METHOD 14XXXP

Phosphorus by Ascorbic Acid Reaction and Photometry

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Acknowledgments

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Disclaimer

This method has been submitted to the U.S. Environmental Protection Agency for use in EPA's water programs but has not been approved for use by EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Introduction

This method consists of convenient, ready to use cell test kits for determination of orthophosphate as phosphorus and can also determine total phosphate levels, when the digestion procedure is applied. This method is based on „Chemical Analysis of Water and Wastewater," EPA methods 365.2 and 365.3, and „Standard Methods for the Examination of Water and Wastewater", 18th edition, 4500-P E. The test kit is suitable for both on-site testing and typical laboratory testing. The test kit consists of pre-measured reagents for both sample preparation and analytical determinations. This method's approach with pre-measured reagents reduces analytical errors, the amount of hazardous waste, and increases occupational safety.

This method incorporates two measuring ranges, capable of analyzing total phosphorus (after digestion) in the range of 0.05 to 25 mg/L. This value can be converted to calculated orthophosphate phosphorus in the range 0.5 to 75.0 mg/L. This broad range can help to reduce the amount of sample manipulation, especially in field applications.

Method 14XXXP

Phosphorus by Ascorbic Acid Reaction and Photometry

1.0 Scope and Application

1.1 This method determines the level of orthophosphate ($O\text{-}PO_4$) in waters and wastewater matrices. In a solution acidified with sulfuric acid, orthophosphate reacts with molybdo-phosphoric acid. Ascorbic acid reduces this to blue phospho-molybdenum blue (PMB), which is then determined photometrically at or near 690 nm. The $O\text{-}PO_4$ result is expressed as mg phosphorus (P)/L, but can be converted to mg $O\text{-}PO_4$-P/L.

1.2 This method is based on prior Environmental Protection Agency (EPA) and association methods for determination of phosphorus (P) (Reference 16.1 and 16.2).

1.3 This method is for use in the United States Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.

1.4 This method is intended for the analysis of P on drinking water and surface waters, ground water, seawater, industrial wastes, and other wastewater matrices.

1.5 The method detection limit (MDL; 40 CFR 136, Appendix B) has been estimated at 0.02 mg P/L.

1.6 The minimum level (ML) for reporting results is 0.05 mg P/L (Section 13.3).

1.7 This method is capable of measuring P in the range of 0.05 to 25 mg/L, and may be extended to higher levels by serial dilution. This range translates into $O\text{-}PO_4$-P concentration range of 0.50 - 75 mg/L.

1.8 Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

2.0 Summary of Method

2.1 Dependent upon the concentration range desired, a one or five-ml aliquot of preserved and appropriately pre-treated sample is measured into a Spectroquant® Phosphate Cell, which contains a pre-measured amount of sulfuric acid. For the determination of total P, a digestion reagent is added (Spectroquant® Reagent P-1K, potassium peroxidisulfate-nitrate solid mixture). The total P cells are digested for 30 minutes at 120°C.

2.2 The next steps of the procedure are the same for total and ortho-phosphorus determinations. The reagents for color formation, ammonium heptamolybdate tetrahydrate-potassium antimony (III) oxide tartrate hemihydrate-sulfuric acid solution and ascorbic acid (Spectroquant® Reagents P-2K and P-3K) are then added.

2.3 The blue complex which is formed, phosphomolybdenum blue (PMB), is determined photometrically at a wavelength at or near 690 nm

2.4 The photometric determination can be conducted on either a Merck Spectroquant® system photometer (References 16.3, 16.4, 16.5 and 16.6), or other photometric device.

2.5 Quality is assured through the use of quality control samples (QCS), calibration of the instrumentation by using calibration test solutions (Section 17, Table 2), and operation of a formal quality assurance program (Reference 16.7).

3.0 Definitions

Definitions for terms used in this method are given in the glossary at the end of the method (Section 18).

4.0 Interferences

4.1 Color and suspended matter may interfere with the photometric measurement. To counter this potential positive interference, prepare a sample blank by adding all reagents except P-2K and P-3K. Samples may also be filtered (after digestion).

4.2 Arsenic acid (AsO_4^{2-}) in concentrations > 0.2 mg/L will react with the molybdate reagent to produce a blue color similar that formed with the phosphate determination (Reference 16.8).

4.3 Sulfide (S^{2-}) in concentrations > 2.5 mg/L will interfere (Reference 16.8).

4.4 Chromate ($Cr_2O_7^{2-}$) in concentrations > 5 mg/L will interfere (Reference 16.8).

5.0 Safety

5.1 This method does not address all safety issues associated with its use. The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical and environmental sample should be regarded as a potential health hazard and exposure should be minimized. 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. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 16.9 and 16.10.

5.2 The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. It is suggested that the laboratory perform personal hygiene monitoring of each analyst using this method and that the results of this monitoring shall be made available to the analyst.

5.3 Samples of unknown origin may possess potentially hazardous compounds. Samples should be handled with care (e.g., under a hood), so as to minimize exposure.

5.4 As samples of unknown origin may contain compounds which could react violently with the reagents, pipette the sample into the cell under a hood, and direct the opening of the cell away from anyone in the area.

5.5 Operate the thermoreactor with the safety shield in place (or behind a suitable shield) in case of severe reaction of sample and reagents, which could result in leakage.

5.6 The Spectroquant® Phosphate Cell Tests are extremely hot after digestion, handle with care when transferring them to the cooling rack. Hold the cells by the cap, or using a test tube holder.

5.7 This method employs the use of Spectroquant® Phosphate Cell Tests containing pre-measured reagents, which limits the handling of hazardous chemicals.

6.0 Equipment and Supplies

NOTE: *Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample collection bottles-1-L borosilicate amber glass or plastic.

NOTE: *Contamination from phosphorus on glassware, usually from commercial detergents, should be avoided. All glassware used should be washed with hot 1:1 HCl and rinsed with deionized water. Treat the glassware all the reagents to remove any remaining traces of phosphorus on the surfaces of the glassware. For best results, consider dedicating glassware for the determination of phosphorus. Cover glassware until it is time for use.*

6.2 Filtration device, acid washed

6.3 Membrane filters, 0.45 µm-pre-wash by soaking in deionized water (e.g. 50 filters in 1L of deionized water for 24 hours).

6.4 Volumetric flasks-various sizes, acid washed.

6.5 Volumetric pipettes-various sizes, acid washed.

6.6 Reaction cells-Spectroquant® Phosphate Cell Test (Section 17.0, Table 1).

6.7 Block digester-capable of maintaining constant temperatures of 120□C- Spectroquant® Thermoreactor, or equivalent.

6.8 Laboratory timer.

6.9 Rack for cells.

6.10 Dry cloths for cleaning reaction cells.

6.11 Photometric device.

6.11.1 Photometer capable of measuring absorbance at or near a wavelength of 690 nm, and with cell compartment for tubes 16 x 100-mm-Merck Spectroquant® system photometer, or equivalent.

6.11.2 Spectrophotometer for use at 690-nm wavelength, with cell compartment for tubes 16 x 100 mm.

7.0 Reagents and Standards

7.1 Deionized water

7.2 Sulfuric acid (H_2SO_4)-conc., ACS grade.

7.3 Sulfuric acid (H_2SO_4)-0.1 N.

7.4 Sodium Hydroxide (NaOH)-0.1 N.

7.5 Spectroquant® Phosphate Cell Test (Spectroquant® Item 14729 or 14543) appropriate to the concentration range selected (Section 17.0, Table 1)-contains sulfuric acid.

7.6 Potassium peroxidisulfate and sodium nitrate-Spectroquant® Reagent P-1K.

7.7 Ammonium heptamolybdate tetrahydrate-potassium antimony (III) oxide tartrate hemihydrate-sulfuric acid solution-Spectroquant® Reagent P-2K.

7.8 Ascorbic acid-Spectroquant® Reagent P-3K.

7.9 Phosphate stock solution (1,000 mg/L)-Spectroquant® Item 19898, or equivalent.

7.10 Phosphate std solution (100 mg/L)-dilute 100 ml of the Phosphate stock solution in a 1-L volumetric flask with deionized water.

8.0 Sample Collection, Preservation, and Storage

8.1 Collect approximately 1-L, or a minimum of 100 ml, of a representative sample in a new plastic or glass bottle, following conventional sampling techniques (Reference 16.11).

8.2 Filter samples for dissolved PO_4^{3-} immediately upon sampling.

8.3 Analyze the samples for PO_4^{3-} within 48 hours, and samples for total P within 28 days of collection**.**

8.4 Preserve samples for total P with H_2SO_4 to a sample pH <2.

8.5 Refrigerate samples at 0 to 4□C from the time of collection until the time of analysis, 40 CFR 136, Table II.

8.6 Collect an additional two aliquots of a sample for each batch (of at least 20 samples) for the matrix spike and matrix spike duplicate.

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program (Reference 16.3). The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the ongoing analysis of laboratory reagent blanks, precision and recovery standards, and matrix-spiked samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data thus generated. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 Analysis of matrix spike and matrix spike duplicate (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.

9.1.3 Analyses of laboratory blanks is required to demonstrate freedom from contamination. The procedure and criteria for blank analyses is described in Section 9.4.

9.1.4 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control. These procedures are described in Sections 9.5 and 9.6.

9.1.5 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.7 and 9.6.3.

9.1.6 Accompanying QC for the determination of P is required per analytical batch. An analytical batch is a set of samples analyzed, to a maximum of 20 samples. Each analytical batch, of up to 20 samples, must be accompanied by a laboratory blank (Section 9.4), and ongoing precision and recovery sample (OPR, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3).

9.2 Initial demonstration of laboratory capability-The initial demonstration of laboratory capability is used to characterize laboratory performance and method detection limits.

9.2.1 Method detection limit (MDL)-The method detection limit must be established for the analyte, using a Phosphate std solution (Sections 7.10). To determine MDL values, take seven replicate aliquots of the diluted Phosphate std solution and process each aliquot through each step of the analytical method. Perform all calculations and report the concentration values in the appropriate units. MDLs should be determined every year or whenever a modification to the method or analytical system is made that will affect the method detection limit.

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 solution (Section 7.10) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the four analyses, compute the average percent recovery (x) and the standard deviation (s, Equation 1) of the percent recovery for P.

Equation 1

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

Where:

n = number of samples

x = % recovery in each sample

s = standard deviation

9.2.2.3 Compare s and x with the corresponding limits for initial precision and recovery in Section 17, Table 3 (Reference 16.12), which lists EPA's proposed standardized QC and QC Acceptance Criteria for Methods in 40 CFR Part 136, Table IB. If s and x meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the precision limit or x falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem, and repeat the test.

9.3 Matrix Spikes-The laboratory must spike, in duplicate, a minimum of five percent of all samples (one sample in each batch of 20 samples). The two sample aliquots shall be spiked with the Phosphate std solution (Section 7.10).

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 P in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 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 P in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard-Phosphate std solution (Section 7.10), or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2 Analyze one sample aliquot out of each set of 20 samples, according to the procedure beginning in Section 11, to determine the background concentration (B) of P.

9.3.2.1 If necessary, prepare a standard solution appropriate to produce a level in

the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per 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 (A).

9.3.3 Calculate the percent recovery (P) of P in each aliquot using the following equation:

Equation 2

$$PO4 - P = 100 * \frac{(A - B)}{T}$$

where:

P=Percent recovery

A=Measured concentration of PO4-P after spiking

B=Measured concentration of PO4-P before spiking

T=True concentration of the spike

9.3.4 Compare the percent recovery of the P with the corresponding QC acceptance criteria in Section 17, Table 3 (Reference 16.12), which lists EPA's standardized QC and QC Acceptance Criteria for Methods in 40 CFR Part 136, Table IB.

9.3.4.1 If the results of the spike fail the acceptance criteria, and the recovery of the Phosphate std solution in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria in Section 17, Table 3 (Reference 16.12), an interference is present. In this case, the result may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method repeat the tests required in Section 9.1.2, and repeat the analysis of the sample and the MS/MSD.

9.3.4.2 If the results of both the spike and the ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample re-analyzed.

9.3.5 Compute relative percent difference (RPD) between the two results (not between the two recoveries) using the following equation:

Equation 3

$$RPD = 100 * \frac{(|D_1 - D_2|)}{(D_1 + D_2)/2}$$

where:

RPD=Relative percent different

D_1=Concentration of PO4-P in the sample

D_2=Concentration of PO4-P in the second (duplicate) sample

9.3.6 The relative percent difference for duplicates shall meet the acceptance criteria in Section 17, Table 3 (Reference 16.12). If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected, and the analytical batch re-analyzed.

9.3.7 As a 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-2s_p to P_a+2s_p. For example, if P_a = 90% and s_p = 10% for five analyses of P, 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 blanks-Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Prepare and analyze a laboratory blank initially (i.e., with the tests in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.

9.4.2 If material is detected in the blank at a concentration greater than the ML (Section 1.6), analysis of samples must be halted until the source of contamination is eliminated and a new blank shows no evidence of contamination. All samples must be associated with an uncontaminated laboratory blank before the results may be reported for regulatory compliance purposes.

9.5 Calibration verification-Verify calibration of the photometric device per Section 10 for each analytical batch of up to 20 samples. If calibration curve linearity differs more than 25%, run a new calibration curve.

9.6 Ongoing Precision and Recovery (OPR)-To demonstrate that the analysis 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 a precision and recovery standard (Sections 7.10) with each analytical batch according to the procedure beginning in Section 11.

9.6.2 Compare the concentration with the limits for ongoing precision and recovery in Section 17, Table 3 (Reference 16.12). If the concentration is in the range specified, the analysis may proceed. If however, the concentration is not in the specified range, the analytical process is not in control. In this event, correct the problem, repeat the analytical batch, and repeat the ongoing precision and recovery test.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous OPR 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 each analyte 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 Quality control sample (QCS)–It is suggested that the laboratory obtain a quality control sample from a source different from the source of the Phosphate std solution used routinely in this method (Section 7.10).

9.8 The standards used for initial precision and recovery (IPR, Section 9.2.2) matrix spikes (MS/MSD, Section 9.3), and ongoing precision and recovery (OPR, Section 9.6) should be identical, so that the most precise results will be obtained.

10.0 Calibration and Standardization

10.1 The Merck Spectroquant® system photometer is shipped factory calibrated (Reference 16.13). Refer to the manufacturer's documents (References 16.3, 16.4, 16.5 and 16.6). The calibration curve can be verified, and the data from this verification can be stored, modified or re-entered at anytime. However, the factory program settings cannot be changed by the user. When appropriate, the manufacturer supplies a new microchip (transponder) containing new calibration data.

10.2 For photometric equipment, other than the Merck Spectroquant® system photometer, plot a calibration curve with a minimum of five (5) data points, from standards prepared from a Phosphate std solution appropriate to the range to be tested. The calibration curve should also include a blank.

10.2.1 For Spectroquant® Phosphate Cell Tests, refer to Section 17.0, Table 2, to prepare standard curves from the Phosphate std solution. The curve should include the lowest and highest concentrations for the range tested.

10.3 Verify the curve, using a calibration standard (mid-point of the curve), with each analytical batch of samples (Section 9.5).

10.4 Run a new calibration curve with each new lot of reagents, or when calibration curve linearity differs more than 25%.

11.0 Procedure

11.1 Choose a Spectroquant® Phosphate Cell Test concentration range appropriate for the sample matrix to be tested, using prior knowledge of the particular waste stream. For the list of test ranges, see Section 17, Table 1.

11.2 If necessary, pH adjust the sample with dilute H_2SO_4 or NaOH to pH between 0 and 10.

11.3 Pipette 1 ml of sample into a Spectroquant® Phosphate Cell Test 14729 or 5 ml of sample for Spectroquant® Phosphate Cell Test 14543 and mix well. For only PO_4^{3-} determination, proceed to Section 11.5.

11.4 Total P determination in both Spectroquant® Phosphate Cell Test Kits.

11.4.1 Preheat the Spectroquant® Thermoreactor at 120□C setting.

11.4.2 Add one dose of P-1K to the cell containing the sample, close with the screw cap.

11.4.3 Place the prepared Spectroquant® Phosphate Cell Test in the preheated Spectroquant® Thermoreactor, and heat at 120□C for 30 minutes.

11.4.4 After the digestion is complete, remove the cells, and place in a cell rack to cool to room temperature.

11.5 Add five drops of P-2K to each cell and shake vigorously to dissolve the solid material.

11.6 Add one dose of P-3K to each cell using the blue dose-metering cap, close tightly with the screw cap and mix.

11.7 Allow five minutes reaction time.

11.8 Determination using Merck Spectroquant® system photometer.

11.8.1 Switch on the Merck Spectroquant® system photometer as per manufacturer's suggestions for operation (References 16.3, 16.4, 16.5 and 16.6).

11.8.2 Place the Spectroquant® Phosphate Cell Tests into the cell compartment with the vertical line aligned with the notch on the instrument, and push down until the cell clicks into place.

11.8.3 Wait as the Merck Spectroquant® system photometer recognizes the bar code. The Spectroquant® Phosphate Cell Test product information is displayed, and the instrument is automatically set to the appropriate wavelength and measuring parameters (bar code recognition of item number, test range, cell format, wavelength, and calibration data).

11.8.4 Record the displayed result as P in mg/L.

11.9 Determination using absorbance mode of Merck Spectroquant® system photometer, or other photometric devices.

11.9.1 Warm up the instrument as per manufacturer's suggestion for operation.

11.9.2 Set the instrument to a wavelength at or near 690 nm.

11.9.3 Zero the instrument with a reagent water / blank which has been prepared in the same manner as the standards and samples.

11.9.4 Place the cell into the cell compartment/cell holder.

11.9.5 Record the absorbance reading from the instrument.

11.9.6 Plot the absorbance reading on the calibration curve, to obtain the concentration P in mg/L.

12.0 Data Analysis and Calculations

12.1 If no pre-dilution was performed upon the sample, no calculation is necessary.

12.2 If pre-dilution was required, calculate the P (mg/L) as follows:

Equation 4

$$PO4-P(mg/L) = A * \frac{V_2}{V_1}$$

where:

A = *Measured concentration of PO4-P from photometer (mg/L)*

V_1 = *Volume of sample used for dilution (ml)*

V_2 = *Final total volume of diluted sample (ml)*

12.3 Report results to two significant digits for concentrations found above the ML (Section 1.6) in all samples. Report results below the ML as <0.05 mg P/L.

13.0 Method Performance

13.1 This method, as equivalent to EPA method 365.3, (Reference 16.2) should achieve the proposed standardized method performance, as cited in Table 1F of the ATP guidelines (Reference 16.12).

13.2 The method detection limit (MDL) study was performed by a single analyst, and has been established at 0.02 mg P/L.

13.3 The minimum level (ML) has been determined as 0.05 mg P/L.

14.0 Pollution Prevention

14.1 The reagents used in this method pose little threat to the environment, when managed properly.

14.2 Reagents should be ordered consistent with laboratory use, to minimize the amount of expired materials to be disposed.

15.0 Waste Management

15.1 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 restriction. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations.

15.2 For further information on waste management, consult „The Waste Management Manual for Laboratory Personnel" and „Less is Better: Laboratory Chemical Management for Waste Reduction," both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

16.0 References

16.1 „Standard Methods for the Examination of Water and Wastewater," 18th Edition, American Public Health Association, 1015 Fifteenth Street, N.W., Washington, D.C. 20005, Method 4500-P E.

16.2 „Methods for the Chemical Analysis of Water and Wastes," 3rd Edition, Environmental Protection Agency, Environmental Monitoring Systems Laboratory–Cincinnati (EMSL–Ci), Cincinnati, Ohio 45268, EPA–600/4-79-020, Methods 365.2 and 365.3.

16.3 Spectroquant® SQ 118 Manual, Merck KGaA, Frankfurter Strasse 250, Darmstadt 64271, Germany, Release July 1998.

16.4 Spectroquant® NOVA 60 Manual, Merck KGaA, Frankfurter Strasse 250, Darmstadt 64271, Germany, Release July 1998.

16.5 Spectroquant® VEGA 400 Manual, Merck KGaA, Frankfurter Strasse 250, Darmstadt 64271, Germany, Release July 1998.

16.6 Spectroquant® NOVA 30 Manual, Merck KGaA, Frankfurter Strasse 250, Darmstadt 64271, Germany, Release July 1998.

16.7 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL-Ci, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

16.8 Spectroquant® Phosphate Cell Test Product Insert, Item 14543 and 14729, Merck KGaA, Frankfurter Strasse 250, Darmstadt 64271, Germany, Release June 1999.

16.9 „OSHA Safety and Health Standards, General Industry," (29CFR 1910), Occupational Safety and Health Administration, OSHA 2206, revised January 1976.

16.10 „Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

16.11 „Standard Methods for the Examination of Water and Wastewater," 18th Edition, American Public Health Association, 1015 Fifteenth Street, N.W., Washington, D.C. 20005, Method 1060.

16.12 Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water-Draft March 1998, Environmental Protection Agency, Office of Water (4303) Washington, DC 20460.

16.13 „German Standard Methods for the Examination of Water, Wastewater, and Sludge," Deutsches Institut fhr Normung e.V., D-10772, Berlin, DIN Method 38402 Part 51, May 1986.

17.0 Tables

Table 1. Product Range, Number, and Usage Information

Range mg/L	Product Number	Sample Volume (ml)
0.5 - 25 (PO_4^{3-}-P)	14729	1
1.5 - 75 (PO_4^{3-})	14729	1
0.05 - 5.0 (PO_4^{3-}-P)	14543	5
0.2 - 15 (PO_4^{3-})	14543	5

Table 2. Calibration Standard Calibration Preparation

Product # **Range (mg/L)**	**Phosphate std Solution Volumes (ml)***	**P Equivalent (mg/L)**
14543 (0.05 - 5.0)	0 - 0.05 - 1 - 2.5 - 3.5 - 5.0	0 - 0.05 - 1.0 - 2.5 - 3.5 - 5.0
14729 (0.50 - 25 mg/L)	0 - 0.5 - 5 - 10 - 20 - 25	0 - 0.5 - 5 - 10 - 20 - 25

* Dilute all working calibration standards to 100 ml in volumetric flasks.

Table 3. Standardized Acceptance Criteria for Performance Tests

Acceptance Criterion	**Section**	**Limit (%)**
Initial precision and recovery	9.2.2	
P Precision (s)	9.2.2.2	44
P Recovery (X)	9.2.2.2	55 - 143
Matrix spike/matrix spike duplicate	9.3	
P Recovery	9.3.4	50 - 148
P RPD	9.3.5	44
Ongoing precision and recovery	9.6	
P Recovery	9.6	50 - 148

18.0 Definitions

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

18.1.1 Symbols

°C	degrees Celsius
>	greater than
<	less than
%	percent

18.1.2 Alphabetical Characters

g	gram
L	liter
mg	milligram
mg/L	milligram per liter
ml	milliliter
nm	nanometer

18.2 Definitions, acronyms, and abbreviations.

18.2.1 Analyte: Phosphorus, the parameter which is analyzed by this method.

18.2.2 Analytical batch: The set of samples analyzed at the same time, to a maximum of 20 samples. Each analytical batch must be accompanied by a laboratory blank (Section 9.4), and ongoing precision and recovery sample (OPR, Section 9.6), a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), and a reagent blank (Section 9.4).

18.2.3 IPR: See initial precision and recovery.

18.2.4 Initial precision and recovery (IPR): Four aliquots of the diluted Phosphate std solution analyzed 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 instrument is modified.

18.2.5 Laboratory blank (method blank): An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, and reagents that are used with samples. The laboratory blank is used to determine if analyte or interferences are present in the laboratory environment, or the reagents.

18.2.6 Matrix spike (MS) and matrix spike duplicate (MSD): Aliquots of environmental sample to which known quantities of the analyte are added in the laboratory. The MS and MSD are prepared and/or analyzed exactly like a field sample. Their purpose is to quantify any additional bias and imprecision caused by the sample matrix. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for background concentrations.

18.2.7 May: This action, activity, or procedural step is neither required nor prohibited.

18.2.8 May not: This action, activity, or procedural step is prohibited.

18.2.9 Memo chip: See transponder.

18.2.10 Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

18.2.11 Minimum level (ML): The lowest level at which the entire analytical system gives a recognizable signal and acceptable calibration point of the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and preparation procedures have been employed.

18.2.12 Must: This action, activity, or procedural step is required.

18.2.13 OPR: See ongoing precision and recovery standard.

18.2.14 Ongoing precision and recovery standard (OPR): A laboratory blank spike with known quantities of analyte. The OPR is treated exactly like a sample. Its purpose is to establish performance of the method by the analyst.

18.2.15 Ortho-Phosphate (PO_4^{3-}): Reactive form of phosphorus, to which all measured P is converted for determination by this procedure.

18.2.16 Phosphate std solution: See phosphorus standard solution.

18.2.17 P: See phosphorus.

18.2.18 Phosphorus standard solution: The 100 mg/L phosphorus standard solution which is used for instrument calibration, matrix spiking, MDL studies, IPR and OPR checks.

18.2.19 Phosphorus (P): The parameter which is analyzed by this method.

18.2.20 Quality control sample (QCS): A sample containing analyte of interest at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that heave been prepared independently from the normal preparation process.

18.2.21 Reagent water: Deionized water demonstrated to be free from phosphate.

18.2.22 Shall: This action, activity, or procedural step is required.

18.2.23 Should: This action, activity, or procedural step is suggested, but not required.

18.2.24 Merck Spectroquant® system photometer: Photometers which contain information about Spectroquant® products. The instruments automatically set to the appropriate wavelength and measuring parameters through bar code recognition of item number, test range, cell format, wavelength, and calibration data.

18.2.25 Spectroquant® Phosphate Cell Test: The pre-measured P reagents, packaged in 16 x 100 mm tubes.

18.2.26 Spectroquant® Thermoreactor: The block digester which can operate at a temperature of 120□C, to effect digestion of the Spectroquant® Phosphate Cell Tests.

18.2.27 Transponder: The memo chip, which contains updated information which may include new methods and updated calibration information for downloading into the Merck Spectroquant® system photometer.