45.1.33 - Vitamins and Other Nutrients / Chemical Methods

AOAC Official Method 995.11
Phosphorus (Total) in Foods

Colorimetric Method
First Action 1995

NMKL-AOAC Method

(Applicable to determination of phosphorus in foods and food ingredients at 0.05-1.00 g/100 g.)

See Table **995.11** for the results of the interlaboratory study supporting the acceptance of the method.

A. Principle

Product is dry-ashed to remove organic material. The acid-soluble phosphate residue forms a blue complex $[(MoO_2 \cdot 4MoO_3)_2 \cdot H_3PO_4]$ with $Na_2M_0O_4$ in the presence of ascorbic acid as reducing agent. Intensity of blue color is measured spectrophotometrically at 823 ± 1 nm.

B. Apparatus

(*Note*: Glassware and crucibles must be cleaned with P-free detergents.)

(**a**) *Spectrophotometer*._ perating at 823 ± 1 nm.

(**b**) *Cuvettes*._1 cm path length or 2.5 cm flow-through.

(**c**) *Analytical balance*._Weighing to 0.1 mg.

(**d**) *Crucibles*._Quartz, ca 50 mL.

(**e**) *Volumetric flasks*._10, 50, 100, and 500 mL.

(**f**) *Muffle furnace*.

(**g**) *Filter paper*._Fast.

(**h**) *Hot plate*.

(**i**) *Water bath*._For boiling, e.g., large saucepan.

(**j**) *Metal basket*._Suitable size for water bath.

(**k**) *Weights*._Metal wire or steel nuts.

(**l**) *Glass rods*._Stable at 525°C.

C. Reagents

(*Note:* All reagents must be analytical grade and must be prepared with distilled H_2O.)

(**a**) *Hydrochloric acid*._Concentrated, 12M.

(**b**) *Zinc oxide*.

(**c**) *Potassium hydroxide solution*._50% (w/v). Dissolve 50 g KOH in 50 mL H_2O.

(**d**) *Sulfuric acid*._Concentrated, 18M.

(**e**) *Sodium molybdate solution ($Na_2MoO_4 \cdot 2H_2O$)*._Carefully mix 140 mL H_2SO_4, (**d**), with 300 mL H_2O in 500 mL volumetric flask. Cool to room temperature and add 12.5 g $Na_2MoO_4 \cdot 2H_2O$. Dilute to volume with H_2O. Mix well.

(**f**) *Ascorbic acid solution*._Dissolve 5 g ascorbic acid in H_2O in 100 mL volumetric flask. Dilute to volume with H_2O. Mix well. Prepare solution on the day of use.

(**g**) *Molybdate-ascorbic acid solution*._Immediately before use add 25 volumes of Na_2MoO_4 solution, (**e**), to 10 volumes of ascorbic acid solution, (**f**), and dilute with H_2O to 100 volumes in volumetric flask. Mix well.

(**h**) *Phosphorus stock standard solution*._1.0 mg P/mL. Dry KH_2PO_4 2 h at 101°C. Dissolve 1.0967 g dried KH_2PO_4 in H_2O in 250 mL volumetric flask. Dilute to volume with H_2O and mix well.

(**i**) *Phosphorus working standard solution*._0.01 mg P/mL. Transfer 5.00 mL P stock standard solution, (**h**), into 500 mL volumetric flask, and dilute to volume with H_2O. Mix well.

(**j**) *Phosphorus solutions for standard curve*._0, 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg P. Using pipet, accurately transfer exactly 0, 1.00, 2.00, 3.00, 4.00, 5.00, and 6.00 mL P working standard solution, (**i**), into separate 50 mL volumetric flasks. Dilute solutions with H_2O to ca 15 mL. (*Note:* Store P standard solutions, (**h**)-(**j**), at ca 5°C to minimize risk of microbial growth. Discard solutions if any haze or turbidity occurs.)

D. Preparation of Test Solution

Accurately weigh 0.5-1.5 g (±1 mg) homogeneous test portion into crucible. To control possible contamination, prepare reagent blank by including an empty crucible in analytical run. Treat reagent blank in the same manner as test portion.

Add 0.5 g ZnO into crucible and mix, using glass rod; leave glass rod in crucible. Dry 1-2 h at ca 110°C. Pre-ash on hot plate until residue is black.

(*Note:* No drying and pre-ashing are needed if furnace used in next step is equipped with a time-temperature regulator.)

Place crucible in muffle furnace at room temperature, and let temperature rise to 525°C. Maintain this temperature 4 h or overnight. When using furnace equipped with a time-temperature regulator, use slow initial increase of temperature to avoid the risk of splashing liquid products.

Remove crucible from oven and let cool to room temperature. To cold crucible, add 5 mL H_2O and 5 mL HCl. Cover crucible with watch glass and boil contents carefully 5 min on hot plate.

Filter contents of crucible into 100 mL volumetric flask. Rinse crucible and inner surface of watch glass with 5 mL hot H_2O. Repeat rinsing 4 times with 5 mL hot H_2O and transfer all rinses through the filter into the volumetric flask.

Cool flask to room temperature, and neutralize solution by adding 50% KOH solution until solution is slightly opalescent [$Zn(OH)_2$]. Add HCl dropwise until opalescence disappears. Add 2 extra drops of HCl. Let solution cool to room temperature and then dilute to 100 mL with H_2O.

Depending on the expected content of P, accurately pipet 1.00-10.0 mL treated solution into 50 mL volumetric flask. Dilute to 15 mL with H_2O. Add 20 mL molybdate-ascorbic acid solution to test solution in 50 mL flask, and also to phosphorus standard solutions, **C**(**j**). Swirl contents carefully.

Place flasks in metal basket. Close each flask with stopper, inserting narrow filter paper strip at the stopper so that flask is not closed too tightly. Place lead wire or stainless steel nut on flask as a weight. Immerse metal basket in vigorously boiling water bath. Keep flasks in water bath exactly 15 min. Cool flasks under tap H_2O to 20-30°C, and then dilute contents to 50 mL with deionized H_2O and mix.

E. Determination

Transfer solutions from **D** to 1 cm cuvettes or flow-cell. Measure absorbance of each solution against reagent blank at 823 ± 1 nm. Measurement must be made within 1 h after the color reaction.

Construct standard curve by plotting absorbances against amounts of P in P standard solutions (0, 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg P). If absorbance of analyte exceeds absorbance of 0.06 mg P, repeat the color reaction, using smaller volume of treated solution.

F. Calculations

Calculate P content as P in test portion (g/100 g) as follows:

$$\text{P, g/100 g} =$$

where V_1 = volume of solution used in the color reaction, mL; V_2 = volume of volumetric flask containing ash test portion, 100 mL; P = amount of P from standard curve corresponding to absorbance of analyte, mg; and W = weigh test portion, mg.

Report results with 2 significant figures (e.g., 1.2, 0.56, or 0.067 g/100 g).

Calculate P content as phosphatide (lecithin, g/100 g) as follows:

$$\text{Phosphatides, g/100 g} = 30 \quad \text{P (g/100 g)}$$

Calculate P content as P_2O_5, g/100 g as follows:

$$P_2O_5\text{, g/100 g} = 2.29 \times \text{P (g/100 g)}$$

References:
J. AOAC Int. **77,** 1557(1994); **79,** 1408(1996).

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Table 995.11: Interlaboratory study results for determination of phosphorus (total) in foods and food ingredients by spectrophotometric method