

(c) *Rubber stoppers.*—Two or three small, solid rubber stoppers to loosen ppt from walls of flask.

#### DETERMINATION

Weigh 3–20 mg sample, depending on P content and whether micro or semi-micro balance is used (max. wt ppt = 50 mg). Weigh in charging tube, if possible, and transfer to Kjeldahl flask. Use porcelain boat for sticky solids and viscous liquids, and glass capillary for volatile liquids.

Add 0.5 ml  $\text{H}_2\text{SO}_4$  followed by 4–5 drops  $\text{HNO}_3$ . Heat on digestion rack to white  $\text{SO}_2$  fumes and cool under tap. Add 4–5 drops  $\text{HNO}_3$ , repeat digestion, and cool under tap. Add 4–5 drops  $\text{HNO}_3$  and again digest to  $\text{SO}_2$  fumes. Cool to room temp.; add 2 ml acid mixt., (a), and 12.5 ml  $\text{H}_2\text{O}$ , rinsing down neck of flask. (If porcelain boat was used to introduce sample, remove boat with Pt wire; if glass capillary was used, filter digestion mixt. to remove capillary. Rinse filter and boat or capillary with 12.5 ml  $\text{H}_2\text{O}$  used to dil. sample.) Place flask on steam bath 15 min. to convert P to  $\text{H}_3\text{PO}_4$ . Remove from steam bath and pipet 15 ml molybdate reagent, (c), into center of sample digest, not down walls of flask. Let stand 2–3 min.; then gently swirl to mix contents, being careful to prevent reagents from splashing on neck of flask. Cover flask and set in dark place overnight.

Condition filter tube as described below and weigh empty tube. Connect tared filter tube to filtration assembly and transfer ppt to filter thru siphon tube. Wash flask alternately with 1–2 ml portions of  $\text{NH}_4\text{NO}_3$  soln, (b), and alcohol. Add 2–3 small rubber stoppers to digestion flask, shake to loosen any ppt, and transfer with  $\text{NH}_4\text{NO}_3$  soln, (b), and alcohol. Disconnect siphon tube; rinse ppt from tip and stopper into filter tube with  $\text{NH}_4\text{NO}_3$  soln, (b), and alcohol. Wash ppt with more  $\text{NH}_4\text{NO}_3$  soln, alcohol, and finally with acetone, and suck dry. Wipe filter tube with chamois skin, place in vertical position in vacuum desiccator contg no desiccant, and evacuate to 1 mm for 30 min. with mechanical vacuum pump in continuous operation. Release vacuum and weigh *immediately* to nearest 0.1 mg. (Rapid weighing is essential because of hygroscopic nature of ppt.)  $\text{Mg ppt} \times 0.014524 \times 100/\text{mg sample} = \%P$ .

#### RESULTS

Seven collaborators reported results for the two samples, Ilidar phosphate and Syntropan. The results are shown in Table 1. In this table,  $n$  is the number of replicates,  $\bar{x}$  is the mean, and  $s$  is the standard deviation of each collaborator's results. For Ilidar phosphate the interlaboratory mean,  $\bar{x}$ , was 9.33 versus the theoretical value of 9.29 per cent, and for Syntropan it was 7.65 versus the theoretical value of 7.64 per cent. This close agreement between the  $\bar{x}$  and theoretical values shows that there are no inherent errors of any magnitude in the proposed method. The standard deviation of the means,  $s_m$ , for Ilidar phosphate was 0.17 per cent, and that for Syntropan was 0.12 per cent; thus interlaboratory precision was satisfactory. Intralaboratory accuracy and precision were also satisfactory. The range of  $\bar{x}$  values was +0.28 and –0.24 per cent from the theoretical value, and the range of  $s$  values was from 0.01 to 0.20 per cent for the fourteen sets of analyses; averages were 0.06 per cent for Ilidar phosphate and 0.11 per cent for Syntropan.

TABLE 1.—*Determination of phosphorus by proposed method*

COLLABORATOR NO.	ILIDAR PHOSPHATE (9.29% P)			SYNTROPAN (7.64% P)		
	n	$\bar{x}$	s	n	$\bar{x}$	s
0	4	9.49	0.03	3	7.59	0.17
29	3	9.37	0.08	3	7.67	0.08
35	3	9.30	0.05	3	7.62	0.07
37	5	9.25	0.14	5	7.58	0.20
49	4	9.05	0.04	4	7.52	0.15
64	3	9.57	0.06	3	7.89	0.11
79	3	9.29	0.03	3	7.65	0.01
$\bar{\bar{x}}$		9.33	(0.06)		7.65	(0.11)
$s_m$		0.17			0.12	

It is recommended\* that the proposed method for phosphorus be adopted as first action.

#### COLLABORATORS

S. J. Tassinari, National Dairy Research Laboratories, Inc.  
 Irving G. Young, International Resistance Company.  
 Esther A. Bass, Hoffman-LaRoche, Inc.  
 Cecil H. Van Etten, Northern Regional Research Laboratory.  
 Ruth B. Kelly, Eastern Regional Research Laboratory.  
 John A. Means, Chas. Pfizer and Company, Inc.  
 G. A. Jones, E. I. DuPont de Nemours and Company.

#### REFERENCES

- (1) OGG, C. L., *This Journal*, 39, 408 (1956).
- (2) *Official Methods of Analysis*, 8th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1955, p. 115.
- (3) STEYERMARK, A., *Quantitative Organic Micro Analysis*, The Blakiston Co., Philadelphia, 1951, pp. 192-198.

\* For report of Subcommittee C and action of the Association, see *This Journal*, 40, 32, 33 (1957).

REPORT ON MICROANALYTICAL DETERMINATION  
OF PHOSPHORUS

By C. L. OGG (Eastern Regional Research Laboratory,\* Philadelphia 18,  
Pa.), *Associate Referee*

A preliminary study of methods for determining phosphorus was conducted last year (1). Collaborators received samples which they were to analyze by the method they were then using. They were asked to submit their analytical data and to return a form giving the details of their procedures.

---

\* A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

The results of last year's study can be summarized as follows: Eleven laboratories collaborated in the study. All used a wet-digestion procedure. Sulfuric-nitric acid oxidation seemed to be superior to sulfuric-perchloric acid. Seven laboratories determined the phosphorus gravimetrically as ammonium phosphomolybdate; four used spectrophotometric procedures. The means obtained by the two methods of analysis did not differ significantly.

The Associate Referee decided to write and test a gravimetric method patterned after the methods used in the preliminary study. This decision was based on the following factors:

(a) A majority of the collaborators used a gravimetric method in the 1955 study.

(b) The results obtained by the gravimetric methods were as good as those by the spectrophotometric methods, and perhaps better.

(c) No special apparatus is required. The apparatus used is also needed for the determination of nitrogen and the halogens.

(d) An official spectrophotometric micro method, originally designed for plant analysis, is already official (2). This method can be used, with appropriate weight and volume adjustments, for all micro samples except those which contain volatile phosphorus compounds such as alkyl phosphonates.

The tentative method followed the procedure described by Steyermark (3) because most of the collaborators had used this or a similar method in 1955. A copy of this procedure and two pure drug samples—Syntropan (3-diethylamino-2,2-dimethylpropyl tropate phosphate) and Ilidar phosphate (6-allyl-6,7-dihydro-5H-dibenz[c,e]azepine)—containing 7.64 and 9.29 per cent phosphorus, respectively, were sent to the collaborators participating in the phosphorus study. Purity of the samples was confirmed by carbon and hydrogen analyses.

#### METHOD FOR PHOSPHORUS

##### REAGENTS

(a) *Nitric-sulfuric acid mixture.*—Slowly pour 420 ml  $\text{HNO}_3$  into 580 ml  $\text{H}_2\text{O}$ ; then slowly add 30 ml  $\text{H}_2\text{SO}_4$ .

(b) *Ammonium nitrate soln.*—2%. Prep. 2% soln of  $\text{NH}_4\text{NO}_3$  in  $\text{H}_2\text{O}$ , add 2 drops  $\text{HNO}_3$ , and store in glass-stoppered bottle. Filter immediately before use.

(c) *Molybdate reagent.*—Dissolve 150 g powd.  $\text{NH}_4$  molybdate in 400 ml  $\text{H}_2\text{O}$  and cool under tap. Place 50 g  $(\text{NH}_4)_2\text{SO}_4$  in liter vol. flask, dissolve in mixt. of 105 ml  $\text{H}_2\text{O}$  and 395 ml  $\text{HNO}_3$ , and cool under tap. Pour cooled molybdate soln slowly into  $(\text{NH}_4)_2\text{SO}_4$  soln with constant stirring and cooling under tap. Dil. soln to 1 l, store in refrigerator 3 days, filter, and store in paraffin-lined, glass-stoppered, brown bottle in refrigerator. Filter reagent immediately before use and check by periodically analyzing std sample.

##### APPARATUS

(a) *Kjeldahl digestion flasks (30 ml), rack, and manifold.*—See 37.10(a) and (c).  
(b) *Filter tubes and filtration assembly.*—See 37.2(c) and (d).