Standard Operating Procedure for Dissolved Reactive Phosphorous (Lachat Method)

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Revision 1

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1.0 Scope and Application

1.1 This method covers the determination of dissolved reactive phosphorous (DRP) in lake water.

1.2 The approximate working range is 1 to 25 µg/L. The method detection limit is 1 µg/L.

2.0 Summary

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.

3.0 Sample Handling and Preservation

3.1 Samples are collected in new glass or plastic containers.

3.2 Samples are filtered and frozen until analysis.

4.0 Interferences

4.1 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant. A silica concentration of 50 mg SiO_2/L is required to produce a 0.0008 mg P/L positive error in orthophosphorous.

4.2 Glassware contamination is a problem in low level phosphorous determinations. Glassware should be washed with 1:1 HCl and rinsed several times with diH_2O. Special glassware (volumetric flasks, graduated cylinders, etc.) has been designated for DRP ONLY use.

4.3 High concentration of ferric ion or arsenate ion can cause error due to competition with the complex for ascorbic acid. Such concentrations are highly unlikely in lake water.

5.0 Apparatus

Lachat QuikChem AE

5.1 Phosphate Manifold (Lachat Manifold #30-115-01-1-B).

5.2 Printer

5.3 XYZ Sampler

6.0 Reagents and Standards

6.1 All *reagents* should be stored in the appropriate bottles and labeled with the following information:

Identity:	Ascorbic Acid
Date:	mm/dd/yy
Initials of Preparer:	M.S.

All *standards* should be stored in the appropriate bottles and labeled as above with the following also included:

Concentration:	100 mg/L

6.2 Use deionized water for all solutions.

6.3 Stock Antimony Potassium Tartrate Solution: In a 1 L volumetric flask, dissolve 3.0 g of antimony potassium tartrate [$K(SbO)C_4H_4O_6 \bullet ½H_2O$] in approximately 800 mL of water. Dilute to the mark and invert three times to mix. Store in a dark bottle.

6.4 Stock Ammonium Molybdate Solution: In a 1 L volumetric flask dissolve 40.0 g of ammonium molybdate tetrahydrate [$(NH_4)Mo_7O_{24}$] in about 400 mL of water. Dilute to the mark and invert to mix.

6.5 Molybdate Color Reagent: In a 1 L volumetric flask containing about 500 mL of water, add 35 mL concentrated sulfuric acid. Swirl to mix. (*Caution: The solution will get hot!*) Add 72.0 mL of the Stock Potassium Tartrate Solution and 213 mL of the Stock Ammonium Molybdate Solution. Dilute to the mark and invert three times to mix. De-gas with helium.

6.6 Ascorbic Acid: In a 1 L volumetric flask dissolve 60.0 g ascorbic acid in about 700 mL water. Dilute to the mark and invert three times to mix. *Degas thoroughly!!* Add 1.0 g sodium dodecyl sulfate [$CH_3(CH_2)_{11}OSO_3Na$]. Mix gently with stir bar; do not shake to mix. *Prepare fresh weekly.*

6.7 Sodium Hydroxide - EDTA Rinse: Dissolve 65 g sodium hydroxide (NaOH) and 6 g tetrasodium ethylenediamine tetraacetic acid (NA_4EDTA) in 1 L of water.

6.8 Preparation of Standards

6.8.1 Stock 100 mg P/L Calibration Standard: Dry a small amount of potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C to constant weight. In a 1 L volumetric flask, dissolve 0.4394 g of dried standard in about 500 mL diH_2O. Dilute to the mark and invert to mix. Store at 4°C.

6.8.2 Intermediate 1.0 mg P/L Calibration Standard: Using a volumetric pipet, pipet 10 mL of the Stock Calibration Standard (6.8.1) into a 1 L volumetric flask. Dilute to the mark and invert to mix. Store at 4°C.

6.8.3 Working Calibration Standards: Prepare standards over the range of analysis. For the working range of 0-25 µg/L; the following standards may be used:

mL Intermediate Solution (6.8.2) diluted to 1 L	Concentration µg P/L
0.0	0.00
2.5	2.50
5.0	5.00
7.5	7.50
10.0	10.00
15.0	15.00
25.0	25.00

Note: Use volumetric flask. Store at 4°C.

6.8.4 Stock 100 mg P/L Control Standard: Dry a small amount of Sodium Phosphate, dibasic anhydrous (Na_2HPO_4) in an oven at 105°C to constant weight. In a 1 L volumetric flask, dissolve 0.458 g of dried standard in about 500 mL water. Store at 4°C.

6.8.5 Intermediate 1.0 mg P/L Control Standard: Using a volumetric pipet, transfer 10.0 mL of the Stock Control Standard (6.8.4) into a 1 L volumetric flask. Dilute to the mark and invert to mix. Store at 4°C.

6.8.6 Working Control Standards: The following concentrations are typical:

	mL Intermediate Standard (6.8.5) diluted to 1 L	Concentration µg P/L
CS-1	9.0	9.00
CS-2	3.0	3.00

Note: Use volumetric flask. Store at 4°C.

7.0 Procedure

7.1 Allow at least 15 minutes for the heating block to warm up to 37°C.

7.2 Samples are pre-filtered and frozen. They should be brought to room temperature prior to analysis.

7.3 Follow the Lachat Daily Operation Procedural SOP.

7.4 At the end of a run, place all lines into the NaOH-EDTA solution (Section 6.7). Pump this

solution for approximately five minutes. Follow with a thorough water rinse.

8.0 Calculations

The computer yields results directly in µg P/L.

9.0 Quality Control

9.1 The minimum acceptable correlation coefficient (r) is 0.995.

9.2 The following items are required with the minimum frequency indicated:

Audit	Type	Frequency	Limits
CS-1	Method	Beg,End,1/40 Samp.	9 ± 4
CS-2	Method	Beg,End,1/40 Samp.	3 ± 2
Reagent Blank	Method	Beg,End,1/40 Samp.	0 ± 1

10.0 Waste Disposal

Effluent from this channel as well as the sample effluent is acidic. It should be disposed of in a yellow labelled waste container.

11.0 Preventive Maintenance

Required maintenance is described in the Lachat Procedural SOP.

12.0 Troubleshooting

12.1 If the baseline drifts and cleaning the system in the prescribed manner does not help, the heating coil tubing may need to be changed.

12.2 An unusually noisy baseline may be due to insufficient purging of air from the reagents. Tiny bubbles tend to develop in the heated tubing and become trapped in the flow cell causing baseline problems.

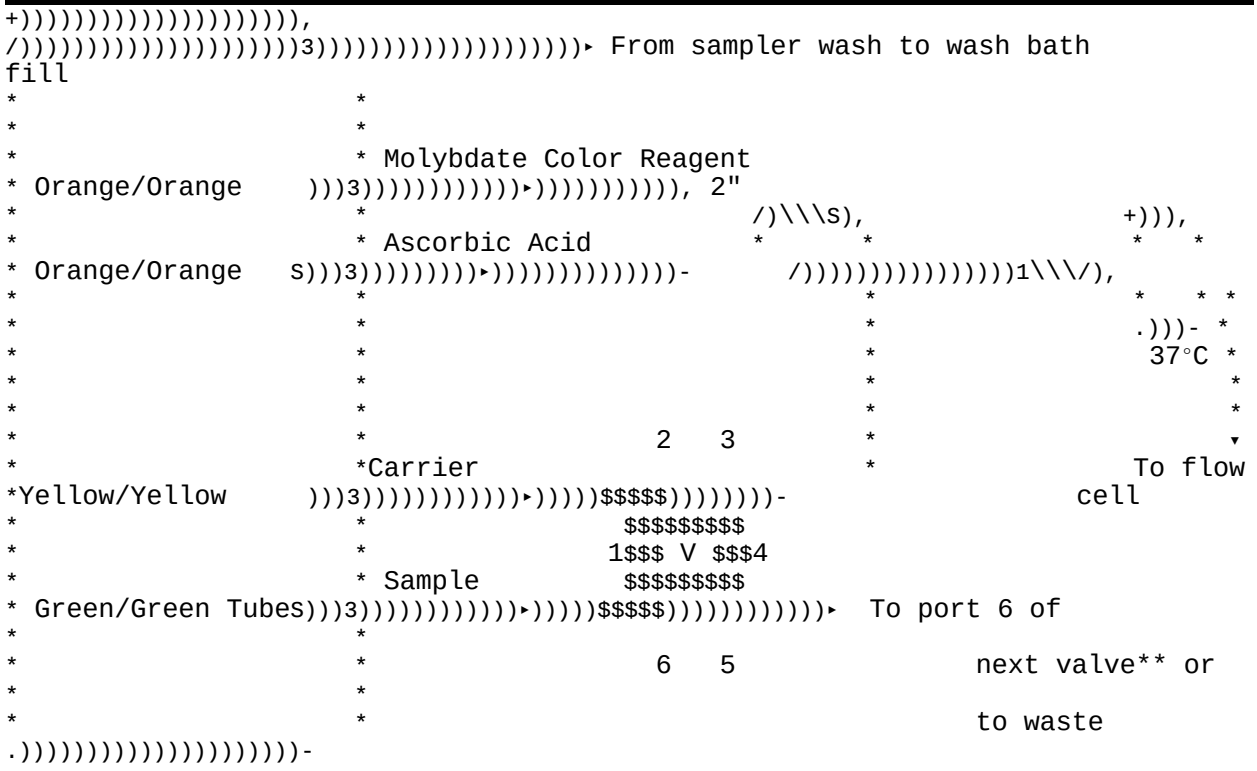
13.0 References

13.1 Lachat Instruments, Method Number. 10-115-01-1-B, Orthophosphate in water, Revision Date April 1992.

13.2 Lachat QuikChem AE Operation Manual.

13.3 GLNPO Soluble Reactive Phosphorous (Orthophosphate). August 1990.

DRP Analytical Manifold



Legend

2.0" \\\: 2.0" Mixing coil (there is 135 cm of tubing on the 2.0" coil support)

Comments

1. Filter used is 880 nm.
2. Sample loop length is 125 cm.
3. All manifold tubing is 0.8 mm (0.032") ID. This relates to a flow of 5.2 µL/cm.
4. The *carrier* is helium degassed diH_2O.
5. Timing: Cycle Period is 36 sec. Inject to start of peak period is 9 sec.

** If more than one channel is being used.

NUTRIENTS SECTION QUALITY CONTROL SHEET

ANALYTE: DISSOLVED REACTIVE PHOSPHOROUS **PROGRAM:** LIMNOLOGY **DATA SET:**_____

DATE	SAMPLE		CHECK STANDARD AUDIT		BLANK AUDIT
	FROM	TO	CH	CL	REAGENT BLANK (LB)
			(13 to 5)	(1 to 5)	(-1 to 1)

COMMENTS:_____

_____

ANALYST:_____DATE:____/____/___ TEAM LEADER:_____DATE:____/____/____