

Phosphorus (Total) in Meat and Sausage Products

Photometric determination using the Phosphorus molybdenum blue method subsequent to fusion melting

Introduction

Phosphorus is an essential nutrient with critical roles in cellular energy metabolism, signal transduction, phosphorylation reactions, and the mineralization of bones and teeth.¹ Major dietary sources include foods of animal origin such as milk, dairy products, meat, poultry, and fish, with additional contributions from cereals and legumes.² Beyond naturally occurring phosphorus, inorganic phosphates (E 338–341, E 343, E 450–452) derived from food additives constitute a significant and increasing proportion of total intake, particularly through processed and convenience foods. In the meat industry, food-grade phosphates are widely applied to enhance water-holding capacity, emulsification, and color stability, thereby improving product quality.³ It is estimated that up to half of daily phosphorus intake in Western diets originates from such additive-derived “hidden phosphorus”.³ Unlike protein-bound phosphorus, which is absorbed at about 60% efficiency, inorganic phosphate salts from additives are almost completely absorbed.⁴

Excessive phosphorus intake has been linked to cardiovascular morbidity and mortality, especially in individuals with chronic kidney disease, making it a broader public health concern. The Scientific Committee for Food established an Acceptable Daily Intake (ADI) for phosphates, expressed as phosphorus, of 40 mg/kg body weight per day.⁵ Additive-derived phosphates pose particular concern because they dissociate readily without enzymatic hydrolysis, unlike organic phosphorus in animal- and plant-derived foods. Plant phosphorus, mostly bound to phytates, has a bioavailability of only 20–30% due to the absence of phytase in humans.⁵ This higher bioavailability of additive phosphorus underscores the need for monitoring intake and exposure, particularly in vulnerable populations.

Accurate quantification of phosphorus in meat and sausage products is therefore necessary to ensure regulatory compliance, dietary exposure assessment, and quality control. Photometric determination using the phosphorus molybdenum blue method subsequent to fusion melting provides a robust and sensitive approach for total phosphorus measurement in complex meat matrices.

Experimental

Method

Most foods containing phosphorus compounds. Especially foods with rich content of proteins like milk and dairy products or meat and poultry are sources of phosphorus compounds.

After combustion of the sample orthophosphate ions react in sulfuric acid with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to Phosphomolybdenum Blue (PMB) that is determined photometrically.

Measuring Range

Spectroquant® Phosphate Cell Test (1.14543)	Test kit	0.2–15.3 mg/L PO ₄ ³⁻
	Method	0.2–15.3 g/kg PO ₄ ³⁻
Spectroquant® Phosphate Cell Test (1.14729)	Test kit	1.5–76.7 mg/L PO ₄ ³⁻
	Method	1.5–76.7 g/kg PO ₄ ³⁻
Spectroquant® Phosphate Test (1.14848):	Test kit	0.015–15.3 mg/L PO ₄ ³⁻
	Method	0.015–15.3 mg/kg PO ₄ ³⁻

Applicable Sample

Meat and Sausage Products

Reagents, Instruments and Materials

Reagents & Test Kits

- Spectroquant® Phosphate Cell Test (**1.14543**)
- Spectroquant® Phosphate Cell Test (**1.14729**)
- Spectroquant® Phosphate Test (**1.14848**)
- Sodium carbonate water-free for analysis (**1.06392**)
- Hydrochloric acid 37% for analysis (**1.00317**)
- Nitric acid 65% for analysis (**1.00452**)
- Water for analysis, EMSURE® (**1.16754**)

Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)
- Spectroquant® Colorimeter Move 100 (**1.73632**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Nova and Prove series.

Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

Instrument Accessories

- Rectangular cells 10 mm (**1.14946**) or
- Rectangular cells 20 mm (**1.14947**) or
- Rectangular cells 50 mm (**1.14944**) or
- Semi-microcells 50 mm (**1.73502**)

Note: Rectangular cells are only necessary if the Spectroquant® Phosphate test **1.14848** is used.

Other Reagents and Accessories

- MQuant® Phosphate Test (**1.10428**)
- Hydrochloric acid 25% for analysis EMSURE® (**1.00316**)
- Analytical balance
- Crucible
- Drying-kiln
- Muffle furnace
- Heating plate
- Glass beaker, 400 mL
- Volumetric flask, 250 and 500 mL

- Volumetric pipette, 25 mL
- Folded filters

Analytical Procedure

Sample preparation

In a crucible mix exactly 5 g of the fine cut sample with 2 g of water-free sodium carbonate. In the drying-kiln pre-dry the content for 30 minutes at 120–130 °C and then decompose it in the muffle at 500 °C for another 30 minutes. After cooling, dissolve the residue with some water for analysis and transfer by rinsing it with water for analysis into a 400 mL glass beaker. Then slowly add 15 mL of hydrochloric acid 37% and 2 mL of nitric acid 65%. Evaporate the mixture to about 10 mL. Transfer by rinsing the residue with water for analysis into a 500 mL standard volumetric flask and fill up to volume with water for analysis. Then mix well and filter off the insoluble parts. Pipette 25 mL off the filtrate, put it into a 250 mL standard volumetric flask, fill up to volume with water for analysis and mix well (=pretreated sample).

Using Cat. No. 1.14543: Procedure and measurement

For more information on the measurement see the packaging insert of the test.

Procedure

- Pipette 5.0 mL pretreated sample into a reaction cell and mix.
- Add 5 drops reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake **vigorously until the reagent is completely dissolved**.
- **Leave to stand for 5 min (reaction time)**, then measure the sample in the photometer.

Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16-mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.14729: Procedure and measurement

For more information on the measurement see the packaging insert of the test.

Procedure

- Pipette 1.0 mL pretreated sample into a reaction cell and mix.
- Add 5 drops reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake **vigorously until the reagent is completely dissolved**.
- **Leave to stand for 5 min (reaction time)**, then measure the sample in the photometer.

Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16-mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.14848: Procedure and measurement

For more information on the measurement see the packaging insert of the test.

Procedure

- Pipette 5.0 mL pretreated sample into a test tube.
- Add 5 drops reagent PO₄-1 and mix.
- Add 1 level blue microspoon (in the cap of the PO₄-2 bottle) reagent PO₄-2 and shake **vigorously until the reagent is completely dissolved**.
- **Leave to stand for 5 min (reaction time)**, then fill the sample into the cell, and measure the sample in the photometer.

Note: For measurement in the **50 mm cell** both the sample volume as well as the quantity of reagents

PO₄-1 and PO₄-2 must be **doubled**. Alternatively, the semi-microcell Cat. No. **1.73502** can be used. It is recommended to measure against an own prepared blank sample (preparation as per measurement sample, but with distilled water instead of sample) to increase the accuracy. Configure the photometer for blank measurement.

Measurement

- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- If a 50 mm cell is used, perform the reagent blank one time for each measurement series. To do this tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the corresponding rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.
- After the reagent blank has been measured, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Calculation

Phosphorus (total) content in mg/kg
 $P = \text{analysis value in mg/L} \times 1000$

Analytical quality assurance

Analytical Quality Assurance (AQA) is recommended before each measurement series.

To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, the ortho-phosphate standard solutions (see section 5 of the respective test instruction) or Spectroquant® CombiCheck 10 or Spectroquant® CombiCheck 20 and 80, respectively can be used. Besides a standard solution with 0.80 mg/L PO₄-P (or 8.0 mg/L PO₄-P or 15.0 mg/L PO₄-P, respectively), CombiCheck 10, 20 and 80 also contain an addition solution for determining sample-dependent interferences (matrix effects).

To view additional notes, visit
[SigmaAldrich.com/qa-test-kits](https://www.sigmaaldrich.com/qa-test-kits).

References

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Featured Products

Description	Cat. No.
Reagent and Test Kits	
Spectroquant® Phosphate Cell Test	1.14543
Spectroquant® Phosphate Cell Test	1.14729
Spectroquant® Phosphate Test	1.14848
Instruments and Accessories	
Spectroquant® VIS Spectrophotometer Prove 100 plus	1.73026
Rectangular cells 10 mm	1.14946
Rectangular cells 20 mm	1.14947
Rectangular cells 50 mm	1.14944
Semi-microcells 50 mm	1.73502
Reagents	
Nitric acid 65% for analysis EMSURE®	1.00452
Hydrochloric acid 37% for analysis	1.00317
Sodium carbonate water-free for analysis	1.06392
Water for analysis EMSURE®	1.16754
Phosphate standard solution traceable to SRM from NIST KH ₂ PO ₄ in H ₂ O 1000 mg/L PO ₄ Certipur®	1.19898

Related Products

Description	Cat. No.
Instruments	
Spectroquant® UV/VIS Spectrophotometer Prove 300 plus	1.73027
Spectroquant® UV/VIS Spectrophotometer Prove 600 plus	1.73028
Spectroquant® colorimeter Move 100	1.73632
Reagents	
MQuant® Phosphate Test	1.10428
Hydrochloric acid 25% for analysis EMSURE®	1.00316

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