Standard Operating Procedure For Total Phosphorous

Revision 0

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1.0 Overview

This procedure describes how to perform laboratory analysis of a water sample for Total Phosphorous.

2.0 Equipment List

___ Acid-washed glassware and pipets
___ 100mL capacity, digestable, screw-top sample tubes
___ Autoclave
___ Spectrophotometer; Shimadzu model UV-1601 with optically matched 5 cm path length cells

3.0 Sample handling protocol

3.1 Samples will be transported to the laboratory in light-blocking polyethylene bottles.
3.2 Samples will be logged in and frozen immediately upon arrival at the laboratory.
3.3 Samples will be analyzed within one year or less according to the needs of the project.

4.0 Analytical Procedure

4.1 Preparation of Samples

4.1.1 Thaw frozen samples in a warm water bath.
4.1.2 Allow to sit upright for a few minutes before removing aliquots.

4.2 Preparation of Reagents

This analysis uses hazardous substances; observe all laboratory safety protocols.

4.2.1 Solutions

4.2.1.1 Phosphorous Process Pre-mix: Dissolve 3.75g Ammonium Molybdate and .085g Antimony Potassium Tartrate in 42mL concentrated Sulfuric acid and an approximately equal volume of deionized water. Allow to return to room temperature and dilute SLOWLY to 500mL with deionized water. Refrigerate until needed. This Pre-Mix is stable for several months.

4.2.1.2 Ascorbic acid solution: Dissolve 2.7g Ascorbic acid in 50 mL deionized water for every 50mL total ascorbic acid solution required for the size of the run. This solution is not stable. Make fresh for each run.

4.2.2 Mixed reagents

4.2.2.1 Mix 4 parts premix to 1 part ascorbic acid solution. Total volume will be (#samples +5) x 3mL. Allow to sit on magnetic stirrer for ½ hour prior to use.

4.3 Preparation of Standard, 10ppm PO4 Solution

4.3.1 Dilute commercially available 1000ppm as PO4 solution to 10ppm using reagent water.

4.4 Preparing Samples for Digestion

4.4.1 Pipet 30mL of sample from sample bottle, without disturbing any material that may have settled out.
4.4.2 Carry standards and blanks through this process as well.
4.4.3 Add one drop of phenolphthalein to each tube. Swirl and look for a pink color.
4.4.4 To any tubes which show a pink color, add enough drops of 5N sulfuric acid to just barely discharge all pink color. This occurs infrequently and the number of drops needed should be noted in the logbook.
4.4.5 Add to each tube, including blanks and standards, 1 scoop equal to .3g of dry Potassium Persulfate.
4.4.6 Loosely cap all tubes.

4.5 Digesting Samples

4.5.1 Check to see that all tubes are loosely capped.
4.5.2 Put sample tubes carefully in steel buckets that will fit in the autoclave being used. All tubes should be very nearly vertical. Rinse and fill autoclave with distilled water just to the level of the rack.
4.5.3 Turn on. Allow to preheat.
4.5.4 Put samples in autoclave, place inner lid on top of tubes to keep caps in place.
4.5.5 Cover, and start tightening screws in pairs. Allow autoclave to vent for 20 minutes.
4.5.6 Close the pressure vent. After another 10 minutes, finish tightening screws. Pressure should begin to build immediately.
4.5.7 The samples should digest at 15lb/sq. inch of pressure for 40 minutes.
4.5.8 Cock the pressure release and allow to return to ambient pressure.
4.5.9 Remove sample bucket with tubes and allow to return to ambient temperature.

4.6 Preparing the spectrophotometer

4.6.1 Power on. Instrument will warm up and go through a self-diagnostic program.

4.6.2 Flush and fill both cells with deionized water. Clean thoroughly with a lint-free lens cloth. Replace cells in holders.

4.6.3 Go through the following keystrokes:

F1- baseline correction
MODE
F1- parameters
F2- set
Phosphorous method
ENTER
START/STOP- to measure
AUTOZERO- to bring to zero absorption
START/STOP- repeat autozero until absorption reads 0.0000.

When using this instrument, clean cell windows of ANY dust, smudges, moisture, etc. before each measurement. The slightest trace of dirt will give an incorrect result. Sample level in the cell must be even with the shoulders of the upright tubes.

4.7 Analyzing samples

Wear appropriate safety gear when handling hazardous materials.

4.7.1 Add one more drop of phenolphthalein indicator to each digestion tube.

4.7.2 Add 500uL Sodium Hydroxide 5N to each tube. Swirl vigorously. The color should just turn pink. If not, continue adding dropwise until liquid remains slightly pink.

4.7.3 Add 3 mL Mixed Reagent to the first tube from an acid-washed pipet. Note time.

4.7.4 Addition of reagent will be in 2 minute intervals, and the elapsed time between addition of reagent and reading in the spectrophotometer will be 21 minutes. Therefore, if the first tube is set up at 2:00, it will be read at 2:21, and the next tube will be set up at 2:02 to be read at 2:23. Record start/end times in the logbook. Rinse pipet between samples with deionized water to prevent contamination.

4.7.5 To fill the spectrophotometer cell with the first sample, remove it carefully from its holder and pour out the deionized water which was used to calibrate the instrument. Rinse with a small amount of sample- it should be the first blank- then fill. Align cell with the same orientation for each reading.

4.7.6 To read sample, press START/STOP on the spectrophotometer and record absorbance in logbook.

4.7.7 After each sample, flush for 10 seconds with distilled water at tap pressure, flush quickly with deionized water from a squeeze bottle, then rinse with a small amount of the next sample to be analyzed.

4.7.8 Log results in a permanently bound logbook.

5.0 Quality Control

5.1 General Quality Control Procedure

5.1.1 Standard calibration curve calculations will be checked with an Outside Check made from a different source of phosphate standard and diluted to approximately the middle of the calibration range. Tolerance for the Outside Check is +/- 10%.

5.2 Duplicate samples

5.2.1 One duplicate sample must be run for every ten samples analyzed. The duplicate sample is split from the same field sample bottle. Otherwise, it is treated the same as the other samples in the batch.

5.2.2 Field duplicates are recommended. These are the responsibility of the sampler and will be treated as a normal sample.

5.2.3 Tolerance is +/- 10% relative difference for duplicates.

5.3 Laboratory-Fortified Samples [SPK]

5.3.1 One SPK sample will be run for every ten samples analyzed. The SPK sample is split from the same field sample bottle, and a known quantity of phosphate is added to test for the presence of matrix interference. This quantity should be between 50% and 200% of the level expected to be present in the sample. Dilution effects will be corrected, and the percent recovery calculated using the formula:
(SPK value -(original value *dilution))/SPK amount

5.3.2 Tolerance is +/- 20% recovery for laboratory-fortified samples.

5.4 Blanks

5.4.1 Blank samples will be made with deionized water and carried through the entire procedure. These will be run at the beginning of the run to check for contamination.

5.5 Detection Limit Checks

5.5.1 Four Detection Limit Checks will be run for each calibration. Dilute stock solution to 8.2 ug/L.

6.0 Calibration

A calibration curve will be calculated from the following standards: Blank, 53.5ug/L, 544ug/L.

7.0 Interferences

Improperly taken samples, specifically, those containing solids such as excess sediment, will show positive error. Solid pieces of organic matter, if included in the digest, will produce an artificially high concentration of phosphorous in the digestate. Suspended sediment in the cell will scatter light and give a false high reading. Interferences can be reduced by removing large particles, but it is ultimately the responsibility of the field sampler to provide samples as free from sediments as possible.