1) Particulate Phosphorus Method Specifications

a) Scope and Application

i) This method is applicable for the determination of particulate phosphorus in water, wastewater, saline, surface, and ground waters by a semi-automated high temperature combustion, HCL extraction technique in the range of 0.01 to 2.0 mg/L of P (Aspila, et al.,1976).

b) Summary of Method (edited)

i) Particulates in a known volume of sample are concentrated on a 0.7 µm glass fiber filter. The filter is combusted at 550 °C to oxidize organic and inorganic phosphorus compounds to orthophosphate and then extracted in dilute HCL. Dissolved orthophosphate in the extract is determined by the ascorbic acid, molybdenum blue method for orthophosphate (Section XX). EPA 365.1.

c) Interferences

i) Silicon concentrations of 100 µg/L or more cause an interference equivalent to approximately 0.04 µg/L of P/L. This interference can be avoided by maintaining an acid concentration of 2.45N H_2SO_4 in the reagents and analysis at 37 ± 2°C.
Comment: SKALAR analysis done at 40°C

ii) Refractive Index correction unnecessary

d) Apparatus and Materials

i) Automated analytical system such as a continuous segmented flow analyzer, flow injection analyzer or discrete analyzer equipped with an autosampler, reagent addition and mixing components, heating bath, colorimeter, phototube and computer-based data system. Add a dilution coil to automatically dilute the sample to 10% with reagent water for orthophosphate analysis as per subsection 3.

ii) ~~Appropriate glassware: see section 1.8~~
~~**NOTE**: Clean all glassware two times with 4N HCl and rinse 9 times with reagent water~~.

Phosphorus -free glassware: All glassware used in the determination must be low in residual phosphate to avoid sample/reagent contamination. Washing with 10% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use critical cleaning liquid detergents instead of or before acid rinsing. A laboratory's glassware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

iii) Autoanalyzer cups. see section 1.8.3

iv) Muffle furnace capable of maintaining temperatures 550 ± 50°C.

v) Whatman® or Environmental Express® glass fiber filters (0.7 µm pore size). 47 or 25 mm OK?

vi) Screw cap centrifuge tubes, 50 mL capacity. Plastic or glass acceptable?

vii) Vacuum filter apparatus with a minimum of 100 mL capacity.

viii) Reagent Grade Water: see Chapter VI, Section 4.2.

~~e) Reagents~~

~~i) Hydrochloric Acid, 1N: In a one-liter volumetric flask, add approximately 800 mL of reagent water. Add 86 mL of concentrated HCl and dilute to one liter with reagent water.~~

~~ii) Sulfuric Acid, 5.0N: To a one-liter volumetric flask, add 400 mL of reagent water. Add 140 mL of concentrated H_2SO_4, mix, cool, and dilute to one liter with reagent water.~~

~~iii) Potassium Antimony Tartrate Solution: In a one-liter volumetric flask, add 800 mL of reagent water. Add 3.0 g of $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$, dissolve, and dilute to volume with reagent water. Store in a glass-stoppered bottle at room temperature.~~

~~iv) Ammonium Molybdate Solution: In a one-liter volumetric flask, add 800 mL of reagent water. Add 40.0 g of ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, dissolve, and dilute to volume with reagent water. Store in a plastic bottle at 4 ± 2°C away from direct sunlight.~~

~~v) Ascorbic Acid: In a one-liter volumetric flask, add 800 mL of reagent water. Add 18.0 g of ascorbic acid, dissolve, and dilute to one liter with reagent water. Dispense 40 mL into clean polybottle and freeze. Thaw overnight in the refrigerator before use.~~

~~vi) Sodium Lauryl Sulfate Solution: In a 100-mL volumetric flask, add 80 mL of reagent water. Add 3.0 g of sodium lauryl sulfate, dissolve, and dilute to 100 mL with reagent water.~~

vii) ~~Working reagent:~~

~~(1) Reagent A: In a 100-mL volumetric flask, add 50 mL of 4.9N H_2SO_4, 5 mL of potassium antimony tartrate solution, 15 mL of ammonium molybdate, and 1.0 mL of sodium lauryl sulfate solution. All reagents must reach room temperature before they are mixed. Add the reagents in the order given and mix the resultant solution after each addition. The combined reagent is stable for 4 hours.~~

~~(2) Reagent B: In a 100-mL volumetric flask, add 30 mL of ascorbic acid, and 0.3 mL of sodium lauryl sulfate solution. All reagents must reach room temperature before they are mixed. Add the reagents in the order given and mix the resultant solution after each addition. The combined reagent is stable for 4 hours.~~

viii) Stock Phosphate Standard Solution: In a one-liter volumetric flask, add 500 mL of reagent water. Add 1.632 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) that has been dried overnight at 105 ± 2°C (stored in a desiccator), dissolve, and dilute to volume with reagent water. Add 1.0 mL of chloroform as a preservative. This solution is only stable for six months at 4 ± 2°C. (1.0 mL = 12.0 µg P)

f) Sample Handling

i) Samples (filter pads) are stored at -20 ± 2°C for a maximum 28 days.

ii) OK to filter in the lab? then what?

g) Procedure

i) Filter and Blank Sample Preparation

CBL: a) Now pre-rinsing for TSS? b) Measures & subtracts blank pad. Rinsed?

ODU: a) Pre-rinse all PP filters, dry. Use TSS filters mostly? b) Blanks?

DCLS: a) Not Pre-rinsed b) Blanks?

ii) Sample preparation:

(1) Field Filtration: Using a forceps transfer a 0.7 µm glass fiber filter, wrinkled side up, onto the base of a vacuum filtration apparatus. Filter a known volume of water through the filter under vacuum pressure (≤ 20 inches Hg) to concentrate particulates on filter pad. Rinse particulates with DI water, continue suction until dry. Freeze at ≤ 20°C

(2) If using a sample filter from the total suspended solids analysis, record the weight of the GF/F filter after the total suspended solids have been determined, proceed to Step 3 or refreeze the filters.

(2) Remove the filters from the freezer and store in a clean container and dry overnight in an oven at 50 ± 2°C. CBL dries only for TSS; DCLS does not dry; ODU dries at 104C.

(3) Place the dried filters in a clean numbered crucible in a muffle furnace and heat for 2 hours at 550 ± 2°C. Cool overnight. CBL & ODU – combusted for 1.5 hrs.

ii) Place the filters in labeled 50-mL plastic screw cap centrifuge tubes, add 10 mL of 1N HCl, cap and shake gently, several times during a 24-hour period. Dilute to 50 mL with reagent water and measure phosphate concentration.

iii) Transfer the supernatant extract to autoanalyzer cups with a Pasteur pipette, and prepare to measure the phosphate concentration as specified in the Orthophosphate method.

iv) ~~Prepare instrument for operation. Obtain a stable baseline with all reagents, feeding reagent water through the sample line.~~

~~v) Prepare a series of standard solutions covering the concentration range of the samples by diluting either the stock or standard solutions with substitute ocean water.~~

~~vi) Analytical sequence: The samples and associated QC samples and standards should be run according to the following sequence.~~

~~(1) Five calibration standards with concentration within the linear range of the test.~~
~~(2) Two method blanks.~~
~~(3) Ten–twenty CBP samples.~~
~~(4) One matrix spike sample.~~
~~(5) One medium concentration calibration standard.~~
~~(6) One method blank.~~
~~(7) Steps 4.7.6.3 – 4.7.6.6 are repeated until are samples are analyzed or QC samples indicate that the system is out of control and recalibration is necessary.~~
~~(8) One high concentration calibration standard.~~
~~(9) One medium concentration calibration standard.~~
~~(10) One low concentration calibration standard.~~

~~vii) Switch sample line from distilled water to sampler and begin analysis.~~

~~viii) Prepare appropriate standard curve by plotting peak heights of processed standards made up with KH_2PO_4, in a 0.2N HCl matrix, against known concentrations. Compute concentration of the samples by comparing sample peak heights with standard curve.~~

NOTE: Subtract the blank background response from the standards before preparing the standard curve. (CBL subtracts the mean reading of filter blanks from the % response.)

ix) Record the stabilized potential of each unknown sample and convert the potential reading to the phosphorus concentration using the standard curve.

h) Quality Control

i) Method detection limits (MDL): Method detection limits should be established using the guidelines in Chapter VI, Section C.

ii) Reference materials: The laboratory must analyze a standard reference material once a year, as available. A laboratory control sample (LCS) for particulates is not commercially available. Prepare a LCS of known concentration in the acid matrix used for the PP extracts.

INDICATOR	ACCEPTANCE LIMITS	ACTION
Correlation Coefficient	≤ 0.995	If < 0.995, evaluate data points. If outside established limits, reject as outlier. Follow rules for establishing a calibration curve
External QC (QCS)	± 10%	Rerun batch if not within acceptance limits. Rejection criteria for batch.
ICV	± 10%	Recalibrate if outside acceptance limits.
CCV	± 10%	If CCV outside acceptance limits, rerun all samples back to last acceptable CCV.
Laboratory Reagent Blank	≤ Quantitation limit	If the LRB exceeds the Quantitation limit, results are suspect. Rerun LRB. If the concentration still exceeds the Quantitation limit, reject or qualify the data.
Laboratory Fortified Blank	80 – 120%	If accuracy as % recovery is outside acceptance limits, the analyte is out of control. Source of problem should be identified and resolved before continuing the analysis.
Laboratory Sample Extract Spike	80 – 120%	If the recovery of any analyte falls outside the designated LFM recovery range and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range the sample should be reported with a "matrix induced bias" qualifier.
Field or Laboratory Duplicate	± 30%.	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analyses.
Dilutor Performance Check	100 ± 10%	If the dilutor check is not within 10% of the target value, then all samples that required dilution must be repeated with manual dilution.

i) Calculations

DCLS - To calculate particulate phosphorus in mg/L:

$$C = E\,x\left(\frac{V_e}{V_f}\right)$$

Where:
E = Concentration determined from linear regression curve (mg/L).
V_e = Extract volume of sample (mL).
V_f = Volume of water filtered (mL).
C = Concentration as corrected for filtrations and extraction (mg/L)

CBL - Phosphorus concentration is calculated using Bran and Luebbe AACE ver.5.22 software. The calculation is based on the following equation:

$$\text{mg P/L} = \frac{[(\%\text{ on AA Chart}) - B] \times F \times V_E}{V_F}$$

Where: B = mean reading of blanks,
F = inverse of the regression slope of the standards,
E V = volume of hydrochloric acid used for extraction (L)
F V = volume of sample filtered (L).

i) References

Aspila, K.I., H Agemian and A.S.Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic, and total phosphate in sediments analysis. 101:187-197.

Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 1193.

Annual Book of ASTM Standards, Part 31, "Water", Standard D 515-72, p 389 (1976).

Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water. 2003 Water-Resources Investigations Report 03–4174 http://nwql.usgs.gov/pubs/WRIR/WRIR-03-4174.pdf

D'Elia, C.F., Magnien, R.E., Zimmermann, C.F., Vaas, P.A., Kaumeyer, N.L., Keefe, C.W., Shaw, D.V., and Wood, K.V., 1987, Nitrogen and phosphorus determinations in estuarine waters: A comparison of methods used in Chesapeake Bay monitoring: University of Maryland Center for Environmental and Estuarine Studies, publication number UMCEES 87-19 CBL, 26 p.