CENTER FOR RESERVOIR AND AQUATIC SYSTEMS RESEARCH (CRASR)

STANDARD OPERATING PROCEDURE #3.0 – Determination of PO_4-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.

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. Autosampler

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 with 10% muriatic acid and triple rinsed with distilled water. Preferable, this glassware should be used only for the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again.

2.4. Special Apparatus

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

2.4.2. Glass calibration vials must be used. Lachat Part No. 21304 for XYZ samplers.

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 12hydrate (Na_2HPO_4*$12H_2O$; 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. DNP Mixed standard (1.0 mg P / L) – The DNP mixed standard is used for the simultaneous determination of PO_4-P, NH_3-N and NO_2-N+NO_3-N in unpreserved 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, 1 ml of Ammonia-N Stock Solution and 1 ml of Nitrate-N Stock Solution into the flask. Dilute to 1000 ml and mix well by inverting flask three times. The DNP Mixed standard may be stored in the refrigerator for up to 48 hours.

4.3. Mixed working standards – Mixed working standards are used as calibration and continuing calibration verification (CCVs) standards in the simultaneous determination of PO_4-P, NH_3-N and NO_2-N+NO_3-N. 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)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	49.75	0.25
10	50	1000	49.50	0.50
25	50	1000	48.75	1.25
50	50	1000	47.50	2.50
100	50	1000	45.00	5.00
250	50	1000	37.50	12.50
500	50	1000	25.00	25.00
1000	50	1000	0.00	50.00

LOW RANGE

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

Mixed working standards may be stored in the refrigerator for up to 48 hours.

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.

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 Volume: In a **1 L** volumetric flask dissolve **40.0 g ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$)] in approximately **800 mL DI water**. Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate. May be stored up to two months when kept refrigerated.

Reagent 2. Stock Antimony Potassium Tartrate Solution

By Volume: In a **1 L** volumetric flask, dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6\cdot 1/2\ H_2O$) or dissolve **3.22 g antimony potassium tartrate** (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\cdot 3H_2O$) in approximately **800 mL DI water**. Dilute to the mark and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate. May be 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 **35.0 mL concentrated sulfuric acid** (CAUTION: The solution will get very hot!) Swirl to mix. When it can be comfortably handled, add **72 mL Antimony Potassium Tartrate Solution** (Reagent 2) and **213 mL Ammonium Molybdate Solution** (Reagent 1). Dilute to the mark and invert to mix. Degas with helium. Prepare fresh weekly.

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 Volume: In a **1 L** volumetric flask, dissolve **60.0 g granular ascorbic acid** (Spectrum catalog no. AS102) in about **700 mL DI water**. Dilute to the mark and invert to mix. Add **1.0 g dodecyl sulfate, sodium salt** ($CH_3(CH_2)_{11}OSO_3Na$ Aldrich catalog no. 86-210-0). Prepare fresh weekly. Discard if the solution becomes yellow. Do not use ascorbic acid powder.

6. Procedure

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

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 NO2-N+NO3-N and PO4-P 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 NO2-N plus NO3-N and PO4-P.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-M, Determination of orthophosphate in waters by flow injection analysis colorimetry.

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 I2601-85.

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

Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

9. Instrument Information

TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

DATA SYSTEM PARAMETERS FOR LACHAT QUIKCHEM 8500 series 2

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

Sample throughput:	30 samples/h, 120 s/sample
Pump Speed:	35
Cycle Period:	120

Analyte Data:

Concentration Units:	mg P/L
Chemistry	Brackish
Inject to BW Baseline Start	10 s
Inject to BW Baseline End	100 s
Inject to BW Integ Start	25 s
Inject to BW Integ End	55 s

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration µg P/L	100	50	25	10	5	1	0

Calibration Rep Handling: Weighted Avg

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None Concentration Scaling: None

Force through zero: No

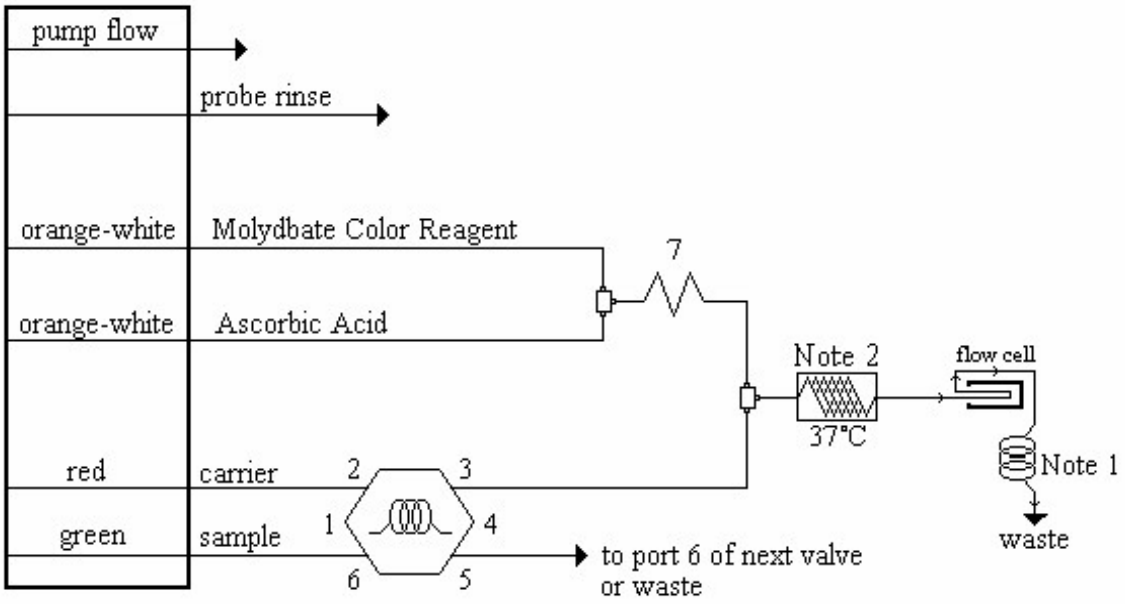
Sampler Timing:

Min. Probe in Wash Period:	59 s
Probe in Sample Period:	50 s

Valve Timing:

Load Time:	0 s
Load Period:	35 s
Inject Period:	85 s

ORTHOPHOSPHATE MANIFOLD DIAGRAM



Carrier: DI water

Manifold Tubing: 0.5 mm (0.022 in) i.d. This is 2.5 µL/cm.

AE Sample Loop: 300 cm x 0.8 mm (0.032 in) i.d. **QC8000**

Sample Loop: 300 cm x 0.8 mm (0.032 in) i.d. **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.

7: 135 cm of tubing on a 7 cm coil support

Note 1: Back pressure loop 300 cm x 0.5 mm (0.022 in) i.d. **Note 2:** 175 cm x 0.8 (0.032 in) i.d.of tubing on the heater.