium molybdate) is commercially available.

11.5   Ascorbic acid, 0.1M: Dissolve 1.76 g ascorbic acid in deionized water and dilute to 100 mL. This solution is stable for approximately 1 week if stored at 4°C.

11.6   Combined reagent: Mix the above reagents (11.2 – 11.5) in the following portions for 100 mL of the mixed reagent. 23 mL 11N H_2SO_4 (11.2), 5 mL antimony potassium tartrate (11.3), 15 mL ammonium molybdate (11.4), 30 mL ascorbic acid (11.5), and enough water to make 100 mL (about 27 mL).

   a.   Each solution should be at room temperature before mixing.
   b.   Mix in the specified order and mix well after each addition.
   c.   If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding.
   d.   The stability of the solution is limited. It should be prepared fresh for each day's run, and used for a maximum of 8 hours.

11.7   Ammonium persulfate: Use ACS reagent grade $(NH_4)_2S_2O_8$. Dissolve in water at concentration of 0.32 g per mL. Prepare volume appropriate to the number of samples that will be run (at 0.25 mL per sample) – see Table 1. Prepare fresh daily. (0.25 mL = 0.08 g $(NH_4)_2S_2O_8$).

   Table 1. Numbers of samples and volume of Ammonium persulfate needed.

Maximum number of samples	$(NH_4)_2S_2O_8$ (g)	Volume (mL)
40	3.2	10.0
100	8.0	25.0
200	16.0	50.0

11.8   Sodium hydroxide solution, 6M: Dilute 31 mL 50% NaOH solution (commercially available) to 100 mL. Store in plastic bottles.

11.9   Sodium hydroxide solution, 1M: Dilute 10 mL 6M NaOH (11.8) with 50 mL water.

11.10 Phosphate stock solution (1,000 mg P/L): This standard is commercially available. Alternately, dissolve 2.197 g anhydrous KH_2PO_4 and dilute to 500 mL in a volumetric flask. 1.00 mL =1.00 mg PO_4^3 - P. Two batches of stock solution are needed, using different sources of phosphate (e.g., different lot numbers from the same supplier or different suppliers). Use one batch to prepare calibration standards and the other to produce quality control standards.

11.11 Phosphate intermediate solution (10.0 mg P/L): Dilute 5.00 mL of the phosphate stock solution (11.10) to 500.0 mL.   1.00 mL = 0.010 mg P.

11.12 Preparation of phosphate calibration and quality control standards: Prepare standards according to Table 2. Use within 24 hours.

11.13 Adenosine-5'-monophosphate monohydrate (MW = 363.24) (AMP) stock solution: 117.9 mg AMP diluted to 100 mL (100 ppm P, 1 mL = 100 mg $P_{organic}$).

11.14 AMP standard: dilute 0.500 mL stock to 250 mL = 0.200 mg/mL (0.200 ppm) P. The AMP standard is used to confirm effective digestion of organic phosphorus. Analyze one AMP test standard per analytical run.

Table 2. Preparation of calibration standards for Total Phosphorus analysis.

Solution	mL intermediate solution (11.11)	Final mL	Concentration (mg PO_4^3-P/L)	Use
LRB	(deionized water)	---	0.000 mg PO_4^3-P/L	Calibration blank
TP-1	0.10	100.0	0.010 mg PO_4^3-P/L	Calibration standard
TP-2	0.20	100.0	0.020 mg PO_4^3-P/L	Calibration standard
TP-3	0.50	100.0	0.050 mg PO_4^3-P/L	Calibration standard
TP-4	1.00	100.0	0.100 mg PO_4^3-P/L	Calibration standard
TP-5	2.00	100.0	0.200 mg PO_4^3-P/L	Calibration standard
TP-6	5.00	100.0	0.500 mg PO_4^3-P/L	Calibration standard
LCC	5.00	250.0	0.200 mg PO_4^3-P/L	Lab control check
*QCC	2.00*	100.0	0.200 mg PO_4^3-P/L	Quality control check

LRB = Lab Reagent Blank; C = Calibration standard; LCC = Laboratory Control Check; QCC = Quality Control * QCC solutions are prepared using alternate phosphate standard solution, i.e., not the same standard used to prepare the calibration standards.

12   Sample collection, preservation, shipment and storage

12.1 Samples are collected in 500-mL plastic bottles and preserved with concentrated sulfuric acid (H_2SO_4) to pH <2 – CAUTION: highly corrosive; will cause chemical burns to exposed skin, wear protective gloves.

12.2 Sample bottles are stored on ice for transport to the laboratory.

12.3 Preserved and refrigerated samples can be stored for up to 28 days.

13   Quality control

13.1 Quality control program: The minimum requirements of the quality control program for this analysis consist of an initial demonstration of laboratory capability and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing check on performance. The laboratory must maintain performance records that define the quality of the data that are generated.

a.   Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Sections 13.4 and 15.1.

b.   Analyses of laboratory blanks are required to demonstrate freedom from contamination.

c.   The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

d.   The laboratory should maintain records to define the quality of data that is generated.

13.2   Initial demonstration of performance. The following must be satisfied before the analytical procedure may be used for samples and before a new analyst may analyze samples.

a.   Method Detection Limit (MDL) – To establish the ability to detect the analyte, the analyst shall determine the MDL by carrying through 7 or more separately prepared reagent blank solutions through the analytical procedure in Section 15. The average value, X, and the standard deviation of the values, s, shall be calculated. The MDL is equal to 3s (3 x standard deviation). The MDL and average value, X, should both be less than or equal to 0.005 mg P/L.

b.   Initial Precision and Recovery – To establish the ability to generate acceptably precise and accurate results, the operator shall perform 10 replicates of a mid-range standard (0.200 mg TP/L), according to the procedure in Section 9. Using the results of the replicates compute the average value, S, and the standard deviation, s, for the analyte. The value of X should be within 10% of the true value. The standard deviation should be less than or equal to 10% of the average value.

13.3   The DIB, LRB, LCC, QCC and AMP should be measured along with the standards at the start of the analytical cycle. The criteria are listed in Table 3.

Table 3. Quality control solution and acceptable ranges.

Solution	Acceptable range	Comments
DIB	< 0.005 mg TP/L	Less than LRB
LRB	≤ 0.005 mg TP/L	Ideally less than or equal to the required detection limit
LCC	0.180 - 0.220 mg TP/L	Within ± 10% of the true value
QCC	0.180 - 0.220 mg TP/L	Within ± 10% of the true value
AMP	0.180 - 0.220 mg TP/L	Within ± 10% of the true value

13.4   With each sample batch of ten samples, the following should also be run
        (acceptance criteria noted):

Table 4. Quality control samples and acceptance criteria.

Solution	Acceptance Criteria
DIB	< 0.001 mg TP/L
LRB	Ideally < 0.001 mg TP/L
LCC	0.180 - 0.220 mg TP/L or 100 ± 10%recovery
LD	greater of ± 20% or ± 0.005 mg TP/L
FD	greater of ± 20% or ± 0.005 mg TP/L
LS-1 and LS-2	both 100 ± 20% recovery

14   Calibration and standardization
        Calibration: Obtain a standard curve by plotting absorbance of standards
(including the reagent blank) versus concentration. The data will be fit to a linear
equation using a spreadsheet program such as Excel.

15   Procedure

15.1   Preparation of laboratory-fortified matrix (LFM): Prepare two LFM samples using
        10-mL aliquots of a water sample (or a smaller aliquot diluted to 10 mL) from the
        same sample. Spike each with 0.200 mL phosphate standard solution. (This
        should increase observed concentration by 0.200 mg/L.) Carry each through the
        sample preparation and analysis procedure (15.3 and following).

15.2   Preparation of samples and standards – digestion: All samples and standards
        (including quality control solutions) should be processed in the same manner.

        a.   Adjust the pH of a well-mixed sample to 6.0 to 8.0 using 6M NaOH and
                1M H_2SO_4 or HCl.
        b.   Transfer 10 mL of a well-mixed sample (or an aliquot of sample diluted to
                10 mL) to a screw-cap culture tube.
        c.   Add 0.25 mL of the $(NH_4)_2S_2O_8$ solution (11.7) and 0.2 mL 5.4 M H_2SO_4 to
                each tube and mix.
        d.   Cap tubes loosely. – It is best to initially tighten the caps, invert the tubes
                a few times to ensure good mixing, and then unscrew the caps until the
                seal just becomes loose.
        e.   Place tubes in rack in either autoclave or pressure cooker.
        f.   For autoclave, follow manufacturer's directions and heat at 121ºC for 30
                minutes.
        g.   For pressure cooker: Add sufficient water to pressure cooker to bring
                water to a depth of at least 5 cm. Heat the pressure cooker on a hotplate
                set to high until the water in the cooker is boiling, as evidenced by a

steady stream of steam emerging from the pressure cooker's vent. Maintain constant boiling (adjusting heat as needed) for 30 minutes. Allow pressure cooker to cool in air for about 30 minutes. After this initial cooling, it is normally possible to open the pressure cooker safely.

h.　Remove the tubes from the autoclave or pressure cooker and cool to 20 – 30ºC.

15.3　Neutralizing digested samples:
a.　Add 0.40 mL 6M NaOH and 1 drop phenolphthalein solution to each sample and mix. *Adjust the volume of 6M NaOH if appropriate.*
b.　Add 6M NaOH until the solution just turns pink, and then add 5.4 M H_2SO_4 until the pink color just clears.

15.4　Spectrophotometer setup:
a.　The spectrophotometer should be allowed to warm up at least 30 minutes prior to the start of measurements.
b.　Set the wavelength to 880 nm.
c.　The spectrophotometer should be set up with a holder appropriate to the size cell used (normally 5 cm cell).

15.5　Color development and measurements:
a.　To the first ten tubes, add 1.5 mL mixed molybdate reagent solution and mix, noting time.
b.　Samples that appear turbid should be centrifuged or filtered.
c.　Just before starting measurements, add mixed molybdate reagent solution to the next ten tubes, again noting time.
d.　Add mixed molybdate reagent to other tubes to maintain an approximately 10 minute interval between time of mixing and measurement.

15.6　Spectroscopic measurements:
a.　Zero the spectrometer using deionized water.
b.　Start measurements approximately ten minutes after addition of the mixed reagent.
c.　Measurements may be continued up to thirty minutes past addition.

16　Data acquisition, calculations, and reporting
16.1　Calculation of concentrations: The concentration of each solution will be calculated based on the linear equation for the regression data. The concentrations will represent the concentration of analyte in the 10-mL aliquot in 15.2.b.

16.2　Calculation of water sample concentrations, corrected for dilution: For samples for which dilution was required, the concentration in the original water sample is calculated using equation 1.

a.　Equation 1: $C_{sample} = C_{analysis} \times (10.0 \text{ mL}/V_{aliquot})$

$V_{aliquot}$

b. Where: C_{sample} is the concentration in the original water sample,
            $C_{analysis}$ is the concentration of the solution as determined in (16.1),
    and $V_{aliquot}$ is the volume of the aliquot diluted to 10 mL in (15.2.b).

16.3   Reporting results: Results should be reported to 0.001 mg TP/L precision.

16.4   The evaluation of MDL and precision require calculation of standard deviation.
       Standard deviations should be calculated as indicated below, where n = number
       of samples, x = concentration in each sample. Note: This is the sample
       standard deviation calculated by the STDEV function in Microsoft Excel.

$$s = \left(\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1} \right)^{\frac{1}{2}}$$

16.5   Calculation of recoveries: Recovery of matrix spike solutions shall be calculated
       as indicated below, where S = concentration observed for spiked sample, U =
       concentration observed for unspiked sample, and 0.200 is the concentration
       increase expected upon spiking. Both S and U are concentrations based on
       (16.1), i.e., not adjusted for dilution of an aliquot. The factor 1.02 corrects for the
       small volume change upon spiking.

$$\% \ recovery = \frac{1.02 \times S - U}{0.200}$$

17   Computer hardware and software
17.1   Word: This document and attached bench sheet are prepared using Microsoft
       Word. The Word document file name for this SOP is: 3010R01 Total P.doc

17.2   Excel: Quality control charts are created using Excel.

18   Method performance
18.1   The desired performance criteria for this measurement are:
       a.   Detection limit: 0.005 mgTP/L
       b.   Precision: ± 20%
       c.   Accuracy: ± 20%
       d.   Minimum Quantification Interval: 0.001 mg TP/L *define in section 6*

18.2   According to EPA 365.2 the applicable range for the method is 0.01 mg TP/L to
       0.5 mg TP/L and may be extended by dilution. EPA 365.2 describes
       performance for undigested orthophosphate samples but not for digested

samples.

19   Pollution prevention

19.1   All wastes from these procedures shall be collected and disposed of according to existing waste policies within the MSU Chemistry Department.

19.2   Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste

20   Data assessment and acceptable criteria for quality control measures

20.1   The analyst should review all data for correctness (e.g., calculations).

20.2   Precision values are calculated for pairs of duplicate analyses. Record the precision values as a percent on the Total phosphorus bench sheet. The desired precision is ± 20%.

20.3   The desired detection limit is 0.005 mgP/L

20.4   Percent recovery is calculated for LCC, QCC and spiked samples. Record these values on the bench sheet. The desired recoveries are; 100 ± 10% for LCC and QCC and 100 ± 20% for spiked samples.

20.5   The completed bench sheet is reviewed by the OEWRI QA/QC manager.

21   Corrective actions for out-of-control or unacceptable data

21.1   Quality control charts will be created for charting precision (REP), accuracy (LCC and LFM) and laboratory reagent blank (LRB) values.

21.2   The results for precision, accuracy and LRB data are compared to the acceptable values for this analysis; ± 20%, 100 ± 10% (LCC), 100 ± 20% (LFM) and 0.005 mgP/L, respectively.

21.3   If a precision value exceeds 20% then the analyst should write in the comments section of the bench sheet: "These data are associated with an out-of-control duplicate analysis. The UCL = 20%." Note: "UCL" is the Upper Control Limit (i.e., 20%).

21.4   If an accuracy value exceeds the UCLs for LCC or LFM, then the analyst should write in the comments section of the bench sheet: "These data are associated with an out-of-control LCC (or LFM) analysis. The limit is 100 ± 10% (or ± 20%)."

21.5   If a LRB value exceeds 0.005 mg/L then the analyst should write in the comments section of the bench sheet: "These data are associated with a LRB value that exceeds the detection limit of 0.005 mg/L."

21.6   If there is sufficient volume of sample remaining for all samples, the analytical batch may be re-analyzed. Quality control data from the first run must be recorded on the control charts.

21.7   If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.

22   Waste management

22.1   The wastes generated in this method are hazardous because of a low final pH. The samples may be neutralized by the addition of water in accumulation container (e.g., a large beaker or flask). Add sufficient amounts of water to adjust the pH to between 2 and 12.5. When the pH is > 5 (for acidic wastes) the solution can be discarded in the laboratory drain followed by an equal volume of water.

22.2   Outdated chemicals are discarded following the procedures of the MSU Environmental Management Department

23   References

23.1   EPA Method 365.2, Phosphorus, All Forms (Colorimetric, Ascorbic Acid, Single Reagent).

23.2   Standard Methods for the Examination of Water and Waste Water, Method 4500-P, Phosphorus, APHA, 19th Edition, 1995, especially B. Sample Preparation, and E. Ascorbic Acid Method.

23.3   "21.0: Determination of Total Phosphorus," EPA Handbook of Methods for Acid Deposition Studies: Laboratory Analysis for Surface Water Chemistry," EPA publication 600/4-87/026, August 1987.

24   Tables, diagrams, flowcharts and validation data

24.1   Tables can be found within this document. There are no diagrams, flowcharts or validation data.

24.2   Appendix A describes the variations from EPA 365.2. See next page.

25 APPENDIX A: Variations from EPA Method 365.2

Sample size: The volume of water sample is reduced from 50 mL to 10 mL and the amounts of sulfuric acid and $(NH_4)_2S_2O_8$ have been adjusted proportionately, as indicated in the table below.

Changes:	EPA 365.2	This Method
Sample size:	50 mL	10 mL
5.4M (11N) sulfuric acid added (mL):	1.0	0.20
Ammonium persulfate added (g):	0.4	0.080

Note that a similar EPA digestion procedure (EPA 1987) employs a 10 mL sample.

pH adjustment: EPA 365.2 indicates that the pH of each digested sample and standard should be adjusted to 7.0 ± 0.2 by addition of 1N NaOH. The method employed here uses a less critical pH adjustment (step 15.2.b) that is, however, consistent with Standard Methods 4500-P B and E. This variation is justified as follows:

- It should be noted that precise adjustment of the pH of an unbuffered solution in this range is difficult – addition of 1 μL (1/50 drop) 1N NaOH to 50 mL deionized water should increase the pH by more than one unit.

- In addition, it is noteworthy that in the same step in which the pH 7.0 ± 0.2 neutralization is specified, the method also states that if a sample is not clear after the neutralization step, 2-3 drops 11N H_2SO_4 should be added – enough acid to drop the pH below 2.0. This suggests that precise pH adjustment is not really critical.

- In addition, the combined reagent contains 2.5N sulfuric acid. Addition of 3 mL of this solution to 50 mL of a pH 7.0 sample (as specified in Method 365.2) results in a solution that is 0.141 N acid, so that the trace excesses of acid or base in samples ranging from pH 6.0 to 8.0 are insignificant.

- Method 365.2 indicates that the pH should be adjusted using 1 N NaOH. In the digestion step, 1.0 mL 11 N acid is added to a 50 mL sample (equivalent to 0.40 mL for a 20 mL sample). Neutralization of this amount of acid with 1 N NaOH requires 11 mL for a 50 mL sample (4.4 mL for a 20 mL sample). This represents a very significant volume change. In this method, the initial step of the neutralization is carried out using 6N NaOH, decreasing the required volume of base considerably.

Addition of ammonium persulfate: In the original method, ammonium persulfate is added as a solid using an appropriate size scoop. As the sample volume is reduced from 50 mL to 10 mL, the amount of ammonium persulfate must be reduced from 0.4g to 0.08 g. However, it would be difficult to add 0.08 g using a scoop. Instead, ammonium persulfate is

delivered as an aqueous solution (0.25 mL of 0.32 g/mL solution). This provides the required amount very reproducibly.

Preparation of combined reagent: EPA 365.2 requires preparation of both 11 *N* and 5 *N* sulfuric acid, for digestion and combined reagent preparation, respectively. In this method, 11 *N* sulfuric acid is used to prepare the mixed reagent, eliminating the need for the 5 *N* sulfuric acid and reducing waste generation.

Detection limits: The desired detection limit for this method is < 0.005 mg TP/L. Method 365.2, an EPA method intended for measuring phosphorus in surface waters, does not specify a detection limit, though it does specify that the method is applicable to samples in the range of 0.01 to 0.5 mg P/L.   In the Precision and Accuracy section, reproducibility for a 0.029 mg P/L orthophosphate sample was ±0.010 mg P/L, suggesting a detection limit substantially higher than 0.005 mg P/L. Note that the performance data described for EPA 365.2 is based on undigested orthophosphate samples.