FEEDS

Comparison of the TRAACS 800 with AOAC Methods for Calcium and Phosphorus in Feeds

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Existing automated methods for calcium and phosphorus in animal feeds have been upgraded to permit determination of protein, calcium, and phosphorus simultaneously from a single sample solution running on a new generation of automated instruments. Minor changes and improvements in chemistry are described, and results using the new method are compared to results using AOAC official atomic absorption (calcium) and gravimetric (phosphorus) methods. For calcium, *t*-tests showed no significant difference at the $P \leq 0.01$ level over a range of concentration levels from 0–25%. For phosphorus, *t*-tests showed no significant difference at the $P \leq 0.01$ level over concentration ranges of 0–2% and 2–6%. Significant differences did exist at a concentration range of 6–18%. The small existing bias in these ranges was shown to be due to irregularities in the phosphorus standard curve fit, caused by protein and/or calcium components in the combined standard solutions.

Currently, AOAC official methods for determining calcium (**968.08**) and phosphorus (**962.02, 968.08**) in animal feeds and are manual methods using atomic absorption and gravimetric techniques, respectively (1). In 1976, Hambleton (2) developed automated simultaneous methods for protein, calcium, and phosphorus, using the protein digest from a block digestor. The analysis used a second-generation autoanalyzer, the Technicon AAII. Recently, Bran + Luebbe (Technicon) Analyzing Technologies, Inc. released the TRAACS 800, a third-generation autoanalyzer. The numerous advantages of TRAACS 800 technology over AAII technology have been discussed previously (3). As with the AAII system, TRAACS methods for calcium and phosphorus can be used individually or as part of a simultaneous system to determine protein, calcium, and phosphorus. The TRAACS protein method has been previously collaborated (4), and is contained in AOAC method **990.02**. The simultaneous, 3 channel system was used for the present study of the equivalency of calcium and phosphorus analyses on the TRAACS 800 with AOAC official methods.

Manifold Improvements

TRAACS calcium chemistry has remained quite similar to the older AAII chemistry with only 2 noteworthy reagent changes. Triton X-100 and sodium acetate replaced Brij-35 and phosphate buffer, respectively.

TRAACS phosphorus chemistry has been altered to include the following improvements. Molybdate, vanadate, and wetting agent are contained in 3 separate reagents. Each is introduced independently into the flow stream and mixed on line. This affords a much greater degree of reagent stability as compared to the single reagent used on the AAII manifold. Wetting agent A has been replaced with Steol, a new phosphorus-compatible wetting agent. Water has been replaced with dilute sulfuric acid as the sample diluent, and the method of dilution has been changed from a resample loop in the AAII system to a dialysis membrane in the TRAACS system. The dilution loop was the least reliable part of the AAII manifold. Not only does the membrane serve as a diluter, it may also filter some components of the donor stream.

Note that the sample wash solution is 6% H_2SO_4 instead of water as indicated in the Bran + Luebbe supplied methods.

TRAACS Method Refinements

Initially, the system was configured as in the protein collaborative study (4). Additional precision was gained in the phosphorus and calcium channels by reducing the sampling rate from 120 to 90 samples/h. However, under these conditions, standards analyzed as samples consistently produced phosphorus and calcium results moderately below their theoretical concentrations (Table 1).

The low standard response problem was first addressed on the phosphorus channel. Acid effects and order-of-analysis effects were examined. An acid effect was noted whereby increasing acid concentration decreased phosphorus instrument response (Table 2). One varying source of acid in the combined standard solutions is the different aliquots of the calcium standard stock solution due to the HCl used in dissolving the $CaCO_3$. To minimize this effect, the amount of HCl in the calcium stock solution was reduced from 12.5 to 2.5 mL HCl/250 mL stock solution, because 2.5 mL HCl is the minimum amount of acid required to dissolve the $CaCO_3$ standard material. Minimizing the amount of acid (and thus the variation in concentration) improved, but did not completely correct, the low bias of standards analyzed as samples (Table 3).

As an additional test, 60 routine feed samples were ana-

Table 1. Standards analyzed as samples (mg/L) using protocol designated in protein collaborative study (4)

Standard number	Theoretical concn	Experimental concn[a]
	Calcium	
1	20	18.94
2	40	40.20
3	80	78.93
4	150	149.53
5	200	199.79
6	250	249.90
	Phosphorus	
1	10	9.39
2	20	19.09
3	40	39.49
4	48	47.53
5	64	63.62
6	80	79.59

[a] Mean value, $n = 10$.

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Table 2. Effect of acid on phosphorus response in solutions of constant phosphorus concentration

Solution number	H_2SO_4, mL/250 mL	Phosphorus,[a] mg/L
1	0	70.08
2	5	69.56
3	10	68.67
4	15	67.70

[a] Mean value, $n = 4$.

lyzed by the official gravimetric method and by the TRAACS method using both sets of standards (2.5 mL and 12.5 mL HCl in the calcium stock standard solution). For lower level (0–6%) phosphorus samples, results from the 2.5 mL HCl standards were in better agreement with gravimetric results than were results from the original standards (Table 4).

The run protocol, or order of analysis, was examined next. It was postulated that the series of null high standard peaks sampled before both the first calibrant and the gain peak could be artificially elevating the standard curve due to uncorrected carryover. An elevation of the standard curve would cause the sample results to be low. Zero concentration standard solution was substituted for the high standard solution in the null peaks preceding both the calibrant sequence and the gain peak. Phosphorus standards (made with 2.5 mL HCl) analyzed with this revised protocol displayed some improvement in agreement (Table 5).

Using the same standards and run protocol, 60 real samples were analyzed by the TRAACS method and the official gravimetric method (Table 6). Average differences were 0.007% for samples in the range 0–2% phosphorus, 0.047% for 2–6% phosphorus, 0.202% for 6–18% phosphorus, and 0.067% overall. While these differences are relatively small, *t*-tests showed a significant difference for samples >6% phosphorus.

Additional experimental work examining standard curve fitting led to the conclusion that irregularities in the lower end of the standard curve were probably the source of the slight bias in phosphorus results. When standards containing only phosphorus were examined, the phosphorus curve fit was improved, and the standards analyzed against themselves indicated that the bias would be corrected in both the right direction and magnitude. However, it was decided that a 3-component standard system was the practical alternative, so further attempts at completely eliminating the remaining phosphorus bias were not pursued.

Table 3. Phosphorus standards analyzed as samples (mg/L) with 12.5 and 2.5 mL HCl in calcium stock standard

Standard number	Theoretical concn	Experimental concn[a]
	12.5 mL HCl	
1	10	9.39
2	20	19.09
3	40	39.49
4	48	47.53
5	64	63.62
6	80	79.59
	2.5 mL HCl	
1	0	0.14
2	15	13.97
3	30	29.13
4	45	44.92
5	60	60.39
6	80	79.65

[a] Mean value, $n = 6$.

Table 4. Routine feed samples ($n = 60$) analyzed by official gravimetric (962.02 C (b)) method and TRAACS method, using 2.5 and 12.5 mL HCl in calcium stock standard

Phosphorus, %	% Mean difference (962.02 − TRAACS)	Significance ($P = 0.05$)
	2.5 mL HCl	
0–2	−0.004	No
2–6	−0.047	No
6–18	−0.202	Yes
	12.5 mL HCl	
0–2	−0.025	Yes
2–6	−0.048	Yes
6–18	−0.153	Yes

On the calcium channel, reducing the acid concentration in the calcium stock standard solution greatly reduced the low bias of standards analyzed as samples (Table 7). Moreover, the combination of the revised protocol and 2.5 mL acid in the calcium stock standard eliminated the bias (Table 8). Under these conditions, there was no difference between the TRAACS method and the official atomic absorption method over a wide range of routine feed samples (Table 9).

It should be noted that the change of phosphorus and calcium null concentrations from high to low standards, if also applied to the protein channel, would technically be a change from what was specified in the protocol used in the TRAACS protein collaborative study. Several experiments were designed to test for any adverse effect on the protein channel.

Standards were analyzed as samples in random order on the protein channel in runs where high as compared to low standards were used for null peaks, at both 90 and 120 samples/h. Means of 4 values for each standard produced as good or better agreement with theoretical values with the low null protocol at each sampling rate.

A group of 39 routine samples were also analyzed using the high vs low null concentrations, again at both 90 and 120 samples/h. *t*-Tests were used to check for any significant difference in results. At 120 samples/h, the mean difference (high minus low null protocols) was 0.05% protein. The *t* value was 0.728, not significantly different at the 95% confidence level. At 90 samples/h the mean difference (high minus low null protocols) was 0.07% protein. The *t* value was 2.143, just significant at the 95% confidence level. However,

Table 5. Phosphorus standards as samples (mg/L) using revised protocol of low nulls

Standard number	Theoretical concn	Experimental concn[a]
1	0	0.22
2	15	14.77
3	30	29.79
4	45	44.83
5	60	60.19
6	80	80.26

[a] Mean value, $n = 6$.

Table 6. Determination of phosphorus in routine feed samples by official gravimetric method and TRAACS method, using 2.5 mL HCl in calcium stock standard and revised protocol of low nulls[a]

Gravimetric[b]	TRAACS[b]	Diff.[b]
0.604	0.601	0.003
0.712	0.699	0.013
0.756	0.750	0.006
0.853	0.879	−0.026
0.862	0.847	0.015
0.866	0.849	0.017
0.870	0.862	0.008
0.929	0.954	−0.025
1.007	1.026	−0.019
1.012	0.996	0.016
1.101	1.080	0.021
1.172	1.167	0.005
1.223	1.206	0.017
1.248	1.246	0.002
1.310	1.288	0.022
1.337	1.352	−0.015
1.389	1.383	0.006
1.454	1.538	−0.084
1.471	1.454	0.017
1.626	1.726	−0.100
1.660	1.727	−0.067
1.711	1.679	0.032
1.826	1.819	0.007
1.884	1.871	0.013
1.885	1.884	0.001
1.986	1.982	0.004
2.037	2.156	−0.119
2.103	2.065	0.038
2.280	2.237	0.043
2.495	2.442	0.053
3.443	3.406	0.037
3.564	3.444	0.120
3.852	3.951	−0.099
3.873	3.926	−0.053
3.939	3.903	0.036
4.368	4.570	−0.202
4.558	4.763	−0.205
4.654	4.549	0.105
4.671	4.889	−0.218
4.995	4.929	0.066
5.172	5.212	−0.040
5.195	5.142	0.053
5.670	5.950	−0.280
5.724	5.916	−0.192
5.912	5.941	−0.029
6.356	6.342	0.014
6.537	6.422	0.115
7.526	7.460	0.066
8.385	8.189	0.196
8.616	8.756	−0.140
9.210	9.189	0.021
9.370	9.567	−0.197
9.612	10.147	−0.535
10.582	10.806	−0.224
10.823	10.973	−0.150
11.690	12.011	−0.321
12.075	12.949	−0.874
12.142	12.503	−0.361
16.675	17.245	−0.570
17.815	17.886	−0.071

[a] *t*-Tests grouped by concn ranges at the $P \leq 0.01$ level. 0–2% (n = 26), no significant difference; 2–6% (n = 19), no significant difference; 6–19% (n = 15), significantly different; 0–18% (n = 60), significantly different.

[b] Percent phosphorus.

Table 7. Calcium standards as samples (mg/L) with 2.5 mL HCl in calcium stock standard

Standard number	Theoretical concn	Experimental concn[a]
1	0	0.16
2	50	49.29
3	100	99.68
4	150	149.90
5	200	199.84
6	250	248.34

[a] Mean value, n = 6.

because the standard deviation of the differences was only half that of the standard deviation of the differences at the 120 samples/h rate, it was judged that there was no practical difference in protein results. The change of null concentration does not introduce a change in protein results as compared to results from the configuration used in the protein collaborative study.

Conclusions

Phosphorus methodology on the TRAACS instrument is in good agreement with the official gravimetric method up to a level of 6% phosphorus. At levels above 6%, there is a slight high bias on the TRAACS. The bias is attributed to imperfect phosphorus curve fitting due to matrix interference in the 3-component mixed standard. TRAACS calcium methodology shows no statistical difference with the official atomic absorption method. Slight changes in sample run order on the TRAACS, which improve calcium and phosphorus results, are shown not to alter official TRAACS protein results.

METHOD FOR CALCIUM IN FEEDS

Principle

Samples are digested with H_2SO_4 in a block digestor and reacted with cresolphthalein in an alkaline medium (pH 10.6). Absorbance of calcium–cresolphthalein complex is measured in a flow cell at 570 nm. Magnesium interference is eliminated by adding 8-hydroxyquinoline.

Apparatus

(a) *Block digestor.*—Capable of maintaining a constant temperature of 410°C.

(b) *Continuous flow autoanalyzer.*—[Bran + Luebbe (Technicon) Analyzing Technologies, Inc. TRAACS 800) with calcium manifold 165-D010-01 (Figure 1).

Reagents

(a) *Wetting agent.*—Add 50 mL methanol to 50 mL Triton X-100[R] (Sigma Chem. Co., PO Box 14508, St. Louis, MO 63178), mix.

(b) *System wash solution.*—Add 1 mL wetting agent (a) to 1 L H_2O, mix.

(c) *0.3M Sodium acetate solution.*—Dissolve 40.8 g sodium acetate in ca 800 mL H_2O. Dilute to 1 L, mix, add 1 mL wetting agent (a), mix.

(d) *Sampler wash solution 6% H_2SO_4.*—Dissolve 60 mL H_2SO_4 in 800 mL H_2O, cool, dilute to 1 L, mix.

(e) *2N HCl solution.*—Add 165 mL HCl to ca 700 mL H_2O, mix, cool, dilute to 1 L, mix.

(f) *CPC solution.*—Pipet 20 mL 2N HCl (e) to 1 L volumetric flask. Add ca 100 mL H_2O. Add 2 g 8-hydroxyquinoline, swirl to dissolve. Add 50 mg accurately weighed *O*-

Table 8. Calcium standards as samples (mg/L) with 2.5 mL HCl in calcium stock standard solution using revised protocol of low nulls

Standard number	Theoretical concn	Experimental concn[a]
1	0	−0.09
2	50	50.00
3	100	100.10
4	150	149.40
5	200	200.20
6	250	250.27

[a] Mean value, $n = 6$.

cresolphthalein complexon sodium salt, swirl to dissolve. Dilute to volume with H_2O, mix. Vacuum filter through 0.45 μm porosity filter, add 1 mL wetting agent (**a**), mix.

(**g**) *AMP solution.*—Add 44.6 g 2-amino-2-methyl-1-propanol to about 800 mL H_2O, dilute to 1 L, mix.

(**h**) *Calcium stock solution, 2.5 mg Ca/mL.*—Add 1.5608 g NIST $CaCO_3$ (assuming a NIST certified value of 40.0442% Ca, dried 2 h at 105°C before use) to 250 mL volumetric flask. Add about 65 mL water and 2.5 mL HCl to dissolve, dilute to volume with H_2O, mix.

Standards

In 6 digestion tubes, prepare reagent blanks as in **976.06(G)**, paragraphs 1 and 2, beginning "Add 9 g K_2SO_4, 0.42 g HgO, and 15 mL H_2SO_4. . .". When spraying tubes after digestion, be sure to allow for addition of appropriate amounts of stock standard(s). Transfer reagent blanks to 250 mL volumetric flasks and pipet 0, 5, 10, 15, 20, 25 mL of Ca stock solution to each. Standards will contain 0, 50, 100, 150, 200, and 250 mg Ca/L. Standards are stable for approximately 2 months.

Determination

Prepare samples as in **976.06(G)**, paragraphs 1 and 2. Use the following parameters to analyze samples: 90 samples/h; 5 sample/wash ratio; no pecking; use base correction; quadratic standard curve fit; base not in calibration curve. Use protocol P,2N,6C,H,2L (10S,I) times *m*, H,2L,2N,G,4N,E, where *m* can be any number from 1–4.

Enter standard concentrations as mg Ca/L. Arrange standards in descending order, with gain peak referenced to the initial high standard. Null peaks should be zero concentration standard solution and internal standard peaks should be high standard solution. Exercise caution when using repeated

Table 9. Determination of calcium in routine feed samples by official atomic absorption method and TRAACS method, using 2.5 mL HCl in calcium stock standard and revised protocol of low nulls[a]

Atomic absorption[b]	TRAACS[b]	Diff.[b]	Atomic absorption[b]	TRAACS[b]	Diff.[b]
0.450	0.506	−0.056	6.870	6.781	0.089
0.470	0.523	−0.053	6.950	6.963	−0.013
0.650	0.710	−0.060	7.110	6.624	0.486
0.660	0.680	−0.020	7.240	7.507	−0.267
0.940	1.009	−0.069	7.310	7.453	−0.143
0.970	1.098	−0.128	7.800	8.509	−0.709
1.120	1.080	0.040	8.630	9.214	0.584
1.410	1.347	0.063	8.660	9.287	−0.627
1.480	1.461	0.019	10.170	10.307	−0.137
1.520	1.344	0.176	10.300	10.188	0.112
1.660	1.781	−0.121	10.310	10.522	−0.212
1.710	1.878	−0.168	10.380	9.729	0.651
1.950	2.005	−0.055	11.430	11.662	−0.232
2.130	2.032	0.098	11.850	12.142	−0.292
2.240	2.229	0.011	11.850	11.685	0.165
2.670	2.608	0.062	12.020	12.124	−0.104
3.270	3.419	−0.149	12.640	12.379	0.261
3.390	3.566	−0.176	12.700	12.749	−0.049
3.430	3.395	0.035	12.880	12.520	0.360
3.430	3.554	−0.124	13.800	13.329	0.471
3.480	3.402	0.078	13.900	13.543	0.357
3.660	3.701	−0.041	15.590	15.186	0.404
3.670	3.665	0.005	16.020	17.138	−1.118
3.840	3.902	−0.062	16.850	16.441	0.409
3.880	4.004	−0.124	16.940	17.274	−0.334
3.940	3.858	0.082	17.200	17.241	−0.041
4.010	4.277	−0.267	17.330	17.823	−0.493
4.020	4.027	−0.007	18.210	17.528	0.682
4.540	4.377	0.163	18.320	18.331	−0.011
5.330	5.352	−0.022	18.910	18.778	0.132
6.030	5.982	0.048	22.400	22.336	0.064
6.380	6.691	−0.311	23.900	23.077	0.823
6.520	6.384	0.136	24.320	24.270	0.050
6.780	6.889	−0.109			

[a] *t*-Tests grouped by concn ranges revealed no significant difference at the $P \leq 0.01$ level. 0–2% ($n = 13$), 2–6% ($n = 17$), 6–12% ($n = 19$), 12–25% ($n = 18$).

[b] Percent calcium.

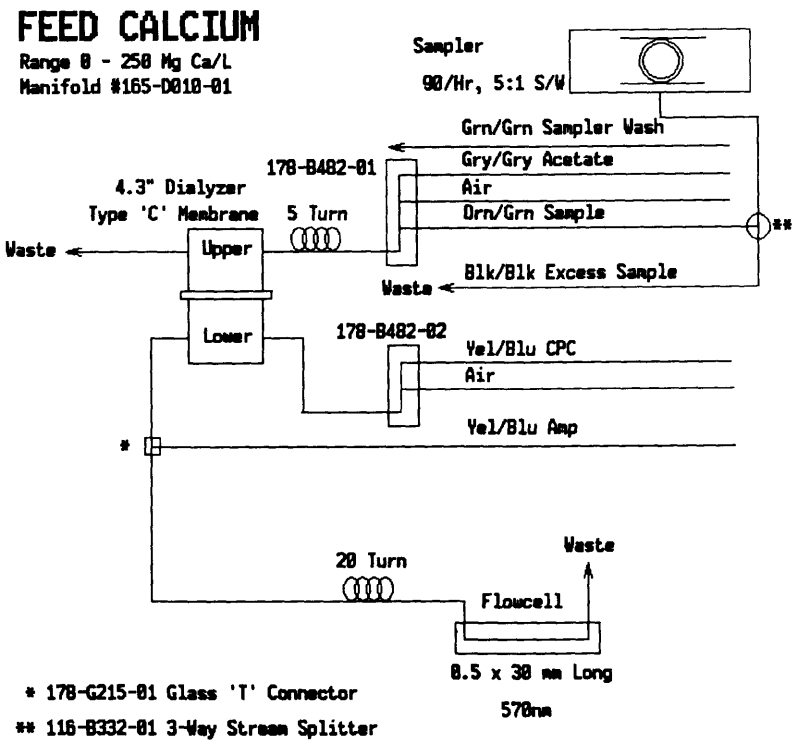

Figure 1. Flow diagram for calcium.

sampling from the same cup, other than for null peaks, because the probe will gradually contaminate the solution.

% Calcium = [calculated sample conc (mg/L) × 0.025]/mg SWT

METHOD FOR PHOSPHORUS IN FEEDS

Principle

Samples are digested on a block digestor with H_2SO_4 and reacted with molybdovanadate in acidic medium. Absor-

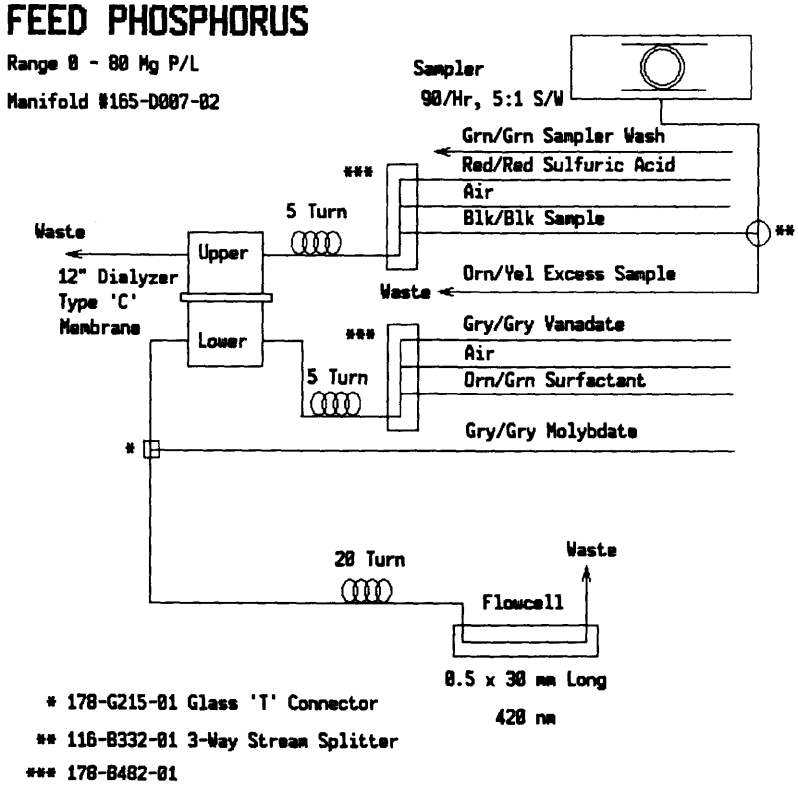

Figure 2. Flow diagram for phosphorus.

bance of phospho-molybdovanadate is measured in a flow cell at 420 nm.

Apparatus

(a) *Block digestor.*—Capable of maintaining a constant temperature of 410°C.

(b) *Continuous flow autoanalyzer.*—[Bran + Luebbe (Technicon) Analyzing Technologies, Inc. TRAACS 800] with phosphorus manifold 165-D007-02 (Figure 2).

Reagents

(a) *Steol 40% w/w solution.*—To 40 g of Steol 650 concentrate (Brand-Nu Laboratories, Inc., Meriden, CT 06450), add 60 mL H_20 slowly, dropwise, with stirring. Mix thoroughly.

(b) *System wash solution.*—Dilute 5 mL HCl to 1 L with H_2O, add 1 mL Steol 40% solution (a), mix.

(c) *Molybdate solution.*—Dissolve 20 g ammonium molybdate in about 800 mL H_2O, adjust pH to 7.0 with NH_4OH (about 10 mL), dilute to 1 L, mix.

(d) *Vanadate solution.*—Add 30 mL H_2SO_4 to about 700 mL H_2O. Add 150 mg ammonium metavanadate and dissolve. Cool, dilute to 1 L, mix.

(e) *H_2SO_4 solution.*—Add 60 mL H_2SO_4 to about 800 mL H_2O, cool, mix, dilute to 1 L, add 1 mL Steol 40% solution (a), mix.

(f) *Surfactant solution.*—Dilute 4 mL Steol 40% solution (a) to 1 L with H_2O, mix.

(g) *Sampler wash solution, 6% H_2SO_4.*—Dissolve 60 mL H_2SO_4 in 800 mL H_2O, cool, dilute to 1 L, mix.

(h) *Phosphorus stock solutions, PI: 0.5 g P/ mL and PII: 2.0 g P/ mL.*—PI: Add 0.4398 g NIST KH_2PO_4 (assuming a NIST certified value of 22.74% P: dried 2 h at 105°C before use) to 200 mL volumetric flask. Stir to dissolve and dilute to volume. PII: Add 0.8795 g NIST KH_2PO_4 (assuming a NIST certified value of 22.74% P: dried 2 h at 105°C before use) to 100 mL volumetric flask. Stir to dissolve and dilute to volume.

Standards

In 6 digestion tubes, prepare reagent blanks as in the first 2 paragraphs of **976.06(G)**, beginning "Add 9 g K_2SO_4, 0.42 g HgO, and 15 mL H_2SO_4. . .". When spraying tubes after digestion, be sure to allow for addition of appropriate amounts of stock standard(s). Transfer reagent blanks to 250 mL volumetric flasks and pipet 0, 15, 30, and 45 mL of PI stock solution (standards 1–4) and 15 and 40 mL of PII stock solution (standards 5 and 6). Standards will contain 0, 15, 30, 45, 60, and 80 mg P/L. Standards are stable for approximately 2 months.

Determination

Prepare samples as in **976.06(G)**, paragraphs 1 and 2. Use the following parameters to analyze samples: 90 samples/h; 5 sample/wash ratio; no pecking; use base correction; quadratic standard curve fit; base not in calibration curve. Use protocol P,2N,6C,H,2L (10S,I) times *m*, H,2L,2N,G,4N,E, where *m* can be any number from 1–4.

Enter standard concentrations as mg P/L. Arrange standards in descending order, with gain peak referenced to the initial high standard. Null peaks should be zero concentration standard solution and internal standard peaks are to be high standard solution. Exercise caution when using repeated sampling from the same cup, other than for null peaks, because the probe will gradually contaminate the solution.

$$\% \text{ Phosphorus} = [\text{calculated sample conc (mg/L)} \times 0.025]/\text{mg SWT}$$

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