Organic Application Note

Nitrogen/Protein in Milk and Liquid Milk Products

Accessories
502-186 Tin Foil Cups, 501-614 Spatula, disposable eyedroppers

Sample Weight
0.2 to 0.25 g

Calibration Standard
502-092 EDTA, Glycine solution (refer to reverse side for detailed instructions), or other suitable standard

Furnace Temperature
950°C

Flow Profile
All High

A. Blank (N)
0.00 (liquids), 0.04 (solids)

Protein Factor
6.38

Crucible Changing Interval
200 to 300 analyses

Procedure
1. Prepare the instrument by following the procedure as outlined in the operator's instruction manual (i.e. check gas supplies, perform any required maintenance, perform leak checks, etc.).
2. Analyze blanks (gas) until a plateau is reached. Analyze three to five additional blanks and set blank using these data.
3. Analyze five EDTA standards (using the 502-186 Tin Foil Cups) at 0.2 g and drift correct (if using the PC option). *NOTE: Each method on PC requires prior calibration with multiple weights of EDTA (0.035 to 0.4 g). If PC is not installed, analyze five EDTA standards and calibrate using the DSP screen menu.*
4. Weigh ~0.25 g glycine solution (~0.1 % N) into a 502-040 Tin Capsule (the capsule is not sealed). Analyze to verify instrument working range.
5. After mixing the sample well, weigh ~0.25 g milk into a 502-040 Tin Capsule (the capsule is not sealed), and analyze. *NOTE: Make sure if autoloader is used, that it is adjusted for slow, smooth movement so sample is not splashed from the capsule.*
6. Analyze a standard at end of set to verify calibration.

Sample	*Weight (g)*		*% Nitrogen*	*% Protein*
Milk (raw)	0.2461		0.597	3.81
	0.2000		0.600	3.83
	0.2125		0.602	3.84
	Mean	**=**	**0.600**	**3.83**
	Std. Dev.	**=**	**0.003**	**0.015**
Whey (raw)	0.2372		0.122	0.78
	0.2306		0.127	0.81
	0.2414		0.128	0.82
	Mean	**=**	**0.126**	**0.80**
	Std. Dev.	**=**	**0.003**	**0.02**

Sample	*Weight (g)*		*% Nitrogen*	*% Protein*
Blend	0.2473		1.745	11.13
	0.2342		1.752	11.18
	0.2244		1.751	11.17
	Mean	**=**	**1.729**	**11.16**
	Std. Dev.	**=**	**0.004**	**0.026**

FP-528

GLYCINE SOLUTION PREPARATION

1. The following formula can be used to make a specific concentration:

$$G = \frac{1}{\frac{[0.18658}{C} - 0.01]}$$

where: C = desired nitrogen concentration as percent
G = grams of glycine powder

Example for 1% solution:

$$G = \frac{1}{\frac{[0.18658}{1.00} - 0.01]} = 5.663$$

NOTE: A quick reference chart, shown below, shows the grams of glycine powder needed to reach given concentrations.

2. Place a flask on the balance and tare. The flask should be large enough to hold 100 ml (where 100 g = 100 ml).
3. Add the amount of glycine calculated in step 1 and record the weight.
4. Tare the balance and add 100 g of distilled water and record the weight.
5. Seal the flask and mix the contents.
6. To figure the exact concentration:

$$\% \text{ Nitrogen} = \frac{G\,(18.658)}{G + W}$$

where: G = weight in grams of glycine recorded in step 3
W = weight in grams of water recorded in step 4

7. If the distilled water is not pure, determining the nitrogen concentration may be necessary.
   a. Using the liquid injector, analyze five samples of distilled water.
   b. Average the nitrogen content of the five samples (A).
   c. Add this average to % nitrogen calculated for the calibration solution.

example: To make a calibration solution of approximately 0.3% nitrogen:
where: G = 1.672 g
W = 99.824 g
A = 0.004%

$$\frac{1.672(18.654)}{(1.672 + 99.824)} + 0.004 = 0.311\%$$

QUICK REFERENCE CONCENTRATION TABLE

Nitrogen Concentration	Grams of Glycine
0.10%	0.539 g
0.30%	1.634 g
0.50%	2.754 g
0.75%	4.188 g
1.00%	5.663 g

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