GUAM ENVIRONMENTAL PROTECTION AGENCY
EMAS ANALYTICAL PROGRAM

STANDARD OPERATING PROCEDURE

DETERMINATION OF ORTHOPHOSPHATE IN WATER BY FLOW INJECTION ANALYSIS COLORIMETRY

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REVISION PAGE

Date	Section	Summary of Changes	Initials
01/27/25	8.1.1 6	Added "The solution must be clear." Added "Solution may be slightly heated for faster dissolution." Revision required due to calibration problems and reactions causing the line in the reaction manifold to turn blue if the Ammonium Molybdate solution is not fully dissolved. Added section 6.4 for preservation of samples	AC
01/29/25	7.1	Changed "Lachat QuikChem 8000 Series FIA+ instrument" to "Lachat QuikChem 8500 Series FIA+ instrument" due to 8000 series out of service.	AC

CONTENTS

1 SCOPE AND APPLICATION

1.1 This method covers the determination of orthophosphate in brackish, seawater, and non-saline waters.

1.2 The method is based on reactions that are specific for the orthophosphate (PO_4^{3-}) ion.

1.3 The applicable range is 0.025 to 1 mg/L for high level orthophosphate concentrations; 15 to 60 ug/L for low levels. The ranges can be extended for high level samples through dilution.

1.4 The quantitation limit for orthophosphate as P is 0.025 mg/L for high levels, and 10 ug/L for low levels.

2 METHOD SUMMARY

2.1 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 ascorbic acid and molybdate reagents are merged on the chemistry manifold and the reagent stream is then merged with the carrier stream. The sample zone appears at the detector less than 10 seconds after injection. The absorbance is proportional to the concentration of orthophosphate in the sample.

2.2 Background absorbance determination is necessary for samples with color absorbance at 880 nm.

3 INTERFERENCES

3.1 Turbidity may be removed by filtering the sample prior to analysis.

3.2 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 mg/L would be required to produce a 1 mg/L positive error in orthophosphate.

3.3 Concentrations of ferric ion (Fe^{3+}) greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent, ascorbic acid.

4 DEFINITIONS

4.1 Analytical Sample – Any sample in which orthophosphate is being determined, excluding standards, method blanks, or QC reference samples.

4.2 Calibration Blank (CB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analyte.

4.3 Calibration Standard (CAL) – A solution prepared by diluting the primary stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

4.4 Field Reagent Blank (FRB) – An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if contamination is occurring in the field environment. Note: Field reagent blanks cannot be used for LD or LFM.

4.5 Field Duplicates (FD) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of field duplicates indicate the precision associated with the sample collection and storage as well as the laboratory procedures.

4.6 Instrument Performance Check (IPC) – A standard containing the analyte of interest that is used to verify the accuracy of analysis and monitor instrument drift. It is analyzed periodically through out an analysis sequence.

4.7 Calibration Verification (CV) solution: Initial (ICV) and Continuing Calibration Verification (CCV) solutions - A known value standard used to verify instrument performance during analysis. It is analyzed to verify that the initial calibration has not changed significantly during the analysis run. The CV fulfills the requirements of the IPC (4.6).

4.8 Laboratory Fortified Blank (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of method analytes are added in the laboratory. The source of LFB must be independent of the calibration standards. LFB is analyzed like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements. The LFB also fulfills the requirements of the QCS (4.14).

4.9 Laboratory Fortified Sample Matrix (LFM) – An aliquot of an analytical sample to which known quantities of the method analytes are added in the laboratory. The LFM

is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

4.10 Laboratory Duplicate (LD) – An aliquot of sample prepared and analyzed separately with identical procedures. Analysis of the sample and LD indicates precision associated with the laboratory procedures, but not with sample collection, preservation or storage procedures.

4.11 Laboratory Reagent Blank (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly like a sample. The LRB is used to detect sample contamination resulting from the procedures used to prepare and analyze the samples in the laboratory environment.

4.12 Linear Calibration Range (LCR) – The concentration range over which the instrument response is linear.

4.13 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

4.14 Quality Control Sample (QCS) – A standard containing orthophosphate that is used to verify the accuracy of the analysis. The method requires that the source of the QCS must be independent of the calibration standards and that the QCS be analyzed quarterly.

4.15 Quantitation Limit (QL) – The concentration at which confidence in the reported value requires no qualifying remarks. The QL, also called as the practical quantitation limit (PQL) is about 5X the MDL and represents a practical and routinely achievable detection limit with a relatively good certainty that any reported value is reliable.

4.16 Stock Standard Solution (SSS) - A concentrated solution containing the method analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4.17 Sample Delivery Group (SDG) – A group of twenty samples or less from the same case that is sent to the laboratory for analysis.

5 HEALTH AND SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Safety precautions must be taken when handling solutions and samples. Protective clothing including lab coats, safety glasses and gloves must always be worn. Contact lenses must not be worn. If solutions come into contact with your skin, wash thoroughly with soap and water. Contact your Supervisor or Health and Safety Coordinator to determine if additional treatment is required.

5.2 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult MSDS.

5.2.1 Antimony Potassium Tartrate

5.2.2 Ammonium Molybdate

5.2.3 Sulfuric Acid

6 SAMPLE HANDLING AND PRESERVATION

6.1 Samples should be collected in precleaned plastic or glass bottles. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

6.2 Samples are received in the EMAS Laboratory by a laboratory staff. Sample IDs, dates and times of collection are verified against the chain-of-custody form. Samples are logged in using the Laboratory Information System (LIMS).

6.3 Maximum holding time is 48 hours for samples stored at 4 ^{o} C.

6.4 If storage is required, add Sulfuric acid to lower the pH $\leq$ 2 and store samples at 4°C. When preserved, maximum holding time is 28 days.

7 EQUIPMENT AND SUPPLIES

7.1 Lachat QuikChem 8500 Series FIA+ instrument, phosphorus manifold and the Lachat QuikChem data system and software

7.2 Analytical Balance, capable of accurately weighing to the nearest 0.0001 g

7.3 Class “S” weights

7.4 Drying oven, capable of being controlled at 140 ± 5 ° C

7.5 Desiccator

7.6 Glassware -- Class A volumetric flasks and pipettes or plastic containers as required.

7.7 Eppendorf air-displacement pipetters capable of delivering volumes ranging from 10 to 100 uL and 100 to 2500 uL with an assortment of high quality disposable pipet tips, or equivalent.

7.7 Disposable syringe filters, 0.45 micron

7.8 10 mL Disposable syringes with luer-lock fittings

8 REAGENTS AND STANDARDS

8.1 Preparation of Reagents

Use ASTM Type II reagent water for all solutions.

8.1.1 Reagent 1. Stock Ammonium Molybdate Solution: In a 1 liter volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in approximately 800 mL reagent water. Dilute to the mark and place on a magnetic stirrer to dissolve. The solution must be clear. The molybdate solid may take up to four hours on a stir plate to dissolve. Solution may be slightly heated for faster dissolution. Store in plastic bottle and refrigerate. Maybe stored up to two months when kept refrigerated.

8.1.2 Reagent 2. Stock Antimony Potassium Tartrate Solution: In a 1 liter volumetric flask, dissolve 3.0 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6.1/2H_2O$) or dissolve 3.22g antimony potassium tartrate (potassium antimonyl tartate trihydrate $K(SbO)C_4H_4O_6.3H_2O$) in approximately 800 mL reagent water. Dilute to the mark and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate. Maybe stored up to two months when kept refrigerated.

8.1.3 Reagent 3. Molybdate Color Reagent: To a 1- liter volumetric flask, add 35.0 mL concentrated sulfuric acid to approximately 500 mL reagent water. (**CAUTION**: The solution will get hot.) Swirl to mix. Add 72 mL Antimony Potassium Tartrate Solution (Reagent 2) and 213 mL Ammonium Molybdate Solution (Reagent 1). Dilute to the mark and invert three times to mix. Store in a dark bottle and refrigerate. Prepare fresh weekly.

8.1.4 Reagent 4. Ascorbic Acid Reducing Solution: In a 250-mL volumetric flask dissolve 15.0 grams granular ascorbic acid in about 150 mL reagent water. Dilute to the mark and shake to mix. Store in a dark bottle and refrigerate. Prepare fresh weekly.

8.1.5 Reagent 5. Sodium Hydroxide – EDTA Rinse: Dissolve 65.0 g sodium hydroxide (NaOH) and 6 g tetrasodium ethylenediamine tetraacetic acid (Na_4EDTA) in about 800 ml reagent water and make up to 1 Liter solution. Prepare fresh annually.

8.1.6 Reagent Water – carrier

8.2 **Preparation of Standards**

8.2.1 **Stock Standards**: Stock standard solutions may be purchased as certified solutions or prepared from ACS grade materials (dried at 110 ºC for at least 2 hours) and are stable for six months when stored at 4 ºC.

8.2.1.1 **Standard 1. Stock Standard 1000 mg/L Phosphorus:** In a 250-mL volumetric flask, dissolve 1.099 g of primary standard grade anhydrous potassium dihydrogen phosphate (KH_2PO_4) that has been dried for 2 hours at 110º C in about 150 mL reagent water. Add 0.5 mL of concentrated sulfuric acid, dilute to 250 mL mark and shake to mix. Prepare fresh annually. Alternatively, a commercially prepared standard can be obtained from a reputable supplier.

8.2.1.2 **Standard 1A: Working Standards Phosphorus for high level concentrations:** The following five calibration standards are prepared using Standard 1 (8.2.1.1), 1000 mg/L phosphorus and diluted with reagent water. Prepare fresh daily.

Calibration Standard	Volume of Stock Standard 1	Final volume
1.00 mg/L	100 uL	100 mL
0.50 mg/L, **CV**	50 uL	100 mL
0.25 mg/L	25 uL	100 mL
0.10 mg/L	10 uL	100 mL
0.05 mg/L	**10 uL**	**200 mL**
Blank	0 uL	100 mL
QL: 0.025 mg/L	12.5 uL	500 mL

QCS (or LFB): 0.50 mg/L --- must be prepared from a second source stock standard.

8.2.1.3 **Standard 1B. Working Stock Standard 5000 ug/L Phosphorus (low level):**

In a 200-mL volumetric flask, add 1 ml stock standard, using Standard 1 (8.2.1.1) to about 100 mL reagent water; add 0.20 mL concentrated sulfuric acid, dilute to the mark and shake to mix. Prepare this standard fresh daily.

8.2.1.4 **Standard 1C. Working Standards Phosphorus (low level):**
The following low level calibration standards are prepared using Standard 1B (8.2.1.3), 1000 ug/L Phosphorus and diluted with reagent water. Prepare fresh daily.

Calibration Standard	Volume of Stock Standard 1B	Final volume
150 ug/L	3 mL	100 mL
100 ug/L,**CV**	2 mL	100 mL
50 ug/L	1 mL	100 mL
25 ug/L	0.5 mL	100 mL
10 ug/L	0.2 mL	100 mL
Blank	0 mL	100 mL

QCS (or LFB): 100 ug/L --- must be prepared from a second source stock standard.

9 QUALITY CONTROL PROCEDURES

9.1 Guam EPA operates a formal quality control (QC) program. The QC program consists of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks, QCS samples and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

Initial Demonstration Proficiency – Each analyst must complete an initial demonstration of proficiency prior to analyzing samples following this method.

9.2.2 MDL – A method detection limit must be confirmed annually and must be <1/2 the QL or corrective action must be initiated.

9.2.3 QCS – a QCS must be prepared and analyzed when beginning the use of this method, on a quarterly basis or as required to meet data-quality needs. The source of the QCS must be independent of the calibration standards. The QCS verifies the calibration standards. Guam EPA Laboratory fulfills the requirements of the QCS with analysis of the LFB.

9.3 Routine Analytical Quality Control

9.3.1 The instrument must be calibrated with a blank and 5 standards. The correlation coefficient of the calibration curve must be ≥ 0.995 or the instrument must be recalibrated.

9.3.2 CV – The accuracy and stability of the calibration shall be verified by the periodic analysis of a CV standard. It must be analyzed at the beginning of an analytical run (the ICV), after every 10 analytical samples (the CCV), and at the end of an analytical run (the closing CCV). The CV solution should be prepared from the same standard stock solutions used to prepare the calibration standards.

The recovery of orthophosphate in the CV is calculated as follows:

$$\%\ R = \frac{M}{T} \times 100$$

Where

%R = percent recovery of the standard
M = measured concentration of orthophosphate, mg/L
T = true concentration of orthophosphate in the CV, mg/L

If the CV recovery exceeds the limits of 90 – 110%, the analysis shall be terminated. The cause of the poor recovery must be determined and the problem corrected. The instrument must be re-calibrated and all samples not bracketed by acceptable CV results must be reanalyzed.

9.3.3 CB (ICB/CCB) – The stability of the baseline must be monitored by analyzing a CB immediately after every CV standard. If the absolute value of the CB result equals or exceeds the QL, the analysis must be terminated. The cause of high CB result must be determined and the problem corrected. The instrument must be re-calibrated and all samples not bracketed by acceptable CB results must be reanalyzed.

9.3.4 QL – The accuracy of the calibration at the reporting limit shall be verified by the analysis of a QL standard. The QL must be analyzed at the beginning of each analytical run, prior to the analysis of environmental samples. The recovery of orthophosphate in the QL is calculated as follows:

$$\%R = \frac{M}{T} \times 100$$

Where

%R = percent recovery of the standard

M = measured concentration of orthophosphate, mg/L
T = true concentration of orthophosphate in the QL, mg/L

If the QL recovery exceeds the limits of 50 – 150%, the analysis shall be terminated. The cause of the poor recovery must be determined and the problem corrected. The instrument must be re-calibrated and all the samples analyzed after the out-of-control QL standard must be reanalyzed. If, after recalibration, the QL recovery still exceeds the 50-150% limits, the calibration standards must be re-prepared and the instrument recalibrated.

9.3.5 LRB – The laboratory must analyze at least one LRB daily or with each batch of 20 or fewer samples of the same matrix, whichever is more frequent. LRB data are used to assess contamination in the laboratory environment. LRB values that exceed the MDL indicate potential laboratory contamination. If the potential contamination significantly impacts the analytical results, the LRB must be re-prepared along with affected samples, and reanalyzed.

9.3.6 LFB – A LFB must be prepared and analyzed with each batch of 20 or fewer samples. The LFB assures that the calibration standards used to calibrate are accurate. The LFB is the QCS. The recovery of orthophosphate in the LFB is calculated as follows:

$$\%R = \frac{LFB}{s} X100$$

Where

%R = percent recovery
LFB = measured concentration of orthophosphate in the LFB, mg/L
s = orthophosphate concentration in the LFB, mg/L

The recovery of orthophosphate in the LFB must be within the 90 – 110% limits. If the recovery exceeds the limits, the analysis system is judged to be out-of-control, and the source of the problem must be identified and resolved before continuing analyses.

9.3.7 LD – Sample homogeneity can affect the quality and interpretation of the data. LD results can be used to assess sample homogeneity.

One LD must be prepared for every 10 routine samples of the same matrix in a sample batch (e.g., 1 LD for a batch containing 1-10 routine samples, 2 LDs for a batch containing 20 routine samples, etc.). Shake the sample selected as the LD, obtain a representative aliquot, and proceed with the sample preparation and analysis, treating the LD sample as a routine sample.

Calculate the relative percent difference (RPD) using the following equation:

$$RPD = \frac{(C_{ld} - C)}{(C_{ld} + C) / 2} \times 100$$

Where

RPD = relative percent difference
C_{ld} = measured orthophosphate in the LD , mg/L
C = measured orthophosphate in the routine sample, mg/L

The relative percent difference (RPD) must be ≤20% for samples with orthophosphate levels greater than or equal to 5X the QL. For other samples, the absolute difference between duplicate results must be less than the QL. If the control limits are exceeded, flag all associated analyte results. Document actions in the **Notes** section of the LIMS analytical results report.

9.3.8 LFM – The LFM is designed to provide information about the effect of sample matrix on the measurement system. One LFM must be prepared for every ten routine samples of the same matrix in a sample batch. The sample chosen as the LD should be used as the sample LFM. Samples identified as field blanks cannot be used for LFM sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than 4X the MDL. Percent recovery may be calculated using the following equation:

$$\%R = \frac{C_{lfm} - C}{s} \times 100$$

Where %R = percent recovery
C_{lfm} = measured concentration of orthophosphate in the LFM, corrected for any dilutions, mg/L
C = measured concentration of orthophosphate in the routine sample, corrected for any dilutions, mg/L
s = expected orthophosphate concentration of the added spike in the LFM, corrected for any dilutions, mg/L

If the value of **C** is less than 4X the value of s, the acceptance window for %R is 75 – 125%. If the recovery falls outside the acceptance window other QC data must be examined to determine if a matrix problem exists. If the laboratory performance for that analyte is in control (i.e., the CV, QL, and the LFB results are acceptable, the poor LFM recovery is most likely matrix related. Lab duplicate results should also be examined to gain additional insight as to whether the matrix components or matrix

heterogeneity are the cause of the unacceptable recovery. In either case, the problem should be discussed in the report and the data user informed that the result for that analyte in the unfortified sample is suspect due either to heterogenous nature of the sample or a matrix effect. Flag any out-of-control analytes. Document actions in the **Notes** section of the LIMS analytical result report.

10. ANALYTICAL PROCEDURES

10.1 CALIBRATION AND STANDARDIZATION – Orthophosphate is determined colorimetrically using the Lachat Automated Ion Analyzer. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements, to maintain quality control data verifying instrument performance.

10.1.1 Instrument Set-up

1) Turn on the Lachat QuikChem 8000 FIA+ instrument and allow the colorimeter to warm up for about 30 minutes.

2) Set up the orthophosphate manifold and allow the heater to warm up to 45^{o}C.

3) Turn on the pump and set the speed to 35 RPM.

4) Download the orthophosphate method in the computer.

5) Pump reagent water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow system to equilibrate until a stable baseline is achieved. Don't forget to place the waste lines into the orthophosphate analysis waste container.

10.1.2 Calibration and Sample Analysis

1) Pour the five calibration standards and the blank into standard tubes and position them in decreasing order in the standards rack at the rear of the autosampler.

2) Load the analytical and QC samples into the samples rack using the sample tubes.

3) The usual sample loading sequence is listed in the following table:

Row	Sample ID	Cup #	Sample Type	Level
1	Cal Std 1	1	Cal Std	1

2	Cal Std 2	2	Cal Std	2
3	Cal Std 3	3	Cal Std	3
4	Cal Std 4	4	Cal Std	4
5	Cal Std 5	5	Cal Std	5
6	Blank	6	Cal Std	6
7	ICV	1	Unknown	0
8	ICB	2	Unknown	0
9	QL	3	Unknown	0
10	LFB	4	Unknown	0
11	LRB	5	Unknown	0
12	Sample 1	6	Unknown	0
13	Sample 1 – LD	7	Unknown	0
14	Sample 1 – LFM	8	Unknown	0
15	Sample 2	9	Unknown	0
16	Sample 3	10	Unknown	0
17	Sample 4	11	Unknown	0
18	Sample 5	12	Unknown	0
19	CCV	13	Unknown	0
20	CCB	14	Unknown	0
21	Sample 6	15	Unknown	0
22	Sample 7	16	Unknown	0
23	Sample 8	17	Unknown	0
24	Sample 9	18	Unknown	0
25	Sample 10	19	Unknown	0
26	Sample 11	20	Unknown	0
27	Sample 11 – LD	21	Unknown	0
28	Sample 11 –LFM	22	Unknown	0
29	Sample 12	23	Unknown	0
30	Sample 13	24	Unknown	0
31	CCV	25	Unknown	0
32	CCB	26	Unknown	0
33	Sample 14	27	Unknown	0
34	Sample 15	28	Unknown	0
35	Sample 16	29	Unknown	0
36	Sample 17	30	Unknown	0
37	Sample 18	31	Unknown	0
38	Sample 19	32	Unknown	0
39	Sample 20	33	Unknown	0
40	CCV	34	Unknown	0
41	CCB	35	Unknown	0

3) Input the information required by the data system such as concentrations, replicates and quality control scheme.

4) Calibrate the instrument by injecting the working standards. The system will analyze the calibration standards and calculate a calibration curve prior to analyzing any of the samples. A correlation coefficient of ≥0.995 is the requirement for the calibration to pass. The system will now automatically analyze the samples loaded in the sample tray.

10.1.3 Post-analysis Review

1) QC Sample Results – Review the results for all QC samples for compliance with the criteria specified in Section 9. If results are not acceptable, take appropriate corrective action.

2) Off-scale Results – Review results for samples that exceed the calibration range. Samples having orthophosphate concentrations larger than the highest calibration standard must be diluted and reanalyzed.

10.1.4 Instrument Shutdown

1) At the end of the run with the pump still turned on, place the color reagent and ascorbic acid transmission lines in to the NaOH-EDTA solution (Reagent 5). Pump this solution for approximately 5 minutes. Then place these lines in reagent water and pump for at least 15 minutes.

2) After the 15 minute period, remove the reagent lines from the reagent water and allow the reagent lines to be purged of the reagent water. Observe the tubing on the manifold – when no liquid is apparent in the tubing the pump can be turned off. Cap all reagents, discard all samples and standards into the appropriate waste containers and turn the power off.

10.2 Data Reduction and Reporting – After set-up and calibration the software reports results for the analyzed solution in units of mg/L (for high levels), or ug/L (for low levels). No further calculations are necessary and values may be reported directly from the data system. All results should be reported using no more than three significant figures; however, no values of less significance than the MDL may be reported. Results less than the MDL should be reported as <MDL values. Values between the MDL and the QL will be flagged as estimated (J flag).

10.2.1 Sample results are entered into the Laboratory Information Management System (LIMS) and analytical results are reported.

10.2.2 Before releasing the results, the laboratory conducts data verification and validation. This is done through peer review of the data and validation by another analyst. The QA Manager makes the final audit and validation prior to the release of the results.

11 DOCUMENTATION

11.1 When samples are received, the laboratory personnel verify that the chain of custody is properly filled out. Laboratory personnel may then receive and sign the chain of custody. A copy of the chain of custody must be included in the data package (Attachment E).

11.2 Each standard and reagent prepared for the analysis is entered in the Inorganic Standards Preparation Logbook (Attachment F) and Inorganic Reagents Preparation Logbook (Attachment G) respectively. Copies of the appropriate page(s) is (are) included in the data package.

11.3 The Omnion FIA Software Report that contains the operator's/analyst's name, calibration and QC data, phosphate as P results in mg/L, sample analysis date and time, client sample IDs/station locations must be included with the data package (Attachment C).

11.4 A QC Summary Report that contains the QC sample results and evaluations must be included in the data package (Attachment D).

11.5 Sample results are entered in the Laboratory Information Management System (LIMS) to facilitate storage and retrieval of data. The LIMS generated report must be included in the data package (Attachment B).

11.6 The data package consists of the following:

Attachment B: Analytical Results Report (LIMS or spreadsheet)
Attachment C: Omnion FIA Software Report
Attachment D: QC Summary Report
Attachment E: Chain of Custody
Attachment F: Inorganic Standards Preparation Logbook
Attachment G: Inorganic Reagents Preparation Logbook

12 REFERENCES

12.1 EPA Method 365.1, Phosphorus, All Forms (Colorimetric, Automated, Ascorbic Acid), Methods for Chemical Analysis of Water and Wastes, U. S. E. P. A., Cincinnati, Ohio, USA.

12.2 QuikChem Method 31-115-01-3-A, Determination of Phosphorus by Flow Injection Analysis Colorimetry.

12.3 Method 4500-P-F, Standard Methods for Examination of Water and Wastewater, 20th Edition, 1998.

Attachment A

Deviations from Reference Method

A. 1 EPA Method 365.1 does not include quality control procedures. This SOP includes routine quality control procedures based on USEPA Region 9 Lab SOP # 596.

Attachment B: Analytical Results Report

(LIMS or Spreadsheet)

GUAM ENVIRONMENTAL PROTECTION AGENCY

EMAS Analytical Program

Parameter Name: **Orthophosphate-P**

Date of Report: 10/31/2024

Method: EPA 365.1

Analyst: R. Paulino and J. Fujihira

#	Lab Sample ID	Date Sampled	Date Analyzed	Location	Dilution Factor	Reporting Limit (mg/L)	Method Detection Limit (MDL), mg/L	Result (mg/L)	Remarks
1	03764-001	9/30/2024	10/28/2024	MZRT-2, Toguan River 1	1	0.025	0.01	**0.305**	
2	03764-002	9/30/2024	10/28/2024	MZRAC, Agachang River 2	1	0.025	0.01	**0.168**	
3	03764-003	9/30/2024	10/28/2024	MZRML, Manell River	1	0.025	0.01	**0.166**	
4	03764-004	9/30/2024	10/28/2024	11SUMAY, Sumay River	1	0.025	0.01	**0.228**	
5	03764-005	9/30/2024	10/28/2024	10LIYOG, Liyog River	1	0.025	0.01	**0.133**	
6	03764-006	9/30/2024	10/28/2024	9AJAYAN, Ajayan River	1	0.025	0.01	**0.107**	
7	03764-007	9/30/2024	10/28/2024	6TINAGO, Tinago River	1	0.025	0.01	**0.105**	
8	03764-008	9/30/2024	10/28/2024	5ASLINGET, Aslinget River 3	1	0.025	0.01	**0.0714**	

Reviewed By: _____

Date Reviewed: 10/31/2024

Approved By: _____

Date Approved: 11/05/2024

Attachment C: Omnion FIA Software Report

Original Run Filename: OM_10-28-2024_06-08-06PM.OMN Created: 10/28/2024 6:08:06 PM
Original Run Author's Signature: [EMASLAB2]
Current Run Filename: 03664 2 of 2 PO4.omn Last Modified: 10/28/2024 6:53:06 PM
Current Run Author's Signature: [EMASLAB2]
Description: Stock Std: STD21-014, HACH Lot# 340221m Exp. Aug, 4 2023
Second source: HACH standard STD23-11 Lot# A3024 1000 mg/L PO4 Exp. Feb 2027

Analyst: J.Fujihira, A.Aflague

Analysis: IWM Study 2024 for PO4

Sample	Rep.	Cup No.	Channel 4 ortho-phosphate (mg/L)	Detection Time	ADF	MDF	Description
1 ppm PO4	1	S9	1.00	10/28/2024@6:09:14			
0.50 ppm PO4	1	S9	0.500	10/28/2024@6:11:58	2.00		
0.25 ppm PO4	1	S9	0.250	10/28/2024@6:14:36	4.00		
0.1 ppm PO4	1	S9	0.100	10/28/2024@6:17:08	10.0		
0.025 ppm PO4	1	S13	0.0250	10/28/2024@6:18:19			
0 ppb PO4	1	S14	0.00	10/28/2024@6:19:28			
ICV 0.5 ppm PO4	1	1	0.479	10/28/2024@6:20:36			
Calibration:			Table/Fig. :				
ICB	1	2	3.67e-4	10/28/2024@6:21:43			
QL 0.025 ppm	1	3	0.0227	10/28/2024@6:22:51			
ICB redo	1	2	5.55e-4	10/28/2024@6:23:58			
LFB 0.5 ppm PO4	1	4	0.531	10/28/2024@6:25:05			
LRB	1	5	5.73e-4	10/28/2024@6:26:13			
LRB redo	1	5	6.14e-4	10/28/2024@6:27:20			
03764-06	1	15	0.107	10/28/2024@6:28:27			
03764-07	1	16	0.105	10/28/2024@6:29:34			
03764-08	1	17	0.0714	10/28/2024@6:30:42			
CCV	1	18	-4.16e-4	10/28/2024@6:31:48			
CCB	1	19	0.0702	10/28/2024@6:32:55			
CCB redo	1	19	0.0545	10/28/2024@6:34:01			
CCV redo	1	18	-7.52e-4	10/28/2024@6:35:08			
CCB redo	1	19	1.39e-3	10/28/2024@6:36:15			
Rinse	1	20	0.0191	10/28/2024@6:37:21			
	2	20	4.18e-3	10/28/2024@6:38:28			
Average:			0.0116				

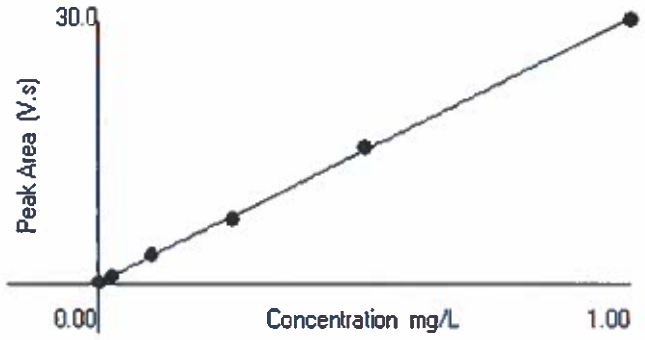
Analyte Properties Table for : 03664 2 of 2 PO4.omn

Property	Channel 4 ortho-phosphate
Concentration Units	mg/L
Calibration Fit Type	First Order
Clear Calibration	Yes
Force through Zero	No
Calibration	1/x
Auto Dilution Trigger	Yes
% of High Standard	110
Quik Chem Method	
Chemistry	Direct/Bipola
Calibration by Height	No
Inject to Peak Start	14
Peak Base Width	45

Table : 1 (ortho-phosphate)

	Known Conc. (mg/L)	Rep	Peak Area (V.s)	Peak Height (V)	% RSD	% Residual	Det. Conc (mg/L)	Detection Date	Detection Time
1	1.00	1	30.0	1.52	0.0	0.5	0.995	10/28/2024	6:09:14 PM
2	0.500	1	15.5	0.774	0.0	-2.5	0.512	10/28/2024	6:11:58 PM
3	0.250	1	7.33	0.369	0.0	3.2	0.242	10/28/2024	6:14:36 PM
4	0.100	1	3.13	0.156	0.0	-1.9	0.102	10/28/2024	6:17:08 PM
5	0.0250	1	0.767	0.0377	0.0	6.3	0.0233	10/28/2024	6:18:19 PM
6	0.00	1	0.114	5.51e-3			1.56e-3	10/28/2024	6:19:28 PM

Figure : 1 (ortho-phosphate)



Area = 30.0 * Conc + 0.0676
Conc = 0.0333 * Area - 2.22e-3
Correlation Coefficient (r) = 0.99995
Weighting : 1/x

Original Run Filename: OM_10-28-2024_05-19-51PM.OMN Created: 10/28/2024 5:19:51 PM
Original Run Author's Signature: [EMASLAB2]
Current Run Filename: 03664 1 of 2 PO4.omn Last Modified: 10/28/2024 6:04:45 PM
Current Run Author's Signature: [EMASLAB2]
Description: Stock Std: STD21-014, HACH Lot# 340221m Exp. Aug, 4 2023
Second source: HACH standard STD23-11 Lot# A3024 1000 mg/L PO4 Exp. Feb 2027

Analyst: J.Fujihira, A.Aflague

Analysis: IWM Study 2024 for PO4

Sample	Rep.	Cup No.	Channel 4 ortho-phosphate (mg/L)	Detection Time	ADF	MDF	Description
1 ppm PO4	1	S9	1.00	10/28/2024@5:22:23			
0.50 ppm PO4	1	S9	0.500	10/28/2024@5:27:04	2.00		
0.25 ppm PO4	1	S9	0.250	10/28/2024@5:29:42	4.00		
0.1 ppm PO4	1	S9	0.100	10/28/2024@5:32:13	10.0		
0.025 ppm PO4	1	S13	0.0250	10/28/2024@5:33:25			
0 ppb PO4	1	S14	0.00	10/28/2024@5:34:34			
ICV 0.5 ppm PO4	1	1	0.504	10/28/2024@5:35:42			
Calibration:			Table/Fig. :				
ICB	1	2	0.0328	10/28/2024@5:36:49			
QL 0.025 ppm PO4	1	3	0.0242	10/28/2024@5:37:56			
LFB 0.5 ppm PO4	1	4	0.543	10/28/2024@5:39:04			
LRB	1	5	0.0128	10/28/2024@5:40:11			
ICB redo	1	2	9.57e-5	10/28/2024@5:41:19			
03764-01	1	6	0.305	10/28/2024@5:42:25			
03764-02	1	7	0.168	10/28/2024@5:43:31			
03764-02 LD	1	8	0.159	10/28/2024@5:44:38			
LRB redo	1	5	1.28e-3	10/28/2024@5:45:45			
03764-02 LFM (0.5 ppm PO4)	1	9	0.717	10/28/2024@5:46:52		2.0	
03764-03	1	10	0.166	10/28/2024@5:47:59			
03764-04	1	11	0.228	10/28/2024@5:49:05			
03764-05	1	12	0.133	10/28/2024@5:50:11			
CCV	1	13	0.482	10/28/2024@5:51:19			
CCB	1	14	0.0315	10/28/2024@5:52:26			
03764-06	1	15	0.110	10/28/2024@5:53:34			
03764-07	1	16	0.111	10/28/2024@5:54:41			
03764-08	1	17	0.0753	10/28/2024@5:55:48			
CCB redo	1	14	2.15e-3	10/28/2024@5:56:56			
CCV	1	18	0.512	10/28/2024@5:58:03			
CCB	1	19	0.102	10/28/2024@5:59:10			
Rinse	1	20	0.0213	10/28/2024@6:00:16			
	2	20	0.0216	10/28/2024@6:01:22			
Average:			0.0215				

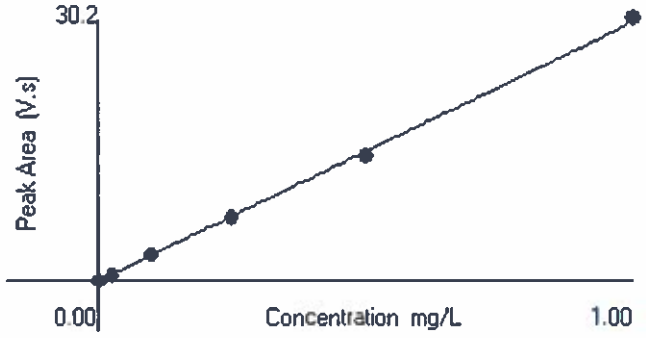
Analyte Properties Table for : 03664 1 of 2 PO4.omn

Property	Channel 4 ortho-phosphate
Concentration Units	mg/L
Calibration Fit Type	First Order
Clear Calibration	Yes
Force through Zero	No
Calibration	1/x
Auto Dilution Trigger	Yes
% of High Standard	110
Quik Chem Method	
Chemistry	Direct/Bipola
Calibration by Height	No
Inject to Peak Start	14
Peak Base Width	45

Table : 1 (ortho-phosphate)

	Known Conc. (mg/L)	Rep	Peak Area (V.s)	Peak Height (V)	% RSD	% Residual	Det. Conc (mg/L)	Detection Date	Detection Time
1	1.00	1	30.2	1.53	0.0	-1.7	1.02	10/28/2024	5:22:23 PM
2	0.500	1	14.5	0.749	0.0	2.6	0.486	10/28/2024	5:27:04 PM
3	0.250	1	7.33	0.378	0.0	1.6	0.246	10/28/2024	5:29:42 PM
4	0.100	1	3.06	0.154	0.0	-1.9	0.102	10/28/2024	5:32:13 PM
5	0.0250	1	0.723	0.0360	0.0	7.7	0.0230	10/28/2024	5:33:25 PM
6	0.00	1	0.106	5.09e-3			2.21e-3	10/28/2024	5:34:34 PM

Figure : 1 (ortho-phosphate)



Area = 29.6 * Conc + 0.0417
Conc = 0.0337 * Area - 1.37e-3
Correlation Coefficient (r) = 0.99993
Weighting : 1/x

Attachment D: QC Summary Report

GUAM EPA LABORATORY
QC Summary Report

GEPA Lab Form: CH-03-01A

Parameter: PO4-P

GEPA Lab Sample Number: 03764 **Analyst:** RP / JF **Date:** 10/28/24

QC Sample	Calculation	Result	Acceptable (Y/N)	Acceptable Range	Notes
ICV	%R = (measured analyte conc. / true analyte conc.) x 100 0.504/0.5 X100 = 100.8 %	100.8 % <QL	Y	90-110%	
ICB		~~0.025~~ JF	Y	<QL	QL = 0.025
QL	%R = (measured analyte conc. / true analyte conc.) x 100 0.0242/0.025 X100= 96.8 %	96.8%	Y	50 - 150%	
LRB		≤MDL	Y	≤MDL < QL *	MDL = 0.01 * for Cl⁻ calibration fits 2nd order polynomial
LFB (or QCS)	%R = (measured analyte conc. / true analyte conc.) x 100 0.543/0.5 X100= 108.6 %	108.6 %	Y	90 - 110%	
LD (1)	RPD = [(meas. analyte conc. in LD - meas. analyte conc. in routine sample) / (mean of LD and routine sample conc.)] x100 0.168-0.159 / 0.164 X100 = 0.054 %	0.054 %	Y	≤ 20% for samples with analyte levels ≥ 5X QL	For other samples, the absolute difference between duplicate results must be <QL. For samples <QL, RPD is not applicable.
LFM (1)	%R = [(meas.analyte conc in LFM - meas. analyte conc. in routine) / (expected analyte conc. of added spike in LFM)] x 100 0.717 - 0.168 /0.5 X100= 109.8 %	109.8 % 96.4 %	Y	If the measured analyte conc in routine sample is <4X the analyte conc of added spike in LFM, %R is 75-125%	For other samples, %R is 90 - 110%
CCV (1)	0.482/0.5 X100 = 96.4 %	~~≤QL~~ JF	Y	90 - 110%	Calculation is same as ICV.
CCB (1)		~~0.025~~ <QL JF	Y	<QL	

Revision: 4 - 02/05/2019 esy
File: qcreport.xls

Peer Reviewed By: _____
Date Reviewed: _____

GUAM EPA LABORATORY
QC Summary Report

GEPA Lab Form: CH-03-01A

Parameter: PO4-P

GEPA Lab Sample Number: 03760 **Analyst:** RP/JF **Date:** 10/28/24

QC Sample	Calculation	Result	Acceptable (Y/N)	Acceptable Range	Notes
LD (2)	RPD = [(meas. analyte conc. in LD - meas. analyte conc. in routine sample) / (mean of LD and routine sample conc.)] x100			≤ 20% for samples with analyte levels ≥ 5X QL	For other samples, the absolute difference between duplicate results must be <QL. For samples <QL , RPD is not applicable.
LFM (2)	%R = [(meas analyte conc in LFM - meas. analyte conc. in routine) / (expected analyte conc. of added spike in LFM)] x 100			90-110%	
CCV (2)	0.512 / 0.5 x100 = 102.4 %	102.4 %	Y	90 - 110%	
CCB (2)		<QL	Y	<QL	
LD (3)	RPD = [(meas. analyte conc. in LD - meas. analyte conc. in routine sample) / (mean of LD and routine sample conc.)] x100			≤ 20% for samples with analyte levels ≥ 5X QL	For other samples, the absolute difference between duplicate results must be <QL. For samples <QL , RPD is not applicable.
LFM (3)	%R = [(meas analyte conc in LFM - meas. analyte conc. in routine) / (expected analyte conc. of added spike in LFM)] x 100			90-110%	
CCV (3)				90 - 110%	
CCB (3)				<QL	
CCV (4)				90 - 110%	
CCB (4)				<QL	

Comments:

Peer Reviewed By: _____
Date Reviewed: _____

Attachment E: Chain of Custody

GUAM ENVIRONMENTAL PROTECTION AGENCY
CHAIN OF CUSTODY RECORD

IWM 2024 Batch 2
PROJECT NAME

ASSIGNED LAB ID # 03764
SDG # G Prelusky
SAMPLER PRINT/SIGN
SAMPLER PRINT/SIGN

FIELD SAMPLE ID	DATE	TIME	COMPOSITE	GRAB	SAMPLE LOCATION	CONTAINER QUANTITY	E. coli	Enterococci	NO3	NO4	TSS	NOTES
FB	9/30/24	8:08		X	Field Blank	1	X	X				
MZRT-2		9:14			Tuguan River 1	1				X		
MZRP-2		9:21			Pigua River 2	1	X					INCI DO
MZRAC		9:38			Achang River 2	2	X			X		
MZRNL		9:46			Manell River	1			X	X		
IISUMAY		9:52			Sumay River	2			X	X	X	INCI DO
IOLIYOG		9:58			Liyog River	3	X		X	X	X	
AGASAYAN		10:04			Agayan River	2	X			X	X	
INRAGB 3		10:11			Agfayan River Mouth	1	X					
INRI 1		10:18			Inarajan River 1	1			X			
GTINAGU		10:25			Tinagu River	1				X		
BASLINGET		10:27			Aslinger River	1				X		
TUM111		10:37			Talofofo Bay	1		X				

GP

RELINQUISHED BY: PRINT	SIGNATURE	DATE	TIME	RECEIVED BY: PRINT	SIGNATURE	DATE	TIME
G Prelusky		9/30/24					
RELINQUISHED BY: PRINT	SIGNATURE	DATE	TIME	RECEIVED BY: PRINT	SIGNATURE	DATE	TIME
				A AFLAGUE			
RELINQUISHED BY: PRINT	SIGNATURE	DATE	TIME	**RECEIVED FOR LAB BY: PRINT**	SIGNATURE	DATE	TIME
						9/30/24	1106

REMARKS

Attachment F: Inorganic Standards Preparation Logbook

GUAM ENVIRONMENTAL PROTECTION AGENCY - EMAS Analytical Program
Inorganic Standard Preparation Logbook

GEPA Standard ID	Standard Description	GEPA Std Receipt Number	Initial Conc. (mg/L, ug/L)	Volume Used (mL, uL)	Final Volume (L, mL)	Final Conc. (mg/L, ug/L)	Preser-vative	Prep Date	Exp Date	Prep By (Initials)	Remarks

Attachment G: Inorganic Reagent Preparation Logbook

GUAM ENVIRONMENTAL PROTECTION AGENCY - EMAS Analytical Program
Inorganic Reagent Preparation Logbook

GEPA Reagent ID	Reagent Description	Reagent 1 (wt or vol used)	Reagent 2 (wt or vol used)	Reagent 3 (wt or vol used)	Final Volume with H2O	Preser-vative (wt, vol)	Prep Date	Exp Date	Prep By (Initials)	Remarks