CENTER FOR RESERVOIR AND AQUATIC SYSTEMS RESEARCH (CRASR)

STANDARD OPERATING PROCEDURE #6.0 – Determination of Total P in Water
Analytical Range: 1 to 1000 μg P/L

Revision 1
10 January 2013

1. Method Description

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex, which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample. Polyphosphates may be converted to the orthophosphate form by sulfuric acid digestion and organic phosphorus may be converted to orthophosphate by persulfate digestion.

2. Equipment and Supplies

2.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

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

2.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

2.3.1. Sampler

2.3.2. Multichannel proportioning pump

2.3.3. Reaction unit or manifold

2.3.4. Colorimetric detector

2.3.5. Data system

2.3.6. Acid washed glassware: All glassware used in the determination of phosphate should be washed in 10% muriatic acid and triple rinsed with distilled water. Preferably, this glassware should only be used in the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again.

2.4. Special Appartus:

2.4.1. Heating Unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)

2.4.2. Autoclave or Hot Plate

3. Sample Collection and Preservation – See CRASR SOP #2 for details.

4. Standards

4.1. Phosphate-P Stock solution (1000 mg P/L) – Add 500 ml deionized water to a 1000 ml volumetric flask. Carefully weigh out 11.5641 g of sodium phosphate dibasic 12-hydrate (Na2HPO4*12H2O; FW=358.14) and add it to the flask. Dissolve material, dilute to 1000 ml, and mix well. Solution may be stored indefinitely in refrigerator if also using a laboratory control standard (LCS) as a calibration verification in analyses. If not using LCS, stock solution should be prepared fresh monthly.

4.2. Phosphate Standard (1.0 mg P / L) – The phosphate standard is used for the determination of PO_4-P in preserved and digested water samples. To prepare, add 500 ml of water to a 1000 ml volumetric flask. Carefully pipette 1 ml of 1000 mg/L Phosphate-P Stock Solution into the flask. Dilute to 1000 ml and mix well. Add 1.0 ml concentrated H2SO4. The phosphate standard may be stored in the refrigerator for up to 28 days.

4.3. Working standards – Working standards are used as calibration and continuing calibration verification (CCVs) standards in the determination of PO4-P in digested samples. Prepare mixed working standards at concentrations (high range) of 5, 10, 25, 50, 100, 250, 500, and 1000 ppb (µg/L) PO_4-P, or (low range) 5, 10, 25, 50, 75, 100, 150, 200 and 250 ppb or (µg/L) PO_4-P. The following table outlines preparation, use pipet:

HIGH RANGE

Target Concentration (µg/L)	Total volume (ml)	DNP Mixed Standard Concentration (µg/L)	H_2SO_4 Volume (ml)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	0.100	49.75	0.25
10	50	1000	0.100	49.50	0.50
25	50	1000	0.100	48.75	1.25
50	50	1000	0.100	47.50	2.50
100	50	1000	0.100	45.00	5.00
250	50	1000	0.100	37.50	12.50
500	50	1000	0.100	25.00	25.00
1000	50	1000	0.100	0.00	50.00

LOW RANGE

Target Concentration (µg/L)	Total volume (ml)	DNP Mixed Standard Concentration (µg/L)	H_2SO_4 Volume (ml)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	0.100	49.75	0.25
10	50	1000	0.100	49.50	0.50
25	50	1000	0.100	48.75	1.25
50	50	1000	0.100	47.50	2.50
75	50	1000	0.100	46.25	3.75
100	50	1000	0.100	45.00	5.00
150	50	1000	0.100	42.50	7.50
200	50	1000	0.100	40.00	10.00
250	50	1000		37.50	12.50

Add concentrated H_2SO_4 to each standard to a final concentration of 0.1%. Acidifying with 2 µL H_2SO_4 per 1mL sample will achieve this concentration. Standards may be stored in the refrigerator for up to 28 days.

4.4. Laboratory Control Standard – A laboratory control standard of 150 ppb should be prepared from a purchased certified aqueous phosphate-phosphorus standard. This standard should be certified by the external source from which it was purchased.

4.5. Organic Phosphorus Standard – A organic phosphorus compound (e.g. RNA) should be digested to determine organic bound phosphorus recovery.

5. Reagents

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube (Lachat Part No. 50100.) Bubble He through the solution for one minute.

Reagent 1. Stock Ammonium Molybdate Solution

By Weight: To a tared **1 L** container add **40.0 g ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$] and **983 g DI water**. Stir for a minimum of four hours. Store in plastic and refrigerate.

Reagent 2. Stock Antimony Potassium Tartrate Solution

By Weight: To a **1 L** dark, tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$) or dissolve **3.22 g antimony potassium tartrate** (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2 \cdot 3H_2O$) and **995 g DI water.** Stir or shake until dissolved. Refrigerate. Maybe stored up to two months when kept refrigerated.

Reagent 3a. Molybdate Color Reagent

By Volume: To a **1 L** volumetric flask add about **500 mL DI water**, and then add **21.0 mL concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 mL Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 mL Ammonium Molybdate Solution** (Reagent 1). Dilute to the mark **DI water** and invert to mix.

Reagent 3b. Molybdate Color Reagent

Hach sells a premixed 1-L bottle of Molybdate Color Reagent (Part no. 52002). This product has a shelf life of at least two months.

Reagent 4. Ascorbic Acid Reducing Solution, 0.33 M

By Weight: To a tared **1 L** container, add **60.0 g granular ascorbic acid and 975 g DI water**. Stir or shake until dissolved. Add **1.0 g dodecyl sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Prepare fresh weekly. Discard if the solution becomes yellow.

Reagent 5. Carrier: Sulfuric Acid, 0.13 M

By Volume: In a **1 L** volumetric flask, add **500 mL DI water** and **7.2 mL concentrated sulfuric acid** (H_2SO_4). Dilute to the mark **DI water** and invert to mix. Degas daily. Prepare fresh weekly.

6. Procedure

6.1. Prepare reagent and standards as described in sections 4 and 5 of this document.

6.2. Transfer 10 ml of each blank, standard, and sample to a digestion tube (borosilicate scinatillation vial with open-top cap). Add 0.095-0.14 g of potassium persulfate ($K_2S_2O_8$) and 0.2 ml of 5.6 M H_2SO_4. Cap samples tightly and mix thoroughly. Autoclave samples for 60 minutes using program 3 on the large autoclave on BSB 3rd floor. Program for autoclave should not include any drying time. After cycle has finished, remove samples from autoclave and allow them to cool to room temperature.

6.2. Set up phosphorus manifold as shown in section 9 of this document (manifold diagram is on the last page of the SOP).

6.3. Make sure power is on to all portions of the instrument (autosampler, manifold pump, and main instrument) then open the Omnion software. On the main screen click on the "Configuration" pull down menu then click on "Autosamplers". When the pop up menu opens, click the button that says "Initialize Autosampler". This should cause the autosampler to re-center itself over the rinse tube then move permanently down into the rinse tube.

6.3. After you have initialized the autosampler, open the total P in waters template by going to the "Run" pull down menu and clicking on "Open". Navigate to the folder Omnion/templates and click on the file named total-P-water.omn. When the file opens, the software will ask if you would like to change the setpoints of the relevant heaters. Click yes. Click again on the "Run" pull down menu, then click "Save As". Navigate to the folder Omnion/Data/Inorganic Nutrients. Save the template as the batch ID (yyyymmddPRJmANL; code on Chain of Custody Form) number associated with the sample set you are running. After the template has been saved, click the "Preview" button on the toolbar. This will allow you to view the baseline signal from the flowcell.

6.3. Secure the pump tubes to the pump by clicking down the tubing shafts. Turn on the pump by pressing the manual flow button on the top left of the pump (blue button). Make sure that the probe rinse pump line is submerged in DI water and that the probe is down in the rinse tube on the autosampler. Put all reagent lines into DI water. Pump DI water through all reagent lines and check for leaks and smooth flow. After any air in the system has passed through the flow cell, the baseline in the preview screen should be completely flat. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. This will probably take at least 15 minutes for all chemicals to come into equilibrium in the mixture.

6.4. Place standards in the sampler according to the slot locations in the method template. Fill in samples with the appropriate duplicates and spikes. All information on duplicate and spike samples has already been set up in the template for the analytical method, the only thing that needs to change are the sample IDs.

6.5. Once all sample data has been entered into the sample sheet, save the run again as described earlier. Once saved, check the baseline to insure that reagents have come through and that the baseline is stable. If stable, press the "Run" button on the tool bar.

6.6. The software will check that the LCS, and that all CCVs, duplicates, and spikes meet appropriate QA/QC criteria. If the LCS fails to meet QA/QC criteria, the run will automatically terminate. However, if the LCS passes and one of the CCVs, duplicates, or spikes fails to meet QA/QC criteria, the run will continue. It is imperative that the analyst check the QA/QC results for all CCVs, duplicates, and spikes and rerun any and all sample sets that do not adhere to QA/QC requirements outlined in CRASR SOP #8.

7. **Quality Control/Quality Assurance –** See CRASR SOP # 8 (Quality Assurance and Quality Control) for details on QA/QC criteria.

8. References

Lachat Method # 10-115-01-1-F, Determination of total phosphorus by flow injection analysis colorimetry (acid persulfate digestion method).

U.S. Environmental Protection Agency, Methods for the Determination of Inorganic Substances in Environmental Samples, EPA-600/R-93/100, August 1993, Method 365.1

Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-85.

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

9. Instrument Information

TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and ***will need to be optimized*** using graphical events programming.

Sample throughput:	60 samples/h, 60 s/sample
Pump Speed:	35
Cycle Period:	60

Analyte Data:

Concentration Units:	µg P/L
Chemistry	Brackish
Inject to BW Baseline Start	10 s
Inject to BW Baseline End	65 s
Inject to BW Integ Start	25 s
Inject to BW Integ End	60 s

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration µg P/L	200	100	50	25	10	3.0	0.0

Calibration Rep Handling:	Average
Calibration Fit Type:	2nd Order Polynomial
Weighting Method:	None

Force through zero:	No

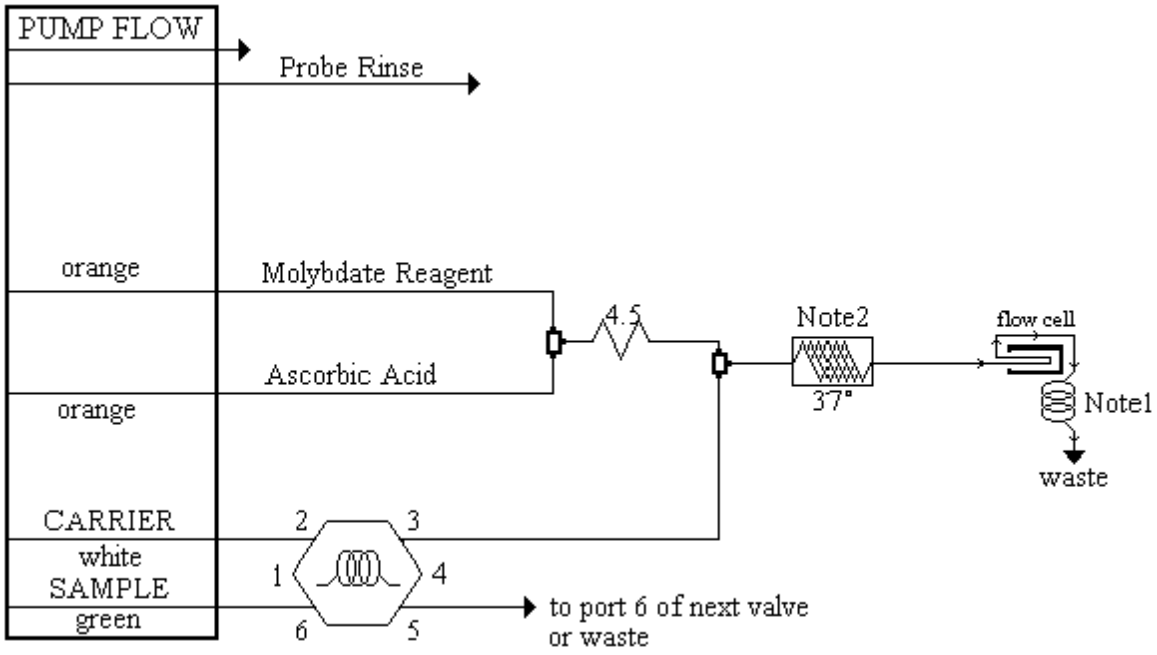
Sampler Timing:

Min. Probe in Wash Period:	10 s
Probe in Sample Period:	23 s

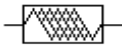
Valve Timing:

Load Time:	0 s
Load Period:	18 s
Inject Period:	42 s

17.3. PHOSPHORUS MANIFOLD DIAGRAM



Carrier: **0.13 M sulfuric acid**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 100 cm
QC8000 Sample Loop: 100 cm
Interference Filter: 880 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at the specified temperature.

4.5: **70** cm of tubing on a 4.5 cm coil support

Note 1: 200 cm back pressure loop, 0.52 mm (0.022 in.) i.d.
Note 2: 175 cm of 0.8 mm i.d. tubing on the heater.