ANALYSIS OF ORTHO PHOSPHATE USING THE BRAN+LUEBBE (AA3/AA500) SEGMENTED FLOW ANALYZER

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1. SCOPE AND APPLICATION

Concentrations of orthophosphate (PO_4^{3-}) in aqueous samples (surface, ground, effluents, influents and saline waters) are determined using Bran+Luebbe (AA3/AA500) segmented flow analyzers. The aqueous sample tests covered by this SOP are W-PO4 and W-PO4-F.

Calibration range	0 – 0.30 mg P/L
Method Detection Limit (MDL)	0.004 mg P/L
Practical Quantitation Limit (PQL)	0.010 mg P/L

Samples containing PO_4^{3-} levels higher than 0.30 mg P/L are diluted prior to analysis.

2. SUMMARY OF THE METHOD

2.1. The described procedure is based on EPA Method 365.1, Rev. 2.0 (1993), the Seal AutoAnalyzer3 method G-146-95 Rev. 3, and the Seal AutoAnalyzer 500 method A-036-19 Rev. 1.

2.2. Orthophosphate reacts with molybdenum (VI) and antimony (III) in an acid medium to form an antimony-phospho-molybdate complex. The complex is reduced with ascorbic acid to form a blue complex that absorbs at 880 nm.

2.3. Interferences: High concentrations of iron may cause precipitation of phosphorous. Samples with background absorbance at the analytical wavelength may interfere. Color correction is used to eliminate sample background interference. High conductivity samples can cause interferences in matrix spike recoveries of orthophosphate. If necessary, samples may be diluted with de-ionized (DI) water prior to analysis to minimize interference effects.

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – SEAL Analytical Segmented Flow Analyzer Systems

Instrument Unique ID	Component	Manufacturer	Model	Application Notes
Bran Luebbe A	Autosampler	SEAL Analytical	XY-3	sample time 36/wash time 36
	Pump	SEAL Analytical	AA3 Pump 4	Pump speed: Normal
	Manifold	SEAL Analytical	AA3	Heater Assembly at 37°C
	Colorimetric Detector	SEAL Analytical	AA3	Flowcell: 10 mm, Filter: 880 nm
	Software	SEAL Analytical	AACE 7.10	
Bran Luebbe E	Autosampler	SEAL Analytical	AS-4	sample time 36/wash time 36
	Pump	SEAL Analytical	AA500	Pump speed: Normal
	Manifold	SEAL Analytical	AA500	Heater Assembly at 37°C
	Colorimetric Detector	SEAL Analytical	AA500	Flowcell: 10 mm, Filter: 880 nm
	Software	SEAL Analytical	AACE 8.02	

3.2. Equipment –

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Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Analytical Balance	Mettler Toledo	MS204TS/0
Adjustable Pipette, 10–100 µL	Eppendorf/Fisher	Research Plus, 3123000047
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher	Research Plus, 3123000080
Volumetric Flask, Class A, Assorted	Fisher (SR)	10-212B; 10-210-5D; 10-212E; 10-212-5L; 10-208M
Graduated Cylinder, Class A, Assorted	Fisher (SR)	08-553A; 08-548D
Glass Beaker, Assorted	Fisher (SR)	02-540E; 02-540G; 02-540K; 02-555-20E
Volumetric Pipette, Class A, Assorted	Fisher (SR)	13-690-110; 13-681-50
Or Equivalent **[1]Note: (SR) designates equipment is typically ordered through the in-house stockroom**		

3.3. Consumables and frequently replaced supplies –

Supplies/Consumables	Vendor[1]	Catalog Number
Pump Tubing, ORN/WHT (0.23 mL/min)	Pulse Instrumentation	170-0596-06
Pump Tubing, BLK/BLK (0.32 mL/min)	Pulse Instrumentation	170-0596-07
Pump Tubing, RED/RED (0.80 mL/min)	Pulse Instrumentation	170-0596-10
Pump Tubing, GRN/GRN (2.00 mL/min)	Pulse Instrumentation	170-0596-14
Pump Tubing, Airbar	SEAL Analytical	117+0540-07
Pump Lubricant and Oil	SEAL Analytical	590-0161-01; 538+9000-01
Pump and Autosampler Cleaning Swabs	SEAL Analytical	AQCSW-20
Pipette tips, Eppendorf, 200 µL	Fisher (SR)	05-403-41
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Eppendorf, 10 mL	Fisher (SR)	05-403-116
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Sample Cups, Polystyrene, 4mL	Fisher (SR)	02-544-4
Standard Rack Tubes, 16 x 100 mm	Fisher	14-962-10D
Centrifuge Tubes, Polypropylene, 15 mL	Fisher (SR)	05-538-59A
Syringe, Polypropylene, Assorted	Fisher (SR)	22-204-500; B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-102; 02-202-100
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Reagent Bottles, Polyethylene, Assorted	Fisher (SR)	05-716-151; 05-719-146; 05-719-145; 05-719-88
Reagent Bottles, Amber Glass, Assorted	Fisher (SR)	05-719-75; 05-719-183
Parafilm	Fisher (SR)	13-374-12
Or Equivalent **[1]Note: (SR) designates supply is typically ordered through the in-house stockroom**		

3.3.1. Pump tubing –

Orthophosphate (channel 1) –

1. Blk/Blk (0.76 mm) Air
2. Red/Red (1.14 mm) Color Reagent (4.2.2.)
3. Wht/Org (0.64 mm) Sample
4. Wht/Org (0.64 mm) Ascorbic acid (4.2.3.)
5. Wht/Org (0.64 mm) DI water

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Color correction (channel 2) -

1. Blk/Blk (0.76 mm) Air
2. Red/Red (1.14 mm) Acid Reagent (4.2.1.)
3. Wht/Org (0.64 mm) Sample
4. Wht/Org (0.64 mm) Ascorbic acid (4.2.3.)
5. Wht/Org (0.64 mm) DI water

Sampler wash line -

1. Grn/Grn (1.85 mm) DI water

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

Note: Wash all glassware with 1:2 HCl and rinse at least three times with DI water.

Note: All reagent preparations must be documented in the reagent and standard logsheet for Ortho Phosphate analysis (NU-RGTSTD_PO4). This excel sheet is located at \\FLDEP1\DEAR\LABS\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Orthos\Forms\. It needs to be updated whenever a reagent is newly prepared. For every analysis file, print the reagent and standard logsheet and place it into the analysis folder.

Note: Reagent and standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the reagent and standard logsheet submitted with each analysis folder. The Certificate of Analysis for the standard reference material and each purchased reagent is maintained in the CertificatesofAnalysis folder. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

4.1. Chemicals –

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
4.1.1.	Sodium Dodecyl Sulfate, SDS	$C_{12}H_{25}NaO_4S$	Fisher Chemical	O2674-25	ACS, ≥95%	N/A	Room Temp
4.1.2.	L-Ascorbic Acid	$C_6H_8O_6$	Fisher Chemical (SR)	A61-100	ACS, ≥99%	N/A	Room Temp
4.1.3.	Antimony Potassium Tartrate Trihydrate	$C_8H_4K_2O_{12}Sb_2*3H_2O$	Fisher Chemical	A867-250	USP, ≥99%	N/A	Room Temp
4.1.4.	Ammonium Molybdate Tetrahydrate	$H_{24}MO_7N_6O_{24}*4H_2O$	Fisher Chemical	A674-500	ACS, ≥95%	N/A	Room Temp
4.1.5.	Cupric Sulfate Pentahydrate	$CuSO_4*5H_2O$	Fisher Chemical	C493-500	ACS, ≥98%	N/A	Room Temp
4.1.6.	Potassium Phosphate Monobasic	KH_2PO_4	Fisher Chemical	P285-500	ACS, ≥99%	N/A	Room Temp
4.1.7.	Sodium Hydroxide	NaOH	Fisher Chemical (SR)	S318-3	ACS, ≥97%	N/A	Room Temp
4.1.8.	Sulfuric acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus ≥95%	N/A	Room Temp

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4.1.9.	Simple Nutrients, WasteWatR™	ALTQC, ERA	ERA	505	See COA	See COA	Room Temp
4.1.10.	Hydrochloric Acid	HCl, For cleaning	Fisher Chemical (SR)	A144S-212	Concentrated, ACS Plus	N/A	Room Temp
4.1.11.	Bleach, Generic	NaOCl, For cleaning	Store Brand (no Clorox)	N/A	No additives, ~6%	N/A	Room Temp
4.1.12.	Deionized (DI) Water	H_2O	In house	N/A	N/A	Daily	N/A
Or Equivalent **[1]Note: (SR) designates chemical is typically ordered through the in-house stockroom**							

4.2. Reagents:

4.2.1. Acid Reagent, 5 N H_2SO_4 –

Sulfuric Acid (4.1.8.)	22 mL
SDS (4.1.1)	2 g
DI water (4.1.12.)	to 1000 mL

To a 1 L flask containing approx. 600 mL DI water, add 22 mL of concentrated H_2SO_4 while stirring. Cool flask in a water bath if needed. Add 2 g of SDS and bring to volume with DI water. Invert to mix thoroughly.

Store in a refrigerator. Holding time, 5 days.

4.2.2. Color Reagent –

Antimony Potassium Tartrate (4.1.3.)	0.10 g
Ammonium Molybdate (4.1.4.)	2.4 g
Sulfuric Acid (4.1.8.)	22 mL
SDS (4.1.1.)	2 g
DI water (4.1.12.)	to 1000 mL

To a 1 L flask containing approx. 500 mL DI water, first add 0.10 g of antimony potassium tartrate followed by 2.4 g ammonium molybdate. Slowly, while swirling, cautiously add 22 mL of concentrated H_2SO_4. Cool flask in a water bath if needed. Add 2 g of SDS and mix well. <u>The order in which the reagents are added is important.</u> Dilute to volume with DI water and invert several times to mix thoroughly.

Store in a refrigerator in an airtight amber container. Holding time, 5 days.

4.2.3. Ascorbic Acid Reagent –

L-Ascorbic Acid (4.1.2.)	11.0 g
DI water (4.1.12.)	to 1000 mL

To a 1 L flask containing approx. 500 mL DI water add 11.0 g L-ascorbic acid. Bring to volume with DI water. Invert to mix thoroughly.

Store in a refrigerator. Holding time, 5 days.

4.2.4. Bleach Cleaning Solution –

Bleach (4.1.11.)	10 mL
DI water (4.1.12.)	to 100 mL

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Prepare a 10% v/v bleach solution by adding 10 mL Bleach to approx. 50 mL DI water. Dilute to volume and mix thoroughly.

Do NOT use bleach with additives (including Clorox brand).

The bleach solution is made fresh for each use.

4.2.5. SDS wash –

Sodium Dodecylsulfate (4.1.1.)	2 g
DI water (4.1.12.)	to 1000 mL

To a 1 L flask containing approx. 600 mL DI water, add 2 g of SDS. Dilute to volume with DI water and invert several times to mix thoroughly.

Store at room temperature. Holding time, 180 days.

4.2.6. Copper Sulfate (Color Correction Cal. (2), Primer, Drift (2)) –

Cupric Sulfate (4.1.5.)	4.6 g
DI water (4.1.12.)	to 1000 mL

To a 1 L flask containing approx. 600 mL of DI water, add 4.6 g of copper sulfate. Bring to volume with DI water. Invert to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

4.2.7. Carrier – DI water (4.1.12.) –

The carrier container is filled with fresh DI water daily. DI water poured fresh every day is also used for all blanks (BLK, CCB) including the method blanks (MBLK).

4.2.8. HCl Cleaning Solution –

HCl (4.1.10.)	100 mL
DI water (4.1.12.)	to 1000 mL

To a 1 L volumetric flask containing approx. 800 mL of DI water, add 100 mL concentrated HCl and bring to volume with DI water. Invert to mix thoroughly.

Store at room temperature.

4.2.9. 1 N NaOH Cleaning Solution –

NaOH (4.1.7.)	40 g
DI water (4.1.12.)	to 1000 mL

To a 1 L volumetric flask containing approx. 800 mL of DI water, add 40 g NaOH pellets and bring to volume with DI water. Invert to mix thoroughly.

Store at room temperature.

4.3. Standards and Spiking Solution:

4.3.1. TP/Orthophosphate 1000 mg P/L stock standard –

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Potassium Phosphate Monobasic (4.1.6.)	4.394 g
DI Water (4.1.12.)	to 1000 mL

Dry at least 5 g of KH_2PO_4 at 105 °C for 12-24 hours. Allow to cool to room temperature in a desiccator before weighing.

To a 1 L flask containing approx. 400 mL DI water, add 4.394 g of the dried potassium phosphate. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

Potassium Phosphate Monobasic molecular weight factor: 0.2276

4.3.2. TP/Orthophosphate 10 mg P/L working standard and spiking solution –

1000 mg P/L stock standard (4.3.1.)	2.0 mL
DI Water (4.1.12.)	to 200 mL

Into a 200 mL flask containing approx. 100 mL DI water pipette 2.0 mL of the 1000 mg P/L stock standard. Dilute to volume with DI water and invert several times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

4.3.3. Orthophosphate working calibration standards –

10 mg P/L working standard (4.3.2.)	see table
DI Water (4.1.12.)	to 500 mL

Prepare standards by pipetting the specified amounts of the 10 mg P/L working standard into 500 mL volumetric flasks, diluting to volume with DI water and inverting several times to mix thoroughly..

Store in refrigerator. Holding time, 30 days.

Standard Concentration (mg P/L)		QC Standard, Alias	Final Volume	Vol of 10 mg P/L standard added (mL)
C1	0.000	DI Water	-	-
C2	0.005		500 mL	0.25
C3	0.010	PQCK1	500 mL	0.50
C4	0.020		500 mL	1.0
C5	0.050		500 mL	2.5
C6	0.100	CHCK1	500 mL	5.0
C7	0.200		500 mL	10.0
C8	0.300	Drift (1)	500 mL	15.0

4.3.4. ALTQC standard –

4.3.4.1. Stock ALTQC Standard –

Simple Nutrients, WasteWatR™ (4.1.9.)	See ERA's instructions
DI Water (4.1.12.)	See ERA's instructions

The supplier for the standard reference material (currently Environmental Resource Associates) differs from the supplier of the calibration standards. This quality control standard is prepared

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from the ampule according to the manufacturer's instructions. For the concentration and expiration date of the ampule, see the manufacturer's certificate.

Store in refrigerator. Holding time, 180 days.

4.3.4.2. Working ALTQC (ICV) –

Stock ALTQC Standard (4.3.4.1.)	refer to certified value
Carrier (4.2.7.)	to 1000 mL

The stock ALTQC standard is diluted appropriately with the carrier so that the concentration falls within the calibration range.

Store in the refrigerator. Holding time, 30 days.

5. SAMPLE PREPARATION, PRESERVATION, AND STORAGE (SEE DEP SOP NU-033)

5.1. Samples are filtered in the field, shipped on ice, and stored ≤ 6 ºC (not frozen). Samples analyzed with improper preservation are reported with the Y qualifier.

5.2. Upon receipt, samples are stored in the Nutrient refrigerators in the receiving room of the DEP laboratory at ≤ 6 °C (not frozen). **Samples must be analyzed for orthophosphate within 48 hours of collection or results will be Q qualified.**

5.3. Remove the samples from the refrigerator following the proper protocols to preserve the samples' chain of custody. Allow samples to come to room temperature before pouring them into the sample cups for analysis.

6. SAMPLE ANALYSIS/QUANTITATION

Note: Read the manufacturer's operation manual before proceeding. See DEP SOP NU-033.

6.1. Detector and Pump Start-Up Procedure:

6.1.1. AA3: Turn on the autosampler, allow needle to return home. Turn on the AA3 pump. Turn on the AA3 Digital Colorimetric detector (heater will take approximately 15 min to come to temperature). Secure the platen.

AA500: Turn on the autosampler, allow needle to return home. Turn on master switch for AA500 system. Platen is secured via software upon in the startup procedure. The platen may be tightened and removed manually if needed.

6.1.2. Double click the **AACE** icon on the desktop to start the main program. Click on **SYS.1 [AA3]** which minimizes it on screen.

6.1.3. AA3: Click **Charting** (make sure that the pump's two rocker switches are set to Run and Normal (speed) respectively) to display Channels 1 and 2 on screen.

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6.2.3.1. Flush the reagent lines with the following reagents by placing the lines sequentially in containers containing the reagents listed below. Line flush reagents (in order):

a. DI water for 10 minutes
b. 2 g/L SDS solution (4.2.5.) for 10 minutes on fast

6.2.4. AA500: Click **Run** tab and select **Start Up**. Select the appropriate start up method.

6.2.4.1. **Wash Start** engages the platen and flushes reagent lines with 2 g/L SDS solution (4.2.5.) for 10 minutes on normal speed.

6.2.4.2. **Complete Start** will engage the platen, flushes reagent lines with 2 g/L SDS solution (4.2.5.) for 10 minutes on normal speed, then flushes lines with reagents.

Note: Keep the sampler wash line in the DI water container.

6.2.5. AA3: Place all reagent tubes into reagent bottles and run for 10 minutes. Right-click and select **Set Base/Offset** (normally 5%), check the baseline is stable.

AA500: Right-click and select **Set Base/Offset** (normally 5%), check the baseline is stable.

6.2.6. While proceeding with the wash procedure (6.2.3. and 6.2.4), batch the samples using the following procedure:

6.2.6.1. Samples with conductivities greater than 5000 µmhos/cm are batched separately. If no conductivity value is given, batch according to the matrix identification in LIMS. Effluents are batched separately.

6.2.6.2. For each batch (maximum of 10 samples), prepare:

- One MBLK (4.2.7.)
- One laboratory fortified blank (LFB) following the same procedure as below substituting carrier (4.2.7.) for the sample.
- A matrix spike plus a duplicate of the QC sample or a duplicate matrix spike.

  The 0.10 mg P/L matrix spikes are prepared by adding 100 µL of the spiking solution (4.3.2.) into 10 mL portions of a selected sample. Spike code LO3.

  Select one sample per batch as the QC sample (to spike and/or analyze in duplicate) that is representative of the samples in the batch. If the selected sample has high PO_4^{3-} level (i.e., the combined sample and spike concentration is outside the calibration range), select another sample to

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spike from the same batch. Alternatively, if all samples are high, the entire batch may be diluted.

Samples may be screened for dilution by adding three drops of color reagent (4.2.2.) followed by two drops of ascorbic acid solution (4.2.3.) to approx. 4 mL of sample in a sample cup. If a blue hue or cloudiness develops the sample may be diluted prior to analysis. It is recommended to start with a two-fold dilution. Dilute further if necessary. Make sure the dilution factor is recorded on the bench sheet (see section 6.2.7.).

6.2.6.3. Additional samples may be added to the run as they become available during the day. Samples may also be added to predefined batches until they contain a maximum of 10 samples.

6.2.7. Once batching is done, start typing up the run – Click **Set Up → Analysis → FDEP Ortho-Phosphate → New Run → Tray Protocol**. Click "OK" to save the run and print the **Main Page tab** for a bench sheet (this printout will also contain the method parameters).

An example run sequence includes:

Primer (Copper sulfate reagent, 4.2.6.)
Drift (1), (0.300 mg/L standard C8, 4.3.3.)
Drift (2), (Copper sulfate reagent, 4.2.6.)
BLK (DI water, 4.2.7.)
Channel 1 Calibrants (STD C1-C8, 4.3.3.)
Channel 2 Calibrant (Copper sulfate reagent, 4.2.6.)
High (0.300 mg/L standard C8, 4.3.3.; this cup along with the next two blanks is used to calculate carryover)
Low (DI water, 4.2.7.)
Low (DI water, 4.2.7.)
BLK
PQCK1 (STD C2, 4.3.3.)
ERAQCPO4A (ALTQC, ICV, 4.3.4.)
CCB (DI water, 4.2.7.)
CHCK1 (STD C5, 4.3.3.)
Batched samples, with an **MBLK**, **LFB**, and spikes for each batch (6.2.6.)
BLK
Baseline (DI water)
CCB
CHCK1
Drift (1)
Drift (2)
BLK
BLK

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End

6.2.8. Place fresh standards in the standards rack on the autosampler. Standards may be kept in the rack covered with parafilm after a run, in case the instrument has to be run more than once in one day.

6.2.9. While waiting for the baseline to stabilize (6.2.5.), pour up the run. Use the printed bench sheet to pour up QC and samples in order. Use clean 4 mL autosampler cups. Rinse each cup with a small amount of sample before filling the cup and placing it in the correct spot on the analysis tray. It is recommended to label each cup with a permanent (Sharpie) marker to avoid confusion.

6.2.10. After pouring up the samples, check the baseline for stability. Make sure it is stable since the baseline cannot be adjusted after starting the run. When ready to start, go to **Run → Start → FDEP Ortho-Phosphate →** select **run** (scroll down). Double click on "**Current Run**" and enter the analyst's initials next to the Operator field followed by Instrument A next to Instrument. Click "**Start**".

6.2.11. Monitor the run for bad peaks (peak shape, air spikes, etc.) and failing QC. Rerun any samples with poor peak shape at the end of the run. Rerun blanks (field blanks, equipment blanks) twice for confirmation if the result in the ongoing run indicates that they may be failing after the final integration is performed when the run is stopped. High samples requiring additional dilutions are also run at the end. Make note of the dilution factors on the bench sheet.

6.2.12. When ready to stop the run, go to **Setup → Analysis → FDEP Ortho-Phosphate**, and double click on **Run → Tray Protocol**. Add the label "End" at the cup number where the run should stop. Make sure that all samples are bracketed by passing CCVs and blanks (see section 8.2.). There should be a blank, CCB, CHCK1, Drift (1), Drift (2), and two blanks for the final Baseline reading (in that order) prior to the cup labeled "End".

6.2.13. AA3: After the run is complete, flush the tubing with DI water for 10 minutes before turning the pump off. Release the pump's platen cover to release the pressure on the pump's tubing.

AA500: After the run is complete, run shut down procedure which flushes the tubing with DI water for 6 minutes on fast before releasing the platen and turning the pump off.

6.3. Color Correction – The presence of organic matter and other colored material in the samples may result in positive interference for orthophosphate analysis. Color correction is carried out by running Acid Reagent (4.2.1.) in Channel 2 simultaneously with Color Reagent (4.2.2.) in Channel 1. Channel 2 is calibrated using Copper Sulfate (Color Correction Reagent, 4.2.6.) as a standard (called primer, Drift (2) and Cal. (2) on the run). Color correction is calculated by subtracting Channel 2 results greater than or equal to 0.0020 mg P/L from the

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Channel 1 sample results. The NU-PO4COLORCORR-1.0 form can be used. Final color corrected results should be written on final report.

Note: The Copper Sulfate Reagent should be approximately the same height in Channel 1 as the highest standard (C8, 0.300 mg/L). This is evaluated periodically and the copper sulfate solution is adjusted accordingly in the Channel 2 Setup for the FDEP Ortho-Phosphate template.

6.4. Run files are automatically exported to the Nutrients drive (see Section 7.4.). Convert the file in QC Manager and save as raw data. Format, calculate, and save as review data. Select uploadable samples and prepare folder for review by a supervisor.

6.5. Quantitation/Calculations:

6.5.1. After completion of the analysis, print a summarized report containing the peaks, calibration graph, and results. The instrument reports analysis results in mg P/L. Retrieve the raw data (called "digits") report and add to the analysis folder.

6.5.2. The QC Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes. For general calculations including RPDs and spike recoveries see the FDEP Quality Manual (Section 5.9).

6.5.3. The individual calibration standards are prepared according to section 4.3.3. The linear calibration curve is calculated by the instrument software and is a part of the analysis report. It includes the correlation coefficient to evaluate the fit and the percent errors of the calibration standards (see Section 8.6.).

6.5.4. Color corrections calculated by subtracting Channel 2 results greater than or equal to 0.0020 mg P/L from the Channel 1 sample results (as described in 6.3) must be manually entered into QC manager in LIMS. Raw results are edited to be the color corrected value. Recalculate to get final LIMS result.

7. DATA ARCHIVAL/VALIDATION

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labelled with the test (W-PO4 and/or W-PO4-F), initials of the analyst, and the file name(s). The file name follows the OPYYMMDDA format, where OP stands for ortho-phosphate, YY is the year, MM is the month, DD is the day of the month, and A indicates the instrument used. Postscripts 2, 3, etc. are used to denote the second, third, etc., runs of the day if necessary. For example, the first run on Feb 24, 2016 would be named OP160224A.

7.2. Each report folder should contain the following – report summary with calibration curve, bench sheet with method parameters, raw data report, reagent and standard logsheet, backlog report with batching documentation, the color correction form

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(if utilized), and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records center for long-term storage according to DEP SOP LB-006.

7.4. Bran+Luebbe (AA3/AA500) instrument data excel transfer files (.txt) for upload to QC Manager are stored in electronic format in the current year's subfolder of BranLuebbeA or BranLuebbeE located on the Nutrients drive at: \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RawData\SegmentedFlowAnalyzers\.

7.5. If available, a Certificate of Analysis for each purchased standard or reagent is maintained in the \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\Orthos\CertificatesofAnalysis\ folder.

7.6. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\FLDEP1\DEAR\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

8. QUALITY CONTROL (SEE DEP SOPS NU-038 AND NU-043)

8.1. For method performance and acceptance criteria for QC measures and corrective action for handling out-of-control or unacceptable data refer to the following DEP SOPs: NU-038 and NU-043.

8.2. A continuing calibration verification, CHCK1 (4.3.3.), and a CCB (4.2.7.) are run as check standards every 10 cups and at the end of each run. Acceptance limits for the recovery of the CHCK1 are 90 – 110%. Acceptance limits for the MBLK and CCB are < 0.004 and > -0.004 mg P/L.

8.3. One initial calibration verification (ICV) standard prepared from a different source than the calibration standards, usually a certified standard reference material, ALTQC (4.3.4.), is analyzed on every analytical run. Acceptance limits for the recovery of the ALTQC are 90 – 110%.

8.4. One practical quantitation limit level standard, PQCK1 (4.3.3.), is analyzed on every analytical run. Acceptance limits for the recovery of the PQCK1 are 70 – 130%. The PQCK1 is also used as a spike for the ongoing MDL and PQL verification.

8.5. An LFB (6.2.6.2.), a matrix spike (6.2.6.2.), and a duplicate sample and/or duplicate matrix spike should be analyzed for every batch of 10 samples. Acceptance limits for the recoveries of the LFB and matrix spike are 90 – 110%. The sample/matrix spike reproducibility should be less than 20% RPD for results above the PQL.

8.6. Linear Calibration Range (LCR) – See Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'.

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- The correlation coefficient of the linear calibration (R^2) must be ≥ 0.995.
- Acceptance limits of the blank level calibration standard calculated results are <0.004 and >-0.004 mg P/L.
- Acceptance limits for the percent error of the 0.005 mg P/L calibrant are –20% to 50%.
- Acceptance limits for the percent error of the PQL level standard are ±30%.
- Acceptance limits for the percent errors of calibration standards above the PQL are ±10%.

8.7. Every day samples are analyzed a new calibration is performed at the beginning of the analysis and the LCR must be verified (See 8.6.). Consecutive runs on one day may use the same calibration.

8.8. The carryover percentage should be less than 2%; high carryover indicates the need for instrument maintenance or troubleshooting. If the carryover is above 2%, instrument maintenance should be performed. Samples must be re-analyzed unless a supervisor has evaluated the analytical run and given approval to report the samples.

8.9. Baselines should be run every 14 peaks. This allows for proper baseline corrections throughout the run. Baseline corrections must appear as "done" on final report. Any run without baseline corrections must be reanalyzed.

8.10. Drifts are run at the beginning and at the end of every run. Drift corrections should appear as "done" on final report. Discuss with a supervisor if Drift corrections do not appear as "done" on final report.

9. MANIFOLD CLEANING, MAINTENANCE

9.1. Observe routine maintenance as recommended by instrument manufacturer, and enter the maintenance performed in the maintenance logbook.

9.2. Weekly Cleaning Procedure –

The following protocol should be done at least every 2 days after samples are analyzed during the instrument shut down procedure.

It should also be done more frequently when more difficult sample matrices are analyzed to prevent contamination from building up in the system. This will help prevent from having to do a full system deep clean more often.

During the instrument daily shut down, once all reagents have been flushed from the system (6.2.13.) and with the pump set to "fast," place all reagent straws in:

- 1:10 HCl (4.2.8.) (incl. wash line and sample probe/line) – 10 minutes
- DI water – 10 minutes
- 1 N NaOH (4.2.9.) – 10 minutes
- DI water – 5 minutes
- SDS wash (NOT incl. wash line and sample probe/line) – 15 minutes
- DI water – 15 minutes

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Continue following the instrument shut down procedure (6.2.13.).

9.3. Monthly Cleaning Procedure –

The following protocol should be done at the **minimum once a month** as preventative maintenance.

It should also be done if instrument has contamination issues that were not resolved through the weekly cleaning procedure (9.2.).

9.3.1. With the pump set to "fast," place all reagent straws in:

- 1:10 HCl (incl. wash line and sample probe/line) – 15 minutes
- DI water – 15 minutes
- 1 N NaOH – 15 minutes
- DI water – 15 minutes
- Bleach Cleaning Solution (4.2.4.) (**NOT** incl. sample probe/line) – 15 minutes
- DI water (incl. sample probe/line) – 30+ minutes

Note: **Do not allow bleach to go through the sample probe!** Bleach will aggressively strip the probe leading to sample breaking and carryover issues. This will lead to the probe needing to be replaced.

9.3.2. With the probe raised, clean the autosampler wash receptable with 10% bleach and a cleaning swab. Rinse thoroughly with DI water. For the AA500, inspect the peristaltic pump tubing for the wash and replace if needed.

9.3.3. Change pump tubing and airbar tubing.

9.4. Troubleshooting Tips –

9.4.1. Drifting or noisy baseline:

- Make sure waste lines from autosampler and colorimeter are not submerged. Submerged waste lines can cause a drifting baseline or air spikes. Replace waste lines if necessary.
- Make sure bubble pattern is even and all bubbles are symmetrical. Straighten tops of reagent straws and ensure no bubbles are inside straws that may be slightly restricting flow.
- When switching from wash solutions to reagents, the air introduced may get stuck before the glass injection joints and between the tubing joints. Remove these bubbles and check if baseline has improved.
- A noisy baseline with fresh reagents and diluent can indicate a contaminated autosampler wash receptacle or sampling probe. Clean the reagent straw/line of the autosampler wash receptable with 10% bleach, the sampling probe with acid and base, and replace the sample line and sample probe if necessary.

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9.4.2. An increase of air spikes in the run usually indicates pump tubing needs to be changed. Glass tubing joints may also need to be replaced if there is a white band and/or bubbles that are getting caught in the joint's gap.

9.4.3. Failing blanks can indicate contamination. There are multiple approaches to fixing this.

- Check that standard and sample tubes are not filled above the autosampler wash receptable line. Ensure that the autosampler wash receptable is filling and emptying appropriately.
- Acid soak with diluted HCl all glassware used to make reagents and standards overnight and replace all bottles. Remake reagents and standards. Acid soak the standards rack glass tubes overnight.
- Clean the system, replace the pump tubing. Replace the probe sample line.
- For deeper contamination issues, it may be necessary to acid soak the reagent straws, replace the tubing connecting glass joints, change the sample probe, replace the Kel-F line from 10-turn coil to detector, and/or replace the reagent and autosampler wash tubing lines (connected to the manifold, autosampler, and reagent straws).

10. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

10.1. Review the Laboratory Health and Safety Plan (accessible at https://floridadep.sharepoint.com/dear/SitePages/Lab Health and Safety.aspx).

10.2. Safety glasses, gloves and lab coats must be worn at all times whilst handling samples and reagents.

10.3. Read the MSDS information for safe reagent and sample handling.

10.4. Sample waste, excess reagents, and waste exiting the flow cell may be poured down the sink while flushing with water.

11. REFERENCES

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

11.1. EPA method 365.1, Rev. 2.0 (1993).

11.2. Bran+Luebbe™ Analyzer Series AA3 System Operation Manual.

11.3. Bran+Luebbe method for orthophosphate analysis 696B-82W.

11.4. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

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11.5. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

11.6. DEP SOP NU-041, Reagent Preparation Procedure.

11.7. DEP SOP NU-042, Standard Preparation Procedure.

11.8. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

11.9. DEP SOP NU-044, Aqueous Extraction of Orthophosphate, Nitrate, Chloride and Sulfate from Soils and Sediments.

11.10. DEP SOP LB-006, Standard operating procedure for records maintenance and storage.

11.11. Quality Manual, Bureau of Laboratories, FDEP.

11.12. Laboratory Health and Safety Plan.

11.13. DEP SOP NU-082, Analysis of Low Level Total Phosphorous Using the Bran+Luebbe Segmented Flow Analyzer.

Appendix of Significant Changes

02/21/2008 Updated SOP to comply with the latest revision of method:_365.1, *Revision 2.0*, August 1993.

02/24/2010 Updated SOP to comply with the latest revision of the Bran_Luebbe method G-146-95, Revision 3.

03/14/2011 Section 8: Verification of the LCR was clarified.

03/30/2012 Starting 04/05/2012, sample with conductivities greater than 10,000 µmho/cm are analyzed undiluted. A section (6.2.6.) was added to describe the sample batching procedure. The manifold cleaning procedure (9.2.) was modified replacing the bleach wash with NaOH and HCl.

04/06/2015 The holding times of the Color Reagent, the Sulfuric Acid Reagent, and the Ascorbic Acid Reagent were changed from 7 days to 5 days. Data archival information was clarified in section 7. Cleaning procedure was amended in section 9.

04/05/2016 Reagent and Standard logsheet information was updated in Sections 4 and 7.2. The 100 mg P/L intermediate stock standard was removed from section 4.3. The holding time for the 10 mg P/L working standard and spiking solution was changed to 30 days. The preparation instructions for the 10 mg P/L working stock solution in section 4.3.2. and the working calibration standards in section 4.3.3. were updated. File paths were updated. The file naming convention in Section 7.1. was corrected.

12/10/2019 Section 4 entries for Copper Sulfate Reagent and acid cleaning solution were modified. In Section 6 the reference to MDL in the run sequence was removed and clarification for the color reagent added. Section 8 was modified to include

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	the acceptance limits for the calibration standards recovery and use of thePQCK1 standard for the ongoing MDL and PQL verification.
05/21/2020	Color correction form NU-PO4COLORCORR-1.0 added to Section 6.3, effective 6/16/2020.
12/10/2020	Bran+Luebbe (AA3/AA500) name updated throughout. In Section 3, the instrumentation, equipment, and supplies were updated and inputted into table format. In Section 4, chemicals were updated and inputted into table format. Preparation procedures for reagents and standards were reformatted. Updates to Section 5 were made to include AA500 analysis procedure. Section 9 Maintenance and Troubleshooting was updated.
01/24/2022	Designated zero calibration point consisting of DI water. Made document consistent regarding the concentration of bleach cleaning solution.
08/18/2023	Updated data drive file path links.

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