



Total Phosphorus, USEPA by Flow Injection Analysis (FIA)

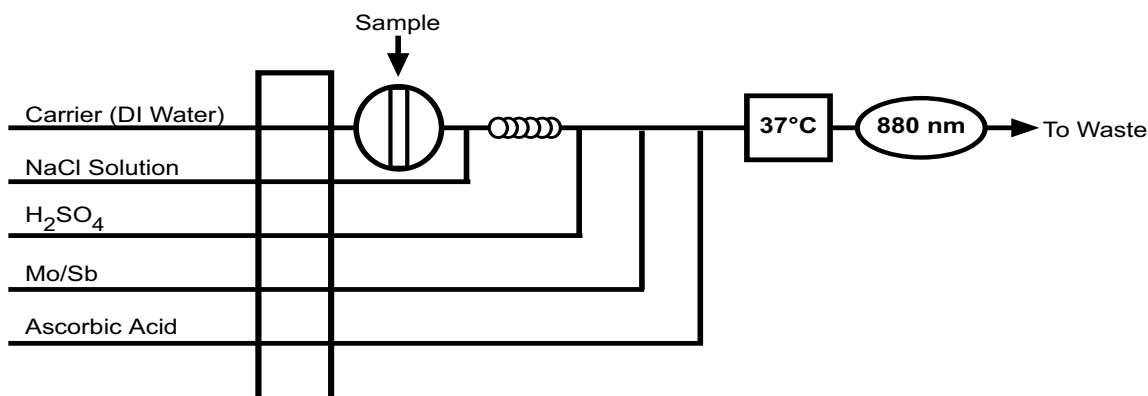
(Cartridge Part #A001558)

1.0 Scope and Application

- 1.1 This method is used for the determination of total phosphorus in drinking, surface and saline waters, domestic and industrial wastes according to USEPA Method 365.1 (Reference 15.4).
- 1.2 The Method Detection Limit (MDL) of this method is 0.005 mg/L phosphorus. The applicable range of this method is 0.02 to 20.0 mg/L phosphorus. The range may be extended to analyze higher concentrations by changing the size of the sample loop.

2.0 Summary of Method

- 2.1 Prior to analysis, samples are digested via Kjeldahl digestion to hydrolyze phosphorus to orthophosphate. Orthophosphate reacts with molybdenum (VI) and antimony (III) in an acidic medium to form an antimony-phosphomolybdate complex. This complex is subsequently reduced with ascorbic acid to form a blue color, and the absorbance is measured at 880 nm (Reference 15.4).
- 2.2 The quality of the analysis is assured through reproducible calibration and testing of the Flow Injection Analysis (FIA) system.
- 2.3 A general flow diagram of the FIA system is shown below (see Section 17.0 for a detailed flow diagram).



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

4.0 Interferences

- 4.1 Ferric iron of 50 mg/L or less, copper of 10 mg/L or less, and silica of 10 mg/L or less do not interfere (Reference 15.4).
- 4.2 Turbid samples may interfere with the photometric detector's ability to measure the true absorbance of the sample. Turbid samples should be filtered prior to analysis to reduce the possibility of such bias.
- 4.3 Samples with background absorbance at the analytical wavelength may interfere.

5.0 Safety

- 5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.
- 5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.
 - 5.3.1 Acetone, C_3H_6O
 - 5.3.2 Ammonium Molybd Tetetrahydrate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
 - 5.3.3 Antimony Potassium Tartrate Hemihydrate, $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$
 - 5.3.4 Ascorbic Acid, $C_6H_8O_6$
 - 5.3.5 Chloroform, $CHCl_3$
 - 5.3.6 Potassium Sulfate, K_2SO_4
 - 5.3.7 Red Mercuric Oxide, HgO
 - 5.3.8 Sulfuric Acid, concentrated, H_2SO_4
- 5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

- 5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.
- 5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

- 6.1 Flow Injection Analysis (FIA) System (OI Analytical Flow Solution 3000) consisting of the following:
- 6.1.1 120-Place Autosampler
 - 6.1.2 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 660-nm optical filter
 - 6.1.3 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software
 - 6.1.4 Orthophosphate/Total Phosphorus, USEPA Cartridge (OI Analytical Part #A001558)
- 6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.
- 6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

7.1 Raw Materials

- 7.1.1 Acetone, C_3H_6O
- 7.1.2 Ammonium Molybdate Tetrahydrate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
- 7.1.3 Antimony Potassium Tartrate Hemihydrate $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$
- 7.1.4 Ascorbic Acid, $C_6H_8O_6$
- 7.1.5 Chloroform, $CHCl_3$
- 7.1.6 Deionized Water, ASTM Type I or II
- 7.1.7 Dowfax® 2A1, Part #A000080
- 7.1.8 Potassium Dihydrogen Phosphate, KH_2PO_4

7.1.9 Potassium Sulfate, K_2SO_4

7.1.10 Red Mercuric Oxide, HgO

7.1.11 Sodium Chloride, $NaCl$

7.1.12 Sulfuric Acid, concentrated, H_2SO_4

7.2 Reagent Preparation

Note: For best results, filter and degas all reagents prior to use.

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparation of degassed reagent water, protect it from reabsorption of atmospheric gases by storing it in a tightly sealed container. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Digestion Reagents

7.2.2.1 1:5 Sulfuric Acid Solution (50 mL)—Cautiously add 10 mL of concentrated sulfuric acid to 40 mL of deionized water while stirring.

Note: The dilution of sulfuric acid in water generates a great amount of heat. Take appropriate precautions.

7.2.2.2 Mercuric Sulfate Solution (100 mL)

7.2.2.2.1 Dissolve 8 g of red mercuric oxide in 50 mL of 1:5 H_2SO_4 .

7.2.2.2.2 Dilute to 100 mL with deionized water in a volumetric flask. Mix well.

Warning: Mercury compounds are highly toxic. Take appropriate precautions.

7.2.2.3 Digestion Solution (1 L)

7.2.2.3.1 Carefully add 200 mL of sulfuric acid to about 700 mL of deionized water.

7.2.2.3.2 Add 133 g of potassium sulfate and stir to dissolve.

7.2.2.3.3 Add 25 mL of mercuric sulfate solution (Section 7.2.2.2).

7.2.2.3.4 Allow to cool.

7.2.2.3.5 Transfer the mixture to a 1-L volumetric flask and dilute to volume with deionized water and mix well.

Note: The dilution of sulfuric acid in water generates a great amount of heat. Take appropriate precautions.

7.2.3 Colorimetric Analysis Reagents

7.2.3.1 Dowfax Start-up Solution (1 L)

7.2.3.1.1 To a 1-L volumetric flask containing 800 mL deionized water, add 2 mL of Dowfax 2A1.

7.2.3.1.2 Dilute to 1,000 mL with deionized water.

7.2.3.2 4% Reagent Sulfuric Acid Solution (2 L)

7.2.3.2.1 Cautiously add 80 mL of concentrated sulfuric acid while stirring to 1,500 mL of deionized water in a 2 L volumetric flask.

Warning: The dilution of sulfuric acid in water generates a great amount of heat. Take appropriate precautions.

7.2.3.2.2 Cool to room temperature and dilute to 2,000 mL with deionized water.

Note: Prepare this reagent several hours before needed to allow plenty of time to cool.

7.2.3.3 Molybdate/Antimony Solution (1 L)

7.2.3.3.1 Dissolve 8 g of ammonium molybdate and 0.2 g of antimony potassium tartrate in 800 mL of deionized water in a 1-L volumetric flask.

7.2.3.3.2 Dilute to 1 L with deionized water.

7.2.3.4 Ascorbic Acid Solution (1 L)

7.2.3.4.1 Dissolve 60 g of ascorbic acid in 600 mL of deionized water in a 1-L volumetric flask.

7.2.3.4.2 Add 2 mL of acetone and dilute 1,000 mL with deionized water.

7.2.3.4.3 Store at 4°C in a polyethylene bottle.

7.2.3.5 Sodium Chloride Diluent (1 L)

7.2.3.5.1 Dissolve 15 g of sodium chloride in 600 mL of deionized water in a 1-L volumetric flask.

7.2.3.5.2 Dilute to 1,000 mL with deionized water.

7.2.3.5.3 Add 2 mL of Dowfax 2A1 to 1 L of solution.

7.2.3.6 Carrier/Sampler Wash, 4% H_2SO_4 (1 L)—4% sulfuric acid solution (Section 7.2.3.2).

Note: If the acid content of the digestates is different than 4%, prepare the sampler wash and the undigested working calibrants at that concentration.

7.3 Calibrant Preparation

7.3.1 Stock 1,000 mg/L Phosphorus (1 L)

7.3.1.1 Dissolve 4.393 g of dry potassium dihydrogen phosphate in approximately 800 mL of deionized water in a 1-L volumetric flask.

7.3.1.2 Dilute the solution to 1,000 mL with deionized water.

7.3.1.3 Mix well.

7.3.2 Intermediate Calibrant 100 mg/L Phosphorus (100 mL)

7.3.2.1 Use a volumetric pipet to add 10 mL of stock calibrant to approximately 80 mL of deionized water in a 100 mL volumetric flask.

7.3.2.2 Dilute the solution to 100 mL with deionized water and mix well. Prepare the intermediate calibrant daily.

7.3.3 Working Calibrants (100 mL)

7.3.3.1 Add the designated volumes of stock calibrant (see Equation 1) to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of deionized water.

7.3.3.2 Dilute each solution to the mark with deionized water and mix well.

EQUATION 1

$$C_1 V_1 = C_2 V_2$$

Where:

C_1 = Concentration (in mg/L) of stock solution (or calibrant)

V_1 = Volume (in L) of stock solution (or calibrant) to be used

C_2 = Desired concentration (in mg/L) of working calibrant to be prepared

V_2 = Final volume (in L) of working calibrant to be prepared

By solving this equation for the volume of stock solution to be used (V_1), the following equation is obtained:

$$V_1 = \frac{C_2 V_2}{C_1}$$

Since the desired concentration (C_2), the final volume (V_2), and the concentration of the stock solution (C_1) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_1) is easily calculated.

7.3.4 Calibrants covering the entire range of this analysis can be prepared from the following table.

Note: This table assumes use of a 100- μ L injection loop.

Final Concentration (mg/L)	Vol. of Inter. Cal. (μ L)	Conc. of Inter. Cal. (mg/L)	Final Volume (mL)
0.02	20	100	100
0.10	100	100	100
0.50	500	100	100
1.00	1,000	100	100
5.00	500	1,000	100
10.00	1,000	1,000	100
20.00	2,000	1,000	100

8.0 Sample Collection, Handling, and Preservation

8.1 Samples should be collected in plastic or glass bottles that have been thoroughly cleaned and rinsed with reagent water (Section 7.2.1).

8.2 The volume of sample collected should be sufficient to insure that a representative sample is obtained, replicate analysis is possible, and waste disposal is minimized.

- 8.3 Determine total phosphorus in unpreserved samples immediately.
- 8.4 Sample analysis should be performed as soon as possible to eliminate loss of analyte. Filter turbid samples prior to analysis. Should storage be required, preserve samples by adding 2 mL of concentrated sulfuric acid per liter of sample and cool immediately. Holding time for acid preserved samples is 28 days from the time of collection (Reference 15.5).

9.0 Quality Control

- 9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.2). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.
- 9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.
- 9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.
- 9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.2. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.
- 9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.
- 9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.
- 9.1.2.2.2 A narrative stating the reason(s) for the modification.
- 9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

- a) calibration (Section 10.4)
- b) calibration verification (Section 9.5)
- c) initial precision and recovery (Section 9.2.2)
- d) analysis of blanks (Section 9.4)
- e) ongoing precision and recovery (Section 9.6)
- f) matrix spike and matrix spike duplicate (Section 9.3)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

- a) sample numbers and other identifiers
- b) analysis dates and times
- c) analysis sequence/run chronology
- d) sample weight or volume
- e) sample volume prior to each cleanup step, if applicable
- f) sample volume after each cleanup step, if applicable
- g) final sample volume prior to injection
- h) injection volume
- i) dilution data, differentiating between dilution of a sample or modified sample
- j) instrument and operating conditions
- k) other operating conditions
- l) detector
- m) printer tapes, disks, and other recording of raw data
- n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

- 9.1.4 Analyses of laboratory reagent blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of an LRB are described in Section 9.4.
- 9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical system is in control. This procedure is described in Section 9.6.
- 9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.
- 9.1.7 Accompanying QC for the determination of total phosphorus is required per analytical batch. An analytical batch is a set of samples analyzed at the same time to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory reagent blank (LRB, Section 9.4), a laboratory control sample (LCS, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial Demonstration of Laboratory Capability

- 9.2.1 Method Detection Limit (MDL)—To establish the ability to detect total phosphorus at low levels, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (Reference 15.1) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 must be achieved prior to practice of this method.
- 9.2.2 Initial Precision and Recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:
 - 9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.
 - 9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery (\bar{x}) and the standard deviation of the percent recovery (s) for total phosphorus. Use Equation 2 for the calculation of the standard deviation of the percent recovery(s).

EQUATION 2

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where:

s = Standard deviation

n = Number of samples

x = Percent recovery in each sample

- 9.2.2.3 Compare *s* and *x* with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of *s* exceeds the precision limit or the value of *x* falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—The laboratory shall spike, in duplicate, a minimum of 10 percent of all samples (one sample in duplicate in each batch of ten samples) from a given sampling site.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of total phosphorus in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of total phosphorus in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of total phosphorus.

9.3.2.1 Spike this sample with the amount of total phosphorus stock solution (Section 7.3.1 or 7.3.2) to produce an orthophosphate concentration in the sample of 5 mg/L. If necessary, prepare another stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of total phosphorus (Section 9.3.1).

- 9.3.2.2 Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking.
- 9.3.3 Calculate the percent recovery of total phosphorus in each aliquot using Equation 3.

EQUATION 3

$$P = \frac{A - B}{T} \times 100$$

Where:

P = Percent recovery

A = Measured concentration of total phosphorus after spiking (Section 9.3.2.2)

B = Measured background concentration of total phosphorus (Section 9.3.2)

T = True concentration of the spike

- 9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.
- 9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch must be reanalyzed.
- 9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 4.

EQUATION 4

$$RPD = \left[\frac{|D_1 - D_2|}{(D_1 + D_2)/2} \right] \times 100$$

Where:

RPD = Relative percent difference

*D*₁ = Concentration of nitrate and/or nitrite in the spiked sample

*D*₂ = Concentration of nitrate and/or nitrite in the spiked duplicate sample

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- 9.3.7 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be reanalyzed.
- 9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (*P_a*) and the standard deviation of the percent recovery (*s_p*). Express the accuracy assessment as a percent recovery interval from *P_a* - 2*s_p* to *P_a* + 2*s_p*. For example, if *P_a* = 90% and *s_p* = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).
- 9.4 Laboratory Reagent Blanks (LRB)—Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.
- 9.4.1 Analyze an LRB initially (i.e., with the tests in Section 9.2) and with each analytical batch. The LRB must be subjected to the exact same procedural steps as a sample.
- 9.4.2 If total phosphorus is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB shows no evidence of contamination.
- 9.5 Calibration Verification—Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the mid-range calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).
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- 9.6 Laboratory Control Sample (LCS)—To demonstrate that the analytical system is in control and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:
- 9.6.1 Analyze an LCS with each analytical batch according to the procedure in Section 10.0.
 - 9.6.2 If the precision and recovery for the LCS are within the acceptance criteria specified in Section 13.0, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.
 - 9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for total phosphorus by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85% to 105%.
- 9.7 Reference Sample—To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institutes of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Configuration and Start-up

10.1 Instrument Configuration

- 10.1.1 Configure the OI Analytical Flow Solution 3000 Analyzer according to the Operator's Manual and verify that each module is properly powered on.
- 10.1.2 Verify that the Orthophosphate/Total Phosphorus, USEPA Cartridge (Part #A001558) is configured as illustrated in the flow diagram shown in Section 17.0.
- 10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

- 10.2.1 Connect the reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2). Start the pump at low speed, allowing the start-up solution to flow through the entire system.
- 10.2.2 Make sure that the flowcell of each detector is purged of all bubbles and the flow is stable and free from surging.
- 10.2.3 Once a stable flow is achieved, connect the reagent pump tubes to their appropriate reagent bottles. Allow these reagents to flow through the entire system, then, once again, verify that the flowcell of each detector is purged of all bubbles.

10.3 Baseline Verification

- 10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.7) for help on creating a Method.
- 10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected total phosphorus concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.7).
- 10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Verify that the baseline does not drift and is free from large fluctuations. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

- 10.4.1 Prepare a series of at least three working calibrants using the total phosphorus stock solutions (Section 7.3.1) according to Equation 1, covering the desired analysis range.
- 10.4.2 Place the calibrants in the autosampler in order of decreasing concentration and analyze each calibrant according to Section 11.0. A calibration curve will be calculated by the WinFLOW software.
- 10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding "true" concentration.
- 10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.6). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Digestion

- 11.1.1 Prepare blanks, calibrants, samples, and QC samples:

Note: The final diluted volume after digestion will be 25 mL.

- 11.1.1.1 For blanks, add 25 mL of deionized water to a digestion tube.
- 11.1.1.2 For calibrants, pipette 25 mL of stock or intermediate calibrant to each tube and rinse it down the side well with deionized water to an approximate total volume of 25 mL.
- 11.1.1.3 For samples, add exactly 25 mL to the tube. If samples are known to be out of range, smaller volumes can be used and the result multiplied by the appropriate dilution factor.

11.1.1.3.1 For example, 10 mL of sample is digested. After digestion and analysis, multiply the result by 2.5.

11.1.1.3.2 For example, 5 mL of sample is digested. After digestion and analysis, multiply the result by 5.

11.1.1.4 For QC samples, prepare the sample according to its instructions and add exactly 25 mL to the tube.

11.1.2 Add 5 mL of digestion solution (Section 7.2.2.3) to each tube and mix all tubes well.

Note: The 5 mL of digestion reagent should be measured accurately to help ensure uniform acid content.

11.1.3 Add two glass boiling rods or a few glass boiling stones to each tube to aid in consistent boiling between tubes.

11.1.4 Place the tubes on the block digester and set the temperature for 160°C. The purpose of this step is to reduce the volume of liquid in the tube before increasing the temperature for digestion. Allow the volume to be reduced to about 5 mL in each tube (approximately 1 hour).

Note: If at any time during the digestion, any amount of liquid boils out of a tube the result will be in error.

11.1.5 Increase the temperature to 380°C for two and one-half hours. When the digestion is complete, the final volume will be about 2–3 mL and the liquid will be clear or straw colored.

Note: If an exhaust system or scrubber is placed on top of the digestion, care must be taken not to draw off significant amounts of the fumes during the high temperature stage. Variable acid contents may result. The digestion can be carried out without an exhaust system if the digestion apparatus is placed in a properly ventilated fume hood.

11.1.6 Remove the tubes from the block and allow to cool. It is preferable not to allow the digestate to crystallize while cooling. A few milliliters of deionized water can be added and mixed with the digestate after cooling the tubes for a few minutes to alleviate the crystallization.

Note: If digests are to be used for TKN determination as well as the total phosphorus determination, ammonia-free water should be used for all dilutions.

Note: If the water is added too soon and the acid is still too hot, the contents of the tube may react vigorously and some material may be lost from the tube.

11.1.7 Dilute the digestate to 25 mL with deionized water. The digestates must be accurately diluted to a known volume in a calibrated container. This is important to help ensure a consistent acid content in the final solutions that will affect the accuracy of the measurement. If the contents of the tube are transferred to a separate container during this step, ensure that all of the digestate is transferred.

11.2 Analysis

- 11.2.1 Begin pump flow with the start-up solution (Section 7.2). Verify a stable baseline.
- 11.2.2 Place all reagents on-line. Pump 5 to 10 minutes. Obtain a stable baseline at 880 nm and autozero before the start of sample run.
- 11.2.3 Load the sampler tray with calibrants, blanks, samples, and QC samples. The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.
- 11.2.4 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the “Fast Forward” button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the sample table.
- 11.2.5 When analysis is complete, pump start-up solution through the system and stop the pump. Release the tension on all pump tubes and power off the system.

11.3 Operating Notes

11.3.1 Reagents

- 11.3.1.1 Ascorbic Acid Stock Solution—Check color of the ascorbic acid reagent. It should be clear liquid, colorless to light amber in color; a darker color indicates poor reagent quality or aged solution. Either remake solution or change to new source of ascorbic acid.
- 11.3.1.2 Sodium Chloride—Do not refrigerate this reagent or the sodium chloride will precipitate out of solution.
- 11.3.1.3 4% Sulfuric Acid—The sampler wash, carrier, and reagent sulfuric acid solution should be made up as a single lot. When samples are digested prior to analysis deionized water blanks should also be digested. If acid concentration in digested samples differ from this method, match the sample’s acidity in the standards, blanks, and sampler wash. Do not change the reagent sulfuric acid concentration.
- 11.3.2 Problems can be isolated to either the cartridge or the digestion by preparing undigested calibrants in 4% acid solution and then running them.
- 11.3.3 If a high background or blank values are observed, carefully evaluate the source of water, cleaning procedure for the digestion glassware, and laboratory environment.
- 11.3.4 When starting up, let system run on start-up solution (Section 7.2) for 10–15 minutes. Add the color reagent and wait for a stable baseline. The pH from the flowcell waste line, should approximate pH 1 when checked with pH paper.
- 11.3.5 If the baseline is noisy:
 - 11.3.5.1 Check the color of ascorbic acid stock solution (Section 11.3.1.1).

- 11.3.5.2 Reagents should be filtered prior to use through a 0.45- μ m membrane filter.
- 11.3.5.3 Acid wash sample cups, reagent bottles, and wash reservoir with 0.1 N HCl before use. Follow with a deionized water rinse.
- 11.3.5.4 Detach flowcell from debubbler and flush the flowcell forcefully with surfactant solution.
- 11.3.6 Carryover can be solved by washing out entire system with a 1.0 N sodium hydroxide solution for 5 minutes. For consistent results, flush system out every day before use and whenever needed. For consistent carryover problems increase the sampler wash time.
- 11.3.7 Abnormal or short peaks:
 - 11.3.7.1 Verify that the solution in the pump tubes is flowing freely and consistently.
 - 11.3.7.2 Verify that the heater is set to 37°C.
- 11.3.8 Frequently occurring air spikes:
 - 11.3.8.1 Clean system out with 1.0 N sodium hydroxide.
 - 11.3.8.2 Using a luer, union, and syringe, clean the flowcell with surfactant solution.
 - 11.3.8.3 Make sure the WHT/WHT Teflon® back pressure coil is installed after the detector outlet tubing.
- 11.3.9 Clear persistent air bubbles in the flowcell by creating back pressure. Pinch off the waste line out of the flowcell for several seconds, then quickly release the line. Repeat if necessary.
- 11.3.10 To prolong usage of pump tubes, be sure to release tension on the platens and flush with deionized water. For long term storage, remove pump tubes from platens.
- 11.3.11 Especially for low ranges, all glassware must be extremely clean. Avoid phosphate based cleaning materials of any kind. Acid rinse all glassware with 1–2 N HCl followed by deionized water before use. For thorough cleaning, soak in 6 N HCl overnight, then rinse thoroughly with deionized water.

12.0 Data Analysis and Calculations

- 12.1 The calibration curve allows for accurate quantitation of the total phosphorus concentration in each sample.
- 12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

13.0 Method Performance

Range:	0.02–20.0 mg/L P
Throughput:	48 samples/hour
Precision:	
0.1 mg/L	<1% RSD
4.0 mg/L	<1% RSD
Method Detection Limit (MDL):	0.005 mg/L P
Percent Recovery:	90–110%

14.0 Pollution Prevention and Waste Management

- 14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.
- 14.2 For further information on waste management, consult Section 13.6 of *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 15.3).

15.0 References

- 15.1 *Code of Federal Regulations*, Part 136, Title 40, Appendix B, 1994.
- 15.2 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*; EPA-600/4-79-019; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.
- 15.3 *Less is Better: Laboratory Chemical Management for Waste Reduction*. Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.
- 15.4 Phosphorus, All Forms. *Methods for Chemical Analysis of Water and Wastewater*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 365.1.
- 15.5 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii.
- 15.6 *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; American Public Health Association: Washington, D.C., 1989; 4–178.
- 15.7 WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

16.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

°C	degrees Celsius
%	percent
±	plus or minus
≥	greater than or equal to

16.1.2 Alphabetical characters

g	gram
L	liter
mg	milligram
mg/L	milligram per liter
µg	microgram
µg/L	microgram per liter
mL	milliliter
ppm	parts per million
ppb	parts per billion
M	molar solution
N	normal solution

16.1 Calibration Blank—A 100-mL volume of CO₂-free reagent water analyzed using the FIA procedure.

16.2 Calibration Standard (CAL)—A solution prepared from the dilution of stock standard solutions. A 100-mL aliquot of each of the CALs are subjected to the analysis procedure. The resulting observations are used to calibrate the instrument response with respect to the analyte concentration.

16.3 Initial Precision and Recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

16.4 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.5 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix that is treated like a sample, including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

- 16.6 **Matrix Spike/Matrix Spike Duplicate (MS/MSD)**—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. The MS/MSD is analyzed like a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot, and the measured values in the MS/MSD must be corrected for the background concentration.
- 16.7 **Minimum Level (ML)**—The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.
- 16.8 **Ongoing Precision and Recovery (OPR)**—See Section 16.4, “Laboratory Control Sample.”

17.0 Figures

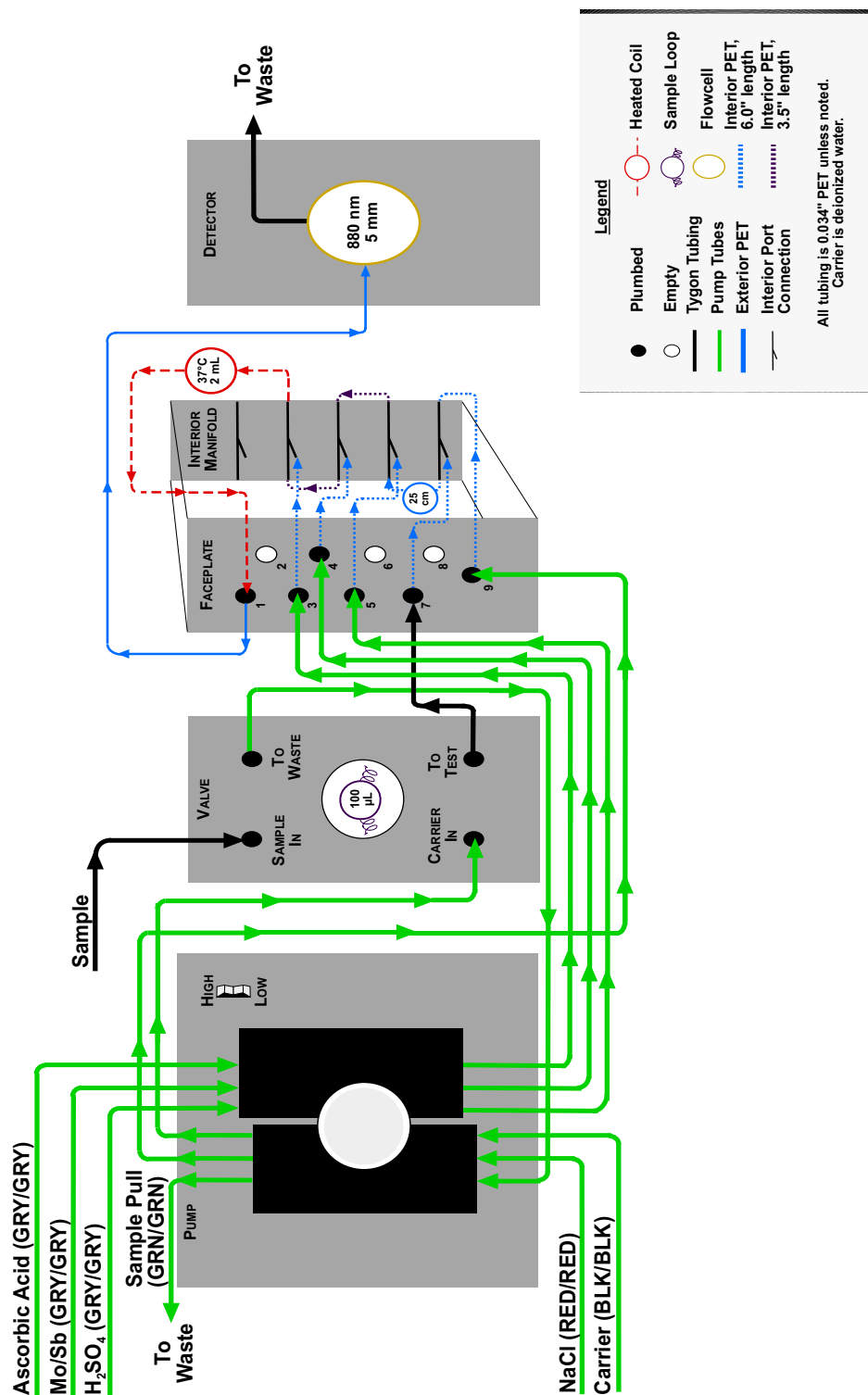


Figure 1. Detailed Flow Diagram for Total Phosphorus by FIA on a Flow Solution 3000, Cartridge Part #A001558

Results were obtained under optimal operating conditions. Actual results may vary depending on sample introduction, cleanliness of sample containers, reagent purity, operator skill, and maintenance of instruments.

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