

## AMMONIUM SALTS of PHOSPHATIDIC ACID

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### SYNONYMS

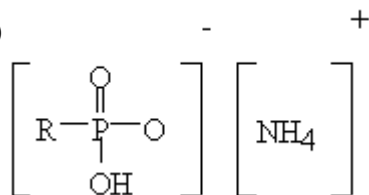
Ammonium phosphatides, Emulsifier YN, Mixed ammonium salts of phosphorylated glycerides; INS No. 442

### DEFINITION

The product consists essentially of a mixture of the ammonium compounds of phosphatidic acids derived from the edible fat (usually partially hardened rapeseed oil). A mono- or diglyceride moiety may be attached to phosphorus. Moreover, two phosphorus esters may be linked together as phosphatidyl phosphatides. The product is prepared by glycerolysis of the fat, followed by phosphorylation with phosphorus pentoxide, and neutralization with ammonia.

The article of commerce may be further specified as to water content, hexane-insoluble matter, inorganic hexane-insoluble matter, pH value and triglyceride content.

Structural formula  
(approximate composition)



where R may be a mono- or di-glyceride moiety

Assay

The phosphorus content is not less than 3.0% and not more than 3.4% by weight; the ammonium N content is not less than 1.2% and not more than 1.5%.

### DESCRIPTION

Unctuous semisolid

### FUNCTIONAL USES

Emulsifier

### CHARACTERISTICS

#### IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water, partially soluble in ethanol and in acetone, soluble in fats

Test for phosphate

Passes tests  
See description under TESTS

Test for fatty acid

Passes tests  
See description under TESTS

Test for glycerol (Vol. 4)

Passes test

#### PURITY

Lead (Vol. 4)

Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

## TESTS

### IDENTIFICATION TESTS

Test for phosphate

Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

Test for fatty acid

Reflux 1 g of the product for 1 h with 25 ml of 0.5 N ethanolic potassium fatty acid hydroxide. Ammonia is evolved from the end of the reflux condenser, recognizable by its odour and by its reaction on moist, red litmus paper. On cooling the residue to 0°, a precipitate of potassium soap is obtained.

## METHOD OF ASSAY

Determination of phosphorus

Absorption spectrophotometry

Reagents and working solutions

Sulfuric acid: sp gr 1.84

Nitric acid: sp gr 1.42

Perchloric acid: 60%, sp gr 1.54

*Vanadate-molybdate solution:* Separately dissolve in water 20 g of ammonium molybdate and 1 g of ammonium vanadate. Mix the two solutions, add 140 ml of concentrated nitric acid and dilute to 1000 ml with water. Mix well.

*Standard phosphate solution:* Stock solution: Dissolve 3.8346 g of potassium dihydrogen phosphate, previously dried at 110°, in water and dilute to 1000 ml; 1 ml of this solution = 2.0 mg P<sub>2</sub>O<sub>5</sub>.

*Standard phosphate working solution:* Transfer 50.0 ml of the standard phosphate solution (1ml = 2.0 mg P<sub>2</sub>O<sub>5</sub>) to a 500 ml volumetric flask and dilute to volume with water and mix well. 1 ml of standard phosphate working solution = 0.2 mg equivalents of P<sub>2</sub>O<sub>5</sub>

Procedure

Weigh accurately 1.5 to 1.6 g of a representative sample into a small glass capsule and transfer to a 300-ml Kjeldahl flask containing 5 ml of sulfuric acid and 10 ml of nitric acid. Heat the flask, gently at first, with continual swirling, and later more strongly over a bare flame. Add further measured amounts of nitric acid from time to time, cooling the flask prior to addition, and continue the heating until the stage where the digest is clear and assumes a golden colour. Cool, add 5 ml of 60% perchloric acid and continue the oxidation until white acid fumes form in the flask. Cool again and add 5 ml of water and continue heating until white fumes are again driven off. Cool, dilute carefully with water, cool again and transfer quantitatively to a 500-ml volumetric flask. Dilute to volume with water and mix well (Test solution).

Carry out a blank digestion exactly as above but omit the sample and use the same volume of acid as required to wet oxidize the sample (Blank digest solution).

Into separate 100-ml volumetric flasks, add by burette:

(a) 25.0 ml of Standard phosphate working solution (= 5.0 mg P<sub>2</sub>O<sub>5</sub>),

(b) 30.0 ml of Standard phosphate working solution (= 6.0 mg P<sub>2</sub>O<sub>5</sub>),

(c) a 25 ml aliquot of the test solution which will contain the equivalent of between 5 and 6 mg P<sub>2</sub>O<sub>5</sub>

Into each of the flasks containing the phosphorus standards, i.e. (a) and (b), transfer an aliquot of the blank digest solution equal in volume to (c), in order to compensate for possible traces of phosphorus derived from the acid digest reagents and which may be present in the Test Solution.

To each add 25 ml of the vanadate-molybdate reagent, mix, dilute to nearly 100 ml with water, mix well, adjust the temperature of the solution to 20°, dilute to the mark with water and re-mix.

After 10 min measure the absorbance of both the 6 mg P<sub>2</sub>O<sub>5</sub> solution and the test solution against the 5 mg standard contained in the blank cell. Use optically matched 1 cm cells and measure at a wavelength of 420 nm, or with an Ilford 604 filter if using a photo-electric colorimeter.

#### Calculation

$$\% \text{ Phosphorus} = \left[ 5 + \frac{A \text{ test}}{A \text{ 6 mg}} \right] \times \frac{0.873}{W}$$

where

A test = absorbance difference between the 5 mg standard and the test solution

A 6 mg = absorbance difference between the 6 mg and 5 mg standards

W = weight of sample taken (g)

#### Determination of ammonium nitrogen

##### Apparatus for steam distillation

The apparatus consists of a 2-L flask fitted with a rubber bung through which pass an approximately 3" length of glass tubing, arranged so that the lower end is near the bottom of the flask, and a shorter L-shaped piece of tubing arranged such that the tube projects about 1/4" below the lower surface of the bung, to act as a steam outlet tube. The flask should be approximately 2/3 filled with distilled water made slightly acid with dilute sulfuric acid TS and contain a few pieces of sintered glass to prevent bumping when the contents of the flask are vigorously boiled to act as a steam generator. A tap funnel may be fitted to the flask if desired to facilitate replenishing the water in the flask between determinations.

The steam outlet tube is connected via a condensation trap to the inlet of a steam distillation head, fitted to a short necked 1-L round bottomed B34 necked flask. The distillation head should be such that the steam inlet tube reaches almost to the bottom of the 1-L flask and the outlet should be fitted with two splash traps, one near the top of the 1-L flask and the other near the top of B19 jointed verticle, single-surface condenser to which the distillation head connects. The vertical condenser should be fitted with an extended outlet tube, able to reach to the bottom of a 500-ml conical flask.

### Reagents

Boric acid solution (2% w/v in water)

Sodium hydroxide solution (40 w/v in water)

0.02N Hydrochloric acid

Mixed indicator: Mix 5.0 of 0.1% w/v alcoholic solution of bromocresol green and 2.0 of a 0.1% w/v alcoholic solution of methyl red and dilute the mixture to 30 ml with 95% alcohol.

Silicone fluid 200/50 MS

### Procedure

Assemble and thoroughly steam out the apparatus. Accurately weigh about 0.2 g of a representative sample of neutral Ammonium salts of phosphatidic acids into a small glass phial (approx. 3/4" diameter, 1/2" deep). Transfer the phial and weighed contents to the distillation flask and add approximately 250 ml distilled water. Connect the distillation head and splash traps to the distillation flask and vertical condenser, and arrange the condenser such that the outlet dips below the surface of 10 ml of 2% boric acid and 1 ml mixed indicator contained in a 500-ml conical flask.

Add to the distillation flask, via a funnel attached by means of a short piece of rubber tubing to the steam inlet tube, 75 ml 40% aqueous sodium hydroxide, and wash in with distilled water. Detach the funnel and connect the steam inlet to the steam supply. (Alternatively, the sodium hydroxide may be added to the flask through a tap funnel, fitted to the distillation flask if preferred and washed in with distilled water. If so a liquid seal should be maintained in the funnel during the addition and distillation). Vigorously steam distil the contents of the distillation flask and collect 200 ml distillate in the boric acid. During the distillation gently agitate the distillation flask if necessary, to avoid the sample being deposited around the upper surfaces of the flask. When the required amount of distillate has been collected, lower the receiving flask, stop the steam supply, and wash down the inside of the condenser, and the outside of the lower end, with a small quantity of distilled water, collecting the washings in the receiving flask.

Titrate the contents of the receiving flask with 0.02N hydrochloric acid. Carry out at least one blank determination in exactly the same way but omitting the sample.

During the distillation difficulty may be experienced with frothing of the contents of the distillation flask. If so, 2 drops of silicone fluid should be added to the distillation flask at the time of adding the sample; and a similar amount included in the blank determination.

### Calculation

1 ml of 0.02 N HCl = 0.2802 mg of nitrogen.

$$\% \text{ Nitrogen} = \frac{(\text{sample titre} - \text{blank titre}) \times 28.02}{(\text{sample wt. in mg})}$$