SECTION D.8  
ORTHOPHOSPHATE, TOTAL AND DISSOLVED

**CEDR Method Codes: PO4F L01**

**PO4W L01**

Scope and Application

This method describes the determination of low-level orthophosphate concentrations in filtered or unfiltered samples taken from fresh and estuarine surface waters.

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

The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection, or discrete mixing apparatus. The analytical range is determined by the instrument used, its configuration and the standard curve that is prepared.

Summary of Method

1. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.
2. After a defined reaction period, either through continuous flow or by timing, the color is measured spectrophotometrically at a wavelength of 800 nm.

Interferences

Color development is pH dependent and it is recommended that samples be in the pH range of 4 to 8.

Turbidity can bias the results through the absorption or scattering of light. A second filtration may be necessary in the determination of dissolved phosphorus (PO4F) to remove this effect.

Refractive Index interferences should be corrected for when analyzing estuarine/coastal samples (EPA 1997). This can be performed by using dual-beam background correction at a different wavelength, or by matching the salinity of the calibration standards and rinse/blank water to the salinity of the samples.

iv) High concentrations of ferric iron (Fe+3) can cause precipitation and loss of orthophosphate.

Apparatus and Materials

Continuous flow automated analytical system equipped with an auto sampler, manifold, proportioning pump, colorimeter, detector (λ = 880 nm) and a computer-based data system. Flow-injection and discrete spectrophotometric instrumentation are considered equivalent to continuous-flow systems when using the same reaction chemistry.

Phosphorus ‑free glassware: All glassware used in the determination must be low in residual phosphate to avoid sample/reagent contamination. Washing with 10-50% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use phosphorus-free detergents instead of, or before acid rinsing. The glassware cleaning procedure will be considered sufficient if all quality control samples are within the expected ranges.

Reagents and Standards

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. For continuous flow analyzers, a surfactant such as FFD-6 or SDS may be added to one or more reagents.

Color reagent solution: Combine proper portions of the following and dilute with reagent water.

1. Ammonium molybdate tetrahydrate ((NH4)6Mo7O24**∙**4H2O),
2. Antimony potassium tartrate (K(SbO)C4H6O6**∙**½H2O or equivalent), and
3. Sulfuric acid (H2SO4).

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

Calibration Standards: Laboratories may purchase or prepare stock and working standards. The initial calibration check standard (ICV) must be purchased or made from a second source.

1. Potassium phosphate monobasic (KH2PO4): Primary standard-grade KH2PO4 (pre-dried for at least 1 hr. at 105°C) and then dissolved in reagent water. This solution is stable for up to 6 months when refrigerated at ≤ 6°C.
2. Prepare a series of standards by diluting suitable volumes of stock solutions with reagent or Artificial Sea Water. Prepare working standards daily, with three or more standards per decade and an additional zero standard. Standards should bracket the expected concentrations of the samples or dilution and reanalysis will be necessary.
3. Reagent water: see Chapter 6, Section C.4.2.
4. Artificial Sea Water (ASW): see Chapter 6, Section C.4.3.   
   1. ASW may be used instead of reagent water to match the salinity of the standards to the salinity of the samples being analyzed. If precipitation occurs, eliminate the magnesium sulfate in the ASW.
   2. When analyzing samples of varying salinities, it may be necessary to prepare standards in a series of salinities to quantify the "salt error", i.e., the shift in the colorimetric response of phosphate due to the change in the ionic strength of the solution. Salinity matching is unnecessary if using a flow injection analyzer or if background correction is built into the instrument.

Sample Handling

1. Samples must be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, keep refrigerated at ≤ 6°C.
2. If samples will not be analyzed within 48 hours of collection, freeze and store them at -20°C or less for a maximum of 28 days.

Procedure

Prepare calibration standards to establish a curve that brackets the expected concentration of samples. Samples above the highest calibration standard may be diluted to fall within the calibration curve. See Chapter 6, Section C.5 for additional calibration requirements.

Sample analysis

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

Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

Analytical sequence: The samples and associated QC samples are typically run according to the following sequence.

* 1. Three or more calibration standards per decade (i.e. per order of magnitude), within the linear range of the instrument.  
     1. An additional calibration standard with zero analyte concentration to estimate the y-intercept.
     2. The lowest standard must have a concentration ≤ PQL or reporting limit.
  2. Initial calibration verification (ICV) standard, traceable to a national standard;
  3. LCS/QCS (if the QCS is a CRM, the ICV standard may be omitted);
  4. Reagent/method blank;
  5. Ten to twenty CBP samples;
  6. One matrix spike sample and one duplicate sample;
  7. One mid-range continuing calibration verification standard (CCV) per decade; and a
  8. Method blank.
  9. Repeat steps (3) e through (3) h until all samples are analyzed or QC samples indicate that the system is out of control and recalibration is necessary.
  10. Reagent/method blank
  11. CCV standard(s)

1. If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected in continuous flow instruments. If the low concentration peak is not clearly defined, it is recommended to reanalyze that sample at the end of the sample run.

Calculations

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 response is the dependent variable.

Results shall be reported in mg PO4-P /L.

Quality Control

Method detection limit (MDL): Method detection limit should be established using the procedures in Chapter 6, Section C.8.

Calibration Checks

The correlation coefficient must be 0.995 or better for the calibration curve to be used.

(2) Results of the initial and continuing calibration verification samples must be within 10% of their expected values.

Method Blank: see Chapter 6, Section C.6.1.

Matrix spike sample: see Chapter 6, Section C.6.4.

Laboratory duplicate: see Chapter 6, Section C.6.3.

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

**Summary of acceptance limits and corrective actions for Orthophosphate QC samples**

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| **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. |
| ICV | ± 10% | Recalibrate if outside acceptance limits. | Beginning of run following standard curve. |
| QCS | ± 10% (EPA 1993)  ± 3 s.d. (NELAC) | 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. |
| CCV | ± 10% | If outside 10%, correct the problem. Rerun all samples following the last in-control CCV. | After every 10-20 samples and at end of batch |
| Laboratory Reagent Blank / Method Blank | ≤ Practical Quantitation Limit (PQL) | If the LRB exceeds the PQL, 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 10-20 samples and at the end of the run. |
| MDL and PQL Verification Spike | Detected ≥ MDL and ≤ PQL (NELAC) | If the spike is not detected, repeat with a higher concentration spike. | Two quarterly low-level spikes, run in separate batches. (EPA: at MDL spike conc.) |
| 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 10-20 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 must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis. | After every 10-20 samples. |

1. References
2. 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.
3. 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
4. American Public Health Association. 2012. “Standard Methods for the Examination of Water and Wastewater”, Method 4500-P F -2011, Automated Ascorbic Acid Reduction Method. Also 4500-P G. Flow Injection Analysis for Orthophosphate.
5. 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.