

SOP-C-162

**Determination of Soil Phosphorus by
Mehlich 3 Extraction**

Revision 0

Approval:


Laboratory Manager

6-29-21
Date


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Texas Institute for Applied Environmental Research

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- I. Identification of the method**
 - a. Soil Analysis of phosphorus (Sims, 2000; SEAL, 2018)
- II. Applicable matrix or matrices**
 - a. Soils and other solids (not TNI accredited)
- III. Limits of detection and quantitation**
 - a. Range 0.2 – 8.0 mg P/L
 - b. Estimate of Reporting Limit 0.2 mg P/L
 - c. Detection limit By USEPA procedure MDL = 0.01 mg P/L
- IV. Scope and application, including parameters to be analyzed**
 - a. This method covers the determination of o-Phosphate–(P) in extracts of soil by extraction with Mehlich 3.
- V. Summary of the method**
 - a. Reaction with acidic molybdate in the presence of antimony forms an antimony phospho-molybdate complex. This complex is chemically reduced by ascorbic acid to an intensely blue complex: phosphomolybdenum blue. The absorbance of this complex is measured photometrically at 880 nm.
- VI. Definitions**
 - a. Extraction – Use of a reagent that displaces the nutrient to be analyzed into the solution so that the nutrient can be analyzed.
 - b. Colorimetric analysis – a wet chemistry analysis procedure based on the reaction between chemicals in reagents and the nutrient of interest to produce a colored product that varies in value depending on the concentration of nutrient in the solution.
 - c. Refer to QAM-Q-101, “Laboratory Quality Control.”
- VII. Interferences**
 - a. More than 50 mg Fe^{3+} /L, 10 mg Cu^{2+} /L, and 10 mg SiO_2 /L cannot be tolerated. High silica concentrations cause positive interference. High iron concentrations can cause precipitation of, and subsequent loss of, phosphorus.
 - b. Arsenate (AsO_4^{3-}) is a positive interference. Arsenates react with the molybdate reagent to produce a blue color similar to that

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formed with phosphate. Concentrations as low as 0.1 mg As(V)/L interfere with phosphate determination.

- c. Bias from background color or turbidity in the extracts is correctable, using sample blanking feature of AQ1 software. However, soil analysts should employ accepted extraction techniques to clarify the extract. For example, activated charcoal can be added to the working extractant. Following extraction and centrifugation, the extract supernatant is filtered using filter paper.

VIII. Safety

- a. Wear proper laboratory attire when conducting this analysis, including safety glasses, gloves, and laboratory coat.
- b. Ammonium fluoride (NH_4F) is toxic if swallowed, in contact with skin, and if inhaled.
- c. EDTA disodium salt dihydrate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) causes serious eye irritation, may be harmful if inhaled.
- d. Concentrated nitric acid (HNO_3) may intensify fire (oxidizer). May be corrosive to metals. Causes severe skin burns and eye damage.
- e. Ammonium nitrate (NH_4NO_3), acetic acid (CH_3COOH), ammonium molybdate tetrahydrate ($(\text{NH}_4)_2\text{MoO}_4$) and antimony potassium tartrate trihydrate ($\text{K}_2(\text{SbO})_2\text{C}_8\text{H}_4\text{O}_{10} \cdot 3\text{H}_2\text{O}$) are Irritating to eyes, skin, and respiratory tract. Harmful if swallowed.
- f. Ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) may cause irritation to eyes and skin.
- g. Potassium dihydrogen orthophosphate (KH_2PO_4) may cause irritation to eyes, skin, or respiratory tract.
- h. Sulfuric acid (H_2SO_4) causes severe burns. Toxic if inhaled or swallowed.

IX. Equipment and supplies

- a. Ponar dredge
- b. AMS sediment sampler or hammer probe
- c. Teflon or Teflon-coated scoop
- d. Balance capable of accurately weighing to the nearest 0.01 g.
- e. Weighing pans and spatulas
- f. Assorted glassware, Class A
- g. Falcon tubes
- h. Shaking stand

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- i. Funnels, filter paper, and filter stand
- j. Filtrate collection vials
- k. SEAL AQ300 autoanalyzer
- l. Laptop computer with SEAL AQ2 software installed.
- m. SEAL sample cups
- n. SEAL reagent wedges
- o. SEAL reaction segments

X. Reagents and Standards

a. Reagents

- i. Ammonium fluoride (NH_4F) and EDTA stock solution: Add 120 mL of DI water to a 200 mL volumetric flask. Add 27.78 g (0.015 M) of NH_4F (CAS 12125-01-8) and mix well. Add 14.61 g (0.25 M) EDTA disodium salt dihydrate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) (CAS 6381-92-6) to the solution. Use phosphorus-free deionized (DI) water to bring the solution to volume in the 200 mL volumetric flask. Mix well and store in a plastic bottle (stock solution for 1,000 samples).
- ii. Mehlich 3 extractant: Add 800 mL of DI water to a 1 L volumetric flask. Dissolve 20 g of ammonium nitrate (NH_4NO_3) (CAS 6484-52-2) in the DI water. Add 4.0 mL NH_4F -EDTA stock solution (X.a.i.) and mix well. Add 11.5 mL (99.5%, 17.4 M) glacial acetic acid (CH_3COOH) (CAS 64-19-7). Add 0.82 mL (68 to 70 %, 15.5 M) of concentrated nitric acid (HNO_3) (CAS 7697-37-2). Add DI water to 1.0 L final volume and mix well (enough extractant for 40 samples). Final pH should be 2.5 ± 0.1 .
- iii. Colorimetric reagents
 - 1. Ammonium molybdate (4%): Dissolve 4 g ammonium molybdate tetrahydrate ($\text{K}_2(\text{SbO})_2\text{C}_8\text{H}_4\text{O}_{10} \cdot 3 \text{H}_2\text{O}$) (CAS 28300-74-5) in approximately 80 mL DI water. Stir at least 1 hour. Dilute to 100 mL with DI water and store in a plastic bottle at >0 to $\leq 6^\circ\text{C}$. Discard if reagent becomes discolored or turbid.
 - 2. Sulfuric acid, 5 N: Add about 400 mL of DI water to a 500 mL beaker. Slowly add 70 mL sulfuric acid, concentrated (H_2SO_4) (CAS 7664-93-9) to the DI water. Caution, the container will become very warm.

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Cool to room temperature and dilute to 500 mL with DI water in a volumetric flask.

3. Antimony potassium tartrate (3 g/L): Add about 400 mL of DI water to a 500 mL volumetric flask. Add 1.5 g antimony potassium tartrate trihydrate tetrahydrate $((\text{NH}_4)_2\text{MoO}_4)$ (CAS 12054-85-2) to the DI water in the volumetric flask. Dilute to 500 mL in volumetric flask and mix well. Store at >0 to ≤ 6 °C in a dark bottle. Prepare fresh monthly.
4. Working phosphate color reagent - To a 100 mL volumetric flask, add 50 mL sulfuric acid, 5 N (x.a.iii.2.) followed by 7 mL antimony potassium tartrate (3 g/L) (x.a.iii.3.) and swirl to mix. Then add 15 mL (4%) ammonium molybdate (x.a.iii.1.). Swirl the contents. Use DI water to bring the volumetric flask to volume and mix well. Store this reagent in a plastic container for up to 3 weeks. Discard if the reagent turns blue or becomes turbid.
5. Working ascorbic acid - Dissolve 0.80 g of ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) (CAS 50-81-7), fine granular, in about 80 mL DI water. Use DI water to dilute to 100 mL in a volumetric flask and mix well. The solution is stable for 1 week if stored at >0 to ≤ 6 °C. Discard if the solution becomes yellowed.

b. Standards

- i. Phosphate stock standard solution (1000 mg P/L) - Dissolve 4.394 g potassium dihydrogen orthophosphate, anhydrous (KH_2PO_4) (CAS 7778-77-0) (dried at 105°C) in about 800 mL DI water in a 1 L volumetric flask. Up to 7 mL sulfuric acid (5 N), may be added for preservation. Use DI water to bring to volume in the 1 L volumetric flask. Store at >0 to ≤ 6 °C.
- ii. Phosphate top standard solution (8 mg P/L) - Transfer 2 mL Phosphate stock standard (100 mg P/L) to a 250 mL volumetric flask. Fill to the mark in the volumetric flask with Mehlich 3 extraction solution and invert to mix. Store at >0 to ≤ 6 °C.

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XI. Sample collection, preservation, shipment and storage

- a. Holding time for air dried soils—180 days.
- b. Sediment collection from rivers and bayous will follow sampling methods as described in “Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods”, Chapter 6, “Collecting Sediment Samples.”
- c. Collection of sediments from locations with deep water accessible only by a bridge, a Ponar dredge will be used. At other locations, where the stream width is narrow and stream depth is relatively shallow, sediment samples will be taken with an AMS® sediment sampler attached to hammer probe or with a Teflon or Teflon-coated scoop.
- d. Soil samples will be collected using an AMS® hammer probe. Soil samples will be collected at 2 depths: 0-15 cm and 15-30.
- e. Soil samples include replicate samples (usually 5 -7) that are collected into a clean bucket, mixed, and subsamples collected into labeled glass jars or plastic bottles.
- f. Prior to soil extraction with Mehlich 3 extracting solution, samples are dried following SOP-C-132, “Preparation of Soil Samples for Analysis on SEAL AQ300.”

XII. Quality Control

- a. All aspects of this procedure comply with QAM-Q-101, “Laboratory Quality Control”

XIII. Calibration and standardization

- a. Use calibration standards to establish the standard curve ($R^2 > 0.98$)
- b. Analyze an ICV and IBV immediately after calibration, and a CCV and CCB following every 10 samples, and at the end of the run.

XIV. Procedure

- a. Extraction of orthophosphate from soil.
 - i. Prepare Mehlich 3 extraction solution (X.a.ii.)
 - ii. Weigh 2.0 g of air-dried soil into a 50 mL Falcon tube.
 - iii. Add 20 mL of Mehlich 3 extraction solution to each Falcon tube.
 - iv. Shake at ≥ 200 rpm for five minutes at a room temperature.

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- v. Filter extracts through Whatman No. 1, 2, or 42 filter paper. Refilter if extracts are not clear.
- vi. Store samples in a refrigerator for 60 days.
- b. Colorimetric analysis of orthophosphate in soil extracts
 - i. Follow procedures described in QAM-I-120, "Operation and Calibration of the SEAL AQ300 Autoanalyzer".
 - ii. A standard curve will be developed automatically by the SEAL AQ300 instrument based on pre-programmed levels of diluting the "standard solution." This standard curve is produced at the beginning of each run.

xv. Data analysis and calculations

- a. Review the graphic display of the output and identify, correct, or resolve any anomalies by examining any problems with the function of the SEAL AQ300 instrument and ensuring all reagents are properly prepared, not outdated, and installed in proper analysis wedges.
- b. Print a copy of the SEAL data sheet for placement in the laboratory logbook.
- c. All data are documented and maintained in accordance with QAM-A-102, "Document and Data Control".
- d. The baseline is within \pm the LOQ value for the method.
- e. All dilution procedures are described in detail.

xvi. Method performance

- a. Method performance: refer to QAM-Q-101, "Laboratory Quality Control".

xvii. Pollution prevention

- a. Pollution prevention: refer to QAM-W-101, "Disposal of Laboratory Waste".

xviii. Data assessment and acceptance criteria for quality control measures

- a. Data assessment and acceptance: refer to QAM-Q-101, "Laboratory Quality Control".

xix. Corrective actions for out-of-control data

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- a. Refer to QAM-Q-101, "Laboratory Quality Control."

XX. Contingencies for handling out-of-control or unacceptable data

- a. Refer to QAM-Q-105, "Corrective Actions."

XXI. Waste Management

- a. All waste is placed into the proper waste receptacle and disposed of in accordance with QAM-W-101, "Disposal of Laboratory Waste".

XXII. References

- a. SEAL. 2018. AQ2 method. AGR-202-A Rev. 2
- b. Sims, J. Thomas. 2000. Soil Test Phosphorus: Mehlich 3. *In: Gary M. Pierzynski (ed.) Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Southern Cooperative Series Bulletin No. # 396.*
<https://sera17dotorg.files.wordpress.com/2015/02/sera-17-methods-for-p-2000.pdf>
- c. TCEQ. "Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods." Texas Commission on Environmental Quality, 2020,
www.tceq.texas.gov/publications/rg/rg-415.
- d. USEPA. 1993. Methods for the Determination of Inorganic Substances in Environmental Samples, USEPA 600/R 93/100, August 1993: Method 365.1, Rev. 2.0.

XXIII. Tables, diagrams, flowcharts and validation data

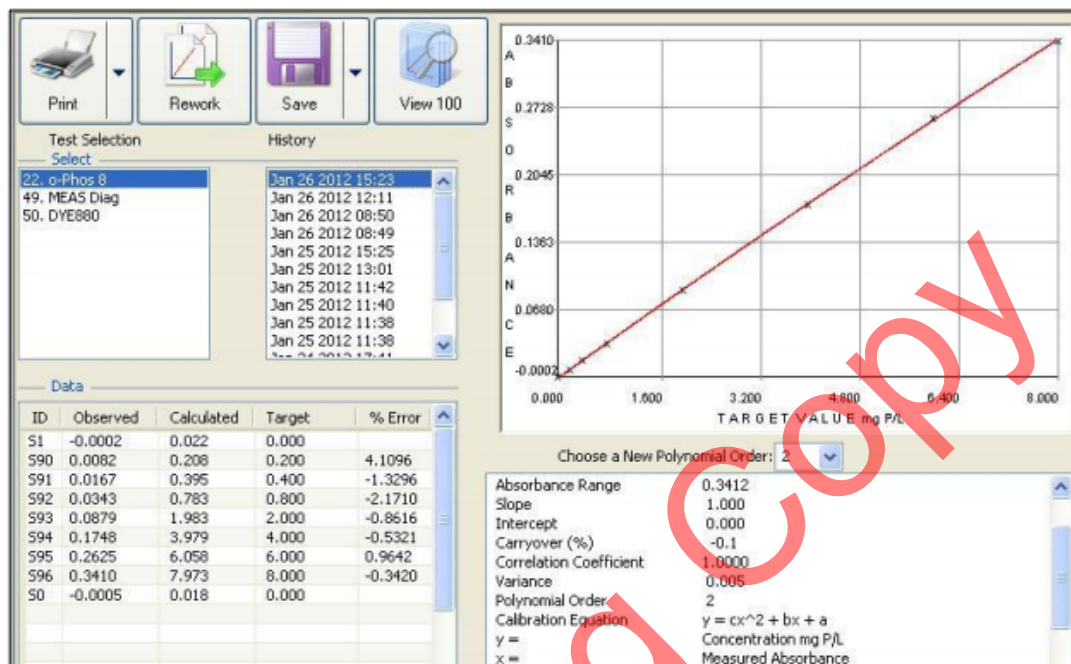
- a. Example Calibration Curve Table

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Attachment 1

Example Calibration Curve Table



DEFINITIONS:

Abs. Range: Difference between the absorbance of the highest standard and the absorbance of the blank

Variance: Calculated as follows:

$$\text{Variance} = \frac{\sum (\text{Deviations, i.e., errors})^2 \times 100}{\sum (\text{Observed values})^2}$$

Carryover: Calculated as follows:

$$\text{Carryover} = \frac{(A - B)}{C}$$

Where,
 A = absorbance of carryover blank (S0)
 B = absorbance of blank (S1)
 C = calibration absorbance range

From: SEAL. 2018. AQ2 method. AGR-202-A Rev. 2