Standard Operating Procedure for Total and Dissolved Phosphorous (Lachat Method)

Grace Analytical Lab
536 South Clark Street
10th Floor
Chicago, IL 60605

June 13, 1994

Revision 1

Standard Operating Procedure for Total and Dissolved Phosphorous (Lachat Method)

1.0 Scope and Application

1.1 This method covers the determination of total phosphorus and total dissolved phosphorous in lake/rain water.

1.2 The approximate working range is 1 to 50 µg/L. The method detection limit is 1 µg/L.

2.0 Summary

2.1 Samples are digested in the presence of sulfuric acid and persulfate to convert or "hydrolyze" polyphosphates and organic phosphorous to orthophosphate.

2.2 The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form 12-molybdophosphoric acid. 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 or acid-washed glass or plastic containers.

3.2 Samples are preserved by addition of 1 mL of H_2S0_4 per liter of sample.

3.3 The preserved samples are stable for at least 28 days when stored at room temperature.

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 0.008 mg P/L positive error in orthophosphorous.

4.2 Glassware contamination is a problem in low level phosphorus 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 TP ONLY use.

4.3 High concentrations of ferric iron 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

5.1 Digestion tubes: Borosilicate Glass 16 x 100 mm Culture Tubes, and digestion caps: White polypropylene screw caps.

5.2 Autoclave

5.3 Automatic pipets with disposable tips calibrated to deliver 8.0 mL and 1.0 mL.

5.4 Lachat QuikChem AE

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

5.4.2 Printer

5.4.3 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 will be stored in appropriate bottles and labeled as above with the following also included:

Concentration:	(100 mg P/L)

6.2 Use deionized water for all solutions.

6.3 0.9 M H_2SO_4: To a 500 mL volumetric flask containing about 400 mLs of diH_2O add 25 mL of concentrated sulfuric acid. Dilute to the mark and invert three times to mix.

6.4 0.28 M Ammonium Persulfate: In a 500 mL volumetric flask, dissolve 31.5 g ammonium persulfate [$(NH_4)_2S_2O_8$] in about 400 ml of water. Dilute to the mark and invert to mix.

6.5 Stock Ammonium Molybdate Solution: In a 1 L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)Mo_7O_{24}$] in approximately 800 mL water. Dilute to the mark and invert three times to mix. Store in plastic and refrigerate.

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

6.7 Molybdate Color Reagent: In a 1 L volumetric flask containing about 500 mL water, add 20.9 mL concentrated sulfuric acid. Swirl to mix. (Caution: The solution will get hot!) Add 72.0 mL of the Stock Antimony 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.

6.8 Ascorbic Acid Reducing Solution: 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. Add 1.0 g dodecyl sulfate, sodium salt ($CH_3(CH_2)_{11}OSO_3Na$). De-gas with helium. Prepare fresh weekly.

6.9 Sulfuric Acid Carrier Solution: In a 1 L volumetric flask containing about 900 mL water, add 9 mL concentrated sulfuric acid (H_2SO_4). Dilute to the mark with water. Invert three times to mix. De-gas thoroughly.

6.10 Sodium Hydroxide - EDTA Rinse: Dissolve 65 g sodium hydroxide (NaOH) and 6 g tetrasodium ethlenediamine tetraacetic acid (Na_4EDTA) in 1 L of water.

6.11 Hydrochloric Acid Rinse: Combine equal parts water and concentrated hydrochloric acid (HCl).

6.12 Preparation of Standards

6.12.1 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 reagent in about 500 mL diH_2O. Add 1.0 mL of concentrated sulfuric acid and dilute to the mark. Store at 4°C.

6.12.2 Intermediate 1.0 mg P/L Calibration Standard: Using a volumetric pipet, pipet 10 mL of the Stock Calibration Standard (6.12.1) into a 1 L volumetric flask. Add 1.0 mL of concentrated sulfuric acid and dilute to the mark. Store at 4°C.

6.12.3 Working Calibration Standards: Prepare standards over the range of analysis. For the working range of 0-50 µg/L, the following standards may be used:

mL Intermediate Solution(6.12.2) 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
25.0	25.00
50.0	50.00

Note: Use volumetric flasks. Preserve the working standards by addition of 1.0 mL of concentrated sulfuric acid and store at 4°C.

6.12.4 Stock 100 mg P/L Control Standard: Dry a small amount of Adenosine-5-Monophosphoric Acid, Disodium salt, [($C_{10}H_{12}N_5O_7PNa_2 \bullet 2H_2O$), F.W - 427.236g/mole, Fluka] in an oven at 105°C to constant weight. Allow to cool to room temperature in a desiccator. In a 1 L volumetric flask, dissolve 1.3793 g of the dried reagent in about 500 mL of water. Add 1.0 mL of concentrated sulfuric acid and dilute to the mark. Store at 4°C.

6.12.5 Intermediate 1.0 mg P/L Control Standard: Using a volumetric pipet, transfer 10.0 mL of the Stock Control Standard (6.12.4) into a 1 L volumetric flask. Add 1.0 mL water, dilute to the mark,and invert to mix. Store at 4°C.

6.12.6 Working Control Standards: The following concentrations are typical:

	mL Intermediate Standard (6.12.5) diluted to 1 L	Concentration µg P/L
	---	---
CS-1	15.0	15.00
CS-2	3.0	3.00

Note: Use volumetric flasks. Preserve the control standards by addition of 1 mL of concentrated H_2SO_4. Store at 4°C.

7.0 Procedure

7.1 Digestion

7.1.1 Do Not Use Commercial Detergents. Soak digestion tubes in 1:1 HCl for one hour, rinse thoroughly with diH_2O and allow to dry completely before use.

7.1.2 Using an automatic pipet with disposable tip, withdraw a 8 mL aliquot of sample. Discard this first portion. Withdraw another 8 mL aliquot and transfer to a digestion tube.

7.1.3 Add 1.0 mL of 0.9M H_2SO_4 (Reagent 6.3), and 1.0 mL of 0.28M Ammonium Persulfate (Reagent 6.4).

7.1.4 Cap the tube tightly and place in metal digestion rack.

7.1.5 Prepare all samples, calibration standards, blanks, and control standards in the same manner.

7.1.6 Place the rack of tubes in an autoclave at 121°C for 30 minutes.

7.1.7 Allow the samples to cool to room temperature before analysis. Redigest any tubes that gain or lose volume.

7.2 Analysis

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

7.2.2 Follow the Lachat Procedural SOP (Typical Daily Operation Section) for the remainder of the analysis.

7.2.3 At the end of a run, place all lines into the NaOH-EDTA solution (6.10). Pump this solution for approximately five minutes. Rinse lines in water and then pump for another five minutes in 1:1 HCl (Reagent 6.11). 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
Rain:			
CS-1	Method	Beg, End, 1/40 Samp.	15 ± 3*
CS-2	Method	Beg, End, 1/40 Samp.	3 ± 2*
Reagent Blank	Method	Beg, End, 1/40 Samp.	0 ± 1
Lab Blank	Method	Beg, End, 1/40 Samp.	0 ± 1
Duplicate	Method	1/40 Sample	$\triangle \leq 1$
Spike	Method	1/40 Sample	100 ± 19%
Lake:			
CH	Method	Beg, End, 1/40 Samp.	15 ± 3*
CL	Method	Beg, End, 1/40 Samp.	3 ± 2*
Reagent Blank	Method	Beg, End, 1/40 Samp.	0 ± 1

*These limit ranges are performance estimates based on data obtained during MDL study.

10.0 Waste Disposal

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

11.0 Preventive Maintenance

Required maintenance is described in the Lachat Procedural SOP.

12.0 Trouble Shooting

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 If negative peaks are observed in some or all of the samples or standards, it is probably due to matrix difference between the carrier and the samples. Check to be sure the carrier was made up properly and that the sulfuric acid addition to the digestate was not unintentionally omitted. Re-digest those samples that exhibited the negative peaks.

12.3 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 may become trapped in the flow cell causing baseline problems.

13.0 References

13.1 Lachat Instruments, Method Number 10-115-01-1-F, Total Phosphorus in Persulfate Digest, Revision Date May 1992.

13.2 Lachat QuikChem AE Operating Manual.

13.3 GLAS Standard Operating Procedure, Total Phosphorus, Low-Level Micro-persulfate digestion. August 1990.

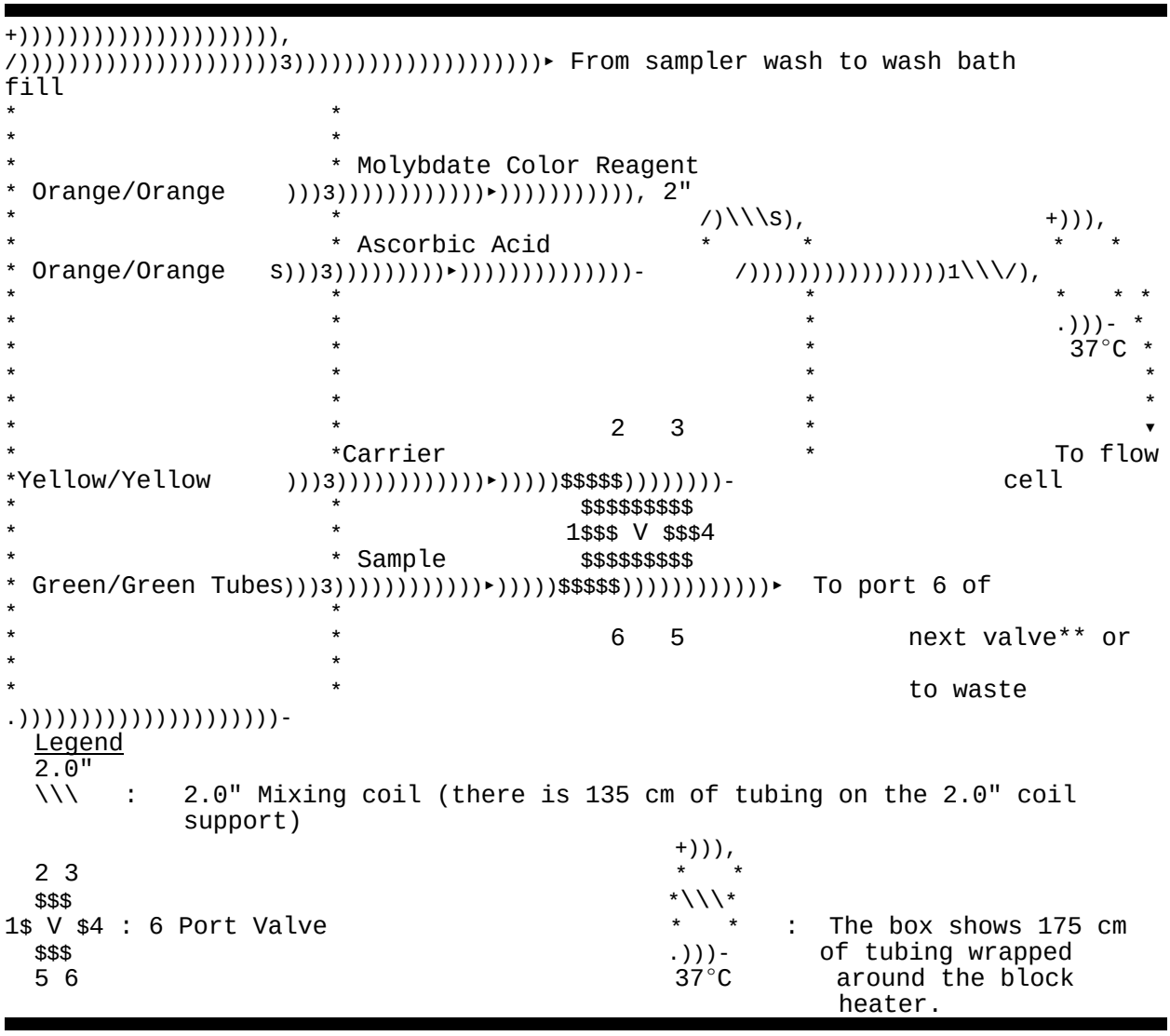

Figure 1. TP/TDP Analytical Manifold

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 Reagent 6.9.
5. Timing: Cycle Period is 44 sec. Inject to start of peak period is 11 sec.

** If more than one channel is being used.

NUTRIENTS SECTION QUALITY CONTROL SHEET

ANALYTE: TOTAL PHOSPHOROUS **PROGRAM:** LIMNOLOGY **DATA SET:**_____

DATE	SAMPLE		CHECK STANDARD AUDIT		BLANK AUDIT
	FROM	TO	CH	CL	REAGENT BLANK (LB)
			(12 to 18)	(1 to 5)	(-1 to 1)

COMMENTS:_____

_____

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

NUTRIENTS SECTION QUALITY CONTROL SHEET

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

DATE	SAMPLE		CHECK STANDARD AUDIT		BLANK AUDIT
	FROM	TO	CH	CL	REAGENT BLANK (LB)
			(12 to 8)	(1 to 5)	(-1 to 1)

COMMENTS:_____

_____

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