



Understanding Phosphorous Method

Introduction – Phosphorous is a very important element when it comes to life on earth. In most fresh water systems it is the limiting nutrient for overall growth. Many wastewater treatment plants actually add phosphorous into their process stream to optimize the biological treatment. For this reason, phosphorous is regulated as a pollutant not because it is detrimental to life but because it can cause overgrowth of certain plant life that will upset the balance in an aquatic habitat. This is a case where too much of a good thing can be harmful.

Approved Methods – There are several different methods that can be used for colorimetric phosphorous determination, but they are all just variations of the same procedure. Persulfate digestion followed by colorimetric analysis tends to be the most commonly used method and is the basis of most of the following discussion.

Orthophosphate (if the digestion procedure is part of the method, do not utilize it. Instead, proceed directly to the colorimetric analysis) –

- Automated Colorimetric:
 - EPA 365.1 Rev 2.0
 - SM 4500-P F-1999 or G-1999
 - AOAX 973.56
- Manual Single Reagent Colorimetric:
 - SM 4500-P E-1999
 - ASTM D515-88(A)
 - AOAC 973.55
- Manual Two Reagent Colorimetric – EPA 365.3
- Ion Chromatography:
 - EPA 300.0 Rev 2.1 or EPA 300.1 Rev 1.0
 - SM4110 B-2000 or C-2000

Total Phosphorous (all methods except those in Standard Methods have the digestion procedure written in, if using any procedures from Standard Methods you must first digest according to SM 4500-P B(5)-1999) –

- Manual Colorimetric:
 - EPA 365.3
 - SM 4500-P E-1999
 - ASTM D515-88(A)
- Automated Colorimetric:
 - EPA 365.1 Rev 2.0
 - SM4500-P F-1999, G-1999, or H-1999
 - AOAC 973.56

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- ICP/AES:
 - EPA 200.7 Rev 4.4
 - SM 3120 B-1999
- Kjeldahl Digestion/Colorimetric:
 - EPA 365.4
 - ASTM D515-88(B)

Method Summary – For total phosphorous a persulfate salt and acid are added to a portion of the sample. This portion is then heated to boiling for a period of time sufficient to convert all the phosphorous in the sample to the orthophosphate form. The analysis is accomplished by reacting with an ammonium molybdate/antimony potassium tartrate solution along with ascorbic acid. This forms molybdenum blue, the intensity of which is proportional to the amount of phosphorous in solution. If only orthophosphate is required, the digestion step is omitted and the analysis proceeds from there with the option to use ion chromatography instead of colorimetry.

What You Should Know – Phosphorous does not exist naturally in its elemental state. It is highly reactive and readily forms compounds under normal environmental conditions. Because of this almost all test methods are designed to detect the phosphate ion, PO₄³⁻. This can make things slightly tricky when reporting your results, you can either report as phosphorous (P) or as phosphate (PO₄³⁻). Most methods, although they detect phosphate, report as phosphorous.

The Method Update Rule from April 2012 added a clarification to the sampling procedure for orthophosphate. It reads as follows “The immediate filtration requirement in orthophosphate measurement is to assess the dissolved or bio-available form of orthophosphorus (i.e., that which passes through a 0.45-micron filter), hence the requirement to filter the sample immediately upon collection (i.e., within 15 minutes of collection).” Therefore, all samples for orthophosphate analysis should be filtered in the field upon collection. Other than the standard refrigeration there is no preservation available for these samples and the holding time is 48 hours. For total phosphorous, the samples should be preserved with sulfuric acid and they have a holding time of 28 days.

Phosphorous is a fairly ubiquitous element, which can make it difficult to achieve clean blanks. It is also easily adsorbed on to glass. Ideally, disposable labware should be used whenever possible to eliminate carryover between sample batches. If glassware is used, preliminary washing is necessary to remove traces of phosphorous that may be present. Wash the glassware with hot 1:1 HCl and rinse with deionized (DI) water. Fill the glassware with DI water and add the appropriate color developing reagents. This process will help remove any last traces of phosphorous that may adhere to the glass. Keep the glassware dedicated solely to phosphorous analysis and cover it when not in use. Do not use commercial detergents as many of them contain phosphates.

You should always prepare standards according to the method of the samples. This means that for total phosphorous, standards must be digested along with the samples. Use the same amount of acid and persulfate as you do with the samples. These constituents will change the color intensity of the samples. You cannot use the same standards for total phosphorous as you do for orthophosphate. They can be made from the same stock, but the end result standards cannot be used interchangeably.



The interferences for phosphorous determination are relatively few. For the colorimetric methods, sample color is an obvious potential problem. Compounds that will interfere with the chemistry of the test include arsenate, nitrite, and hexavalent chromium. Because arsenic is in the same group as phosphorous on the periodic table it reacts similarly and will form a similar blue compound. Arsenic as arsenate will cause a positive interference at concentrations as low as 0.1 mg/L. NO₂⁻ and Cr⁶⁺ will start to cause negative interference at 1mg/L. With total phosphorous the digestion procedure typically eliminates any color interferences. Digestion will increase the potential for interference from arsenic due to the formation of arsenate.

Most colorimetric phosphorous methods quickly reach a plateau on the upper end of their detection. It is not at all unusual on a calibration for phosphorous reaching 0.5 mg/L to have a sample read just over 1.0 mg/L without a dilution and require up to a 50x dilution to finally be within range to read. If interferences are suspected, dilutions should be performed prior to any digestion process. This will help to minimize the effect of the interferant. Calibration curves from an IC system or orthophosphate won't plateau quite as sharply, but samples should still be diluted within the range of the curve to ensure valid reporting.

The color developing reagent for phosphate determination has a very short lifespan. It is only stable for a maximum of 4 hours. There is a method that allows you to have two separate reagents that are stable for weeks to months and then add them in separate amounts to the sample for color development. This is the 'two reagent' version of the method. However, the automated version contains provisions to keep the reagents separate and mix them just prior to contacting the sample in the reagent board, essentially giving the same time flexibility.



Method Procedure

Note – This is not intended to be a standalone method and does not address all safety or quality control aspects that may be required. Please consult your local regulations to comply with all requirements.

1. Collect your sample in a [250 mL HDPE container](#). If the sample is for orthophosphate [filter](#) within 15 minutes of collection, do not add preservative, and proceed to step 7. If the sample is for total phosphorous [preserve with sulfuric acid](#).
2. Preheat your [HotBlock](#) to achieve a sample temperature of around 100 C. No specific temperatures are given in the methods, just the instructions to ‘boil gently.’
3. Measure 50 mL of sample into a [digestion vessel](#).
4. Add 1 mL 11 N [sulfuric acid](#) solution and 0.4 g ammonium persulfate or 0.5 g [potassium persulfate](#) to each sample.
5. Boil gently for at least 30 minutes, but do not boil down past 10 mL. According to Standard Methods, some organophosphorous compounds may take as long as 1.5-2 hours to completely digest.
6. Remove samples from the HotBlock and allow them to cool to ambient temperature.
7. Different methods may have requirements regarding pH adjustment at this step. Comply with these conditions and if necessary bring the sample up to a final volume of 50 mL.
8. Set up your favorite analyzer according to the manufacturer’s instructions for phosphorous using the [appropriate reagents](#). In most cases it is the same set up for total phosphorous and orthophosphate. Flow injection analyzers typically need a surfactant, most often sodium dodecylsulfate, in the ascorbic acid reagent. Discrete analyzers do not need this additive.

Don’t forget your [calibration standards](#).

We all like things that make life easier. Was this document helpful? Or do you...disagree with something? Have something to add? Contact me at DavidS@envexp.com to let me know what you think.