Organic Application Note

Nitrogen/Protein in Milk Using the Manual Injector

Accessories 601-470-110 Manual Injector

Sample Weight ~0.5 g

Calibration Standard Glycine Solution (see reverse side for detailed instructions), or other suitable standard

Furnace Temperature 950°C

Flow Profile All High

Atmospheric Blank (N) 0.00

Protein Factor 6.38

Crucible Changing Interval 200 to 300 analyses using 614-961-110 Porous Crucible

Analysis Time 4 minutes

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 glycine solution standards @ ~0.5 g using the procedure outlined in the manual liquid injector kit instructions (see 601-470-901, operation steps 5 to 10), 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. After mixing sample well, weigh ~0.5 g milk into a syringe. Analyze using the procedure outlined in the manual liquid injector kit instructions (see 601-470-901, operation steps 5 to 10). *NOTE: After injecting the sample, tap the syringe before removing to release the last drop of sample. Place syringe on balance with original tare to check weight. If some sample remains in syringe, adjust sample weight to correct for this difference.*
5. Analyze a glycine solution standard at the end of the set to verify calibration.

Sample	*Weight (g)*	*% Nitrogen*	*% Protein*
Milk, Reduced Fat 2%	0.4584	0.527	3.36
	0.4348	0.526	3.36
	0.4429	0.531	3.39
	0.4653	0.535	3.41
	0.5002	0.527	3.36
	0.4638	0.529	3.38
	0.4925	0.539	3.44

Sample	*Weight (g)*	*% Nitrogen*	*% Protein*
	0.5131	0.534	3.41
	0.4854	0.536	3.42
	0.4686	0.535	3.41
	0.5073	0.534	3.41
	0.4953	0.534	3.41
Average =	**0.532**		**3.40**
Std. Dev. =	**0.004**		**0.03**

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

LECO Corporation • 3000 Lakeview Ave. • St. Joseph, MI 49085-2396
Phone: 800-292-6141 • Fax: 269-982-8977
info@leco.com • www.leco.com • *ISO-9001 No. FM 24045*

Form No. 203-821-166 *7/03-REV1*