Orthophosphate

a) Scope and Application

i) This is a procedure outline for the determination of low level orthophosphate concentrations normally found in estuarine and/or coastal waters.

ii) The method detection limit (MDL) is determined on a yearly basis. This MDL is defined as the student t multiplied by the standard deviation of at least seven replicates of a low level estuarine sample, or reagent water matrix.. The range is determined by the instrument used, its configuration, and the standard curve that is used. A more detailed explanation of the MDL can be found in 40 CFR part 136 appendix C.

iii) This method should be used by analysts experienced in the use of colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

iv) The reaction chemistry described may be used with segmented flow, flow injection, manual spectrophotometric and discrete instrumentation.

b) Summary of Method

i) Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid, which is measured colorimetrically. The color is proportional to the phosphorus concentration.

ii) After a defined reaction period, either through continuous flow or by timing, the color is measured spectrophotometrically.

c) Interferences

i) Color development is pH dependant so it is recommended that samples are in the pH range of 4 to 8.

ii) Sample turbidity should be removed by filtration prior to analysis.

iii) Refractive Index interferences should be corrected for when analyzing estuarine/coastal samples. This can be performed by using dual beam correction for background, faster time of flight with flow injection, or matching the salinity of the calibration standards and rinse/blank liquid to the salinity of the samples. Up to 20% salinity, error is less than 1%.

iv) High iron concentrations can cause precipitation and loss of orthophosphate.

d) Apparatus and Materials

i) Continuous flow automated analytical system equipped with an autosampler, manifold, proportioning pump, colorimeter, phototube or recorder or computer based data system. Flow injection, discrete, and manual spectrophotometric analysis would be considered equivalent when using the same reaction chemistry.

ii) Phosphorus -free glassware: All glassware used in the determination must be low in residual phosphate to avoid sample/reagent contamination. Washing with 10% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use critical cleaning liquid detergents instead of or before acid rinsing. A laboratory's glassware cleaning method

will be considered sufficient if all quality control samples are within the expected ranges.

e) Reagents

i) Stock reagent solutions: The specific recipe for these reagents is generally instrument dependent, and may change due to the concentration of the samples being analyzed. In this SOP the chemicals needed for the reaction will be listed, but not the specific amounts. If continuous flow is being utilized an appropriate surfactant like FFD-6 is added to one or more of the reagents.

(1) Color reagent solution: Combine proper portions of ammonium molybdate tetrahydrate, antimony potassium tartrate, and sulfuric acid and dilute with reagent water.

(2) Ascorbic Acid solution: Dissolve the proper amount of ascorbic acid powder in to reagent water. It can be used as a separate reagent or combined with (1) in proper proportion to make a single reagent test. When combined it is only good for one day.

ii) Reagent water: see Chapter VI, Section 4.2.

iii) Artificial Sea Water (ASW): see Chapter VI, Section 4.3. This can be used for the matrix at an appropriate salinity for the samples being analyzed. If precipitation occurs eliminate the magnesium sulfate.

iv) Standards should bracket the expected concentration of the samples. Prepare a minimum of 5 standards to cover the working range. Standards should be dissolved in DI water and made fresh for each run.

v) When analyzing samples of varying salinities, it is also recommended that standards be prepared in a series of salinities in order to quantify the "salt error," the shift in the colorimetric response of ammonia due to the change in the ionic strength of the solution. This becomes necessary when no method of background correction is built into the system.

f) Sample Handling

i) Samples must be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, then refrigeration at 4 ± 2°C is acceptable.

ii) If samples will not be analyzed within 48 hours of collection, the sample must be stored at -20°C or less for a maximum of 28 days.

g) Procedure

i) Calibration: Standard curve to bracket the concentration of expected samples must be analyzed.

ii) Sample analysis

(1) If samples have not been freshly collected and are frozen, thaw the samples to room temperature.

(2) Allow both colorimeter and recorder to warm up for 30 minutes or the specific instrument recommendation. Obtain a steady instrument state that is necessary for the instrument to be ready to collect data.

(3) Use a sampling rate which ensures reliable results.

(4) Analytical sequence: The samples and associated QC samples and standards should be run according to the following sequence.

a. Five calibration standards with concentration within the linear range of the test.

b. Reagent/method blank and blank spike.

c. Initial calibration verification

d. QCS

e. Ten CBP samples.

f. One matrix spike sample and duplicate.

g. One medium concentration calibration standard.

h. One method blank.

i. Steps 5.7.2.4.3 - 5.7.2.4.6 are repeated until samples are analyzed or QC samples indicate that the system is out of control and recalibration is necessary.

j. Method blank

k. Calibration verification sample

L QCS

(5) If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected. It is recommended that if there is not a clearly defined low concentration peak, that the sample be reanalyzed at the end of the sample run.

iii) Calculations

(1) Orthophosphate concentrations are calculated from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variable and their corresponding signals are the dependent variable.

(2) Results should be reported in mg orthophosphate-p /L.

h) Quality Control

i) Method detection limits (MDL): Method detection limits should be established using the procedures in Chapter VI, Section C.8.

ii) Calibration

(1) Linear calibration range: Calibration standards should bracket the range of CBP samples.

(2) Correlation coefficient: The correlation coefficient must be 0.995 or better for the calibration curve to be used.

iii) Method Blank: see Chapter VI, Section C.6.1.

iv) Matrix spike sample: see Chapter VI, Section C.6.4.

v) Laboratory duplicate: see Chapter VI, Section C.6.3.

vi) Reference materials: The laboratory must analyze a standard reference material or some other second source performance check with each run.

vii) Summary table for QC parameters:

INDICATOR	**ACCEPTANCE/ACTION LIMITS**	**ACTION**	**FREQUENCY (BATCH)**
Correlation Coefficient	≥ 0.995	If < 0.995, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.	1 per batch if acceptable.
QCS (EPA)	± 10%	If QCS value is outside ± 10% of the target value, reject the run, correct the problem and rerun samples.	Beginning of run following the ICV.
QCS (NELAC)	± 3s (See multirules)	See Multirules actions.	Beginning of run following the ICV.
ICV	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
CCV	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 10 samples and at end of batch
Laboratory Reagent Blank / Calibration Blank[a]	≤ Method Quantitation Limit	If the LRB exceeds the quantitation limit, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	Following the ICV, after every 10 samples and at the end of the run.
Method Quantitation Limit (MQL) The concentration of the lowest standard.	Determine the standard deviation at the concentration of the lowest standard. The MQL will be ±3s.	When the value is outside the predetermined limit and the ICV is acceptable, reanalyze the sample. If the reanalysis is unacceptable, increase the concentration and reanalyze. If this higher concentration meets the acceptance criteria, raise the reporting limit for the batch.	Beginning of run following the LRB
Laboratory Fortified Sample Matrix	± 20%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	After every 10 samples
Laboratory Duplicate	± 20%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample	After every 10 samples.

		must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	

i) References

(a) EPA 1993. "Methods for the Determination of Inorganic Substances in Environmental Samples,'' NERL–CI, EPA/600/R–93/100, August, 1993. Method 365.1, Rev. 2.0, orthophosphate (as P) - Automated, spectrophotometric.

(b) Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125, 217 p. Method ID: I-2523-85

(c) MacDonald, R.W. and F.A. McLaughlin. 1982. The effect of storage by freezing on dissolved inorganic phosphate, nitrate, and reactive silicate for samples from coastal and estuarine waters. Water Research, 16:95-104.

(d) 40 CFR 136, Appendix A and 136.6