Application Note

Instrument: FP628

Determination of Nitrogen/Protein in Grains, Feeds, and Pet Food

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Introduction

Protein is one of the most significant nutrient components. The accurate and precise determination of protein not only plays a role in the characterization of nutritional or dietary value in feed materials, but is also key to the economic value of these materials. Protein in feed products is most commonly calculated using the measured nitrogen in the sample and a multiplier (protein factors vary according to the sample matrix).

The LECO FP628 is a combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a vertical quartz furnace for the sample combustion process resulting in an analysis time of 3.5 minutes with no metal oxidizer reagents in the primary or secondary furnace. A thermoelectric cooler removes the moisture in the combustion gas without the use of chemical reagents. A 3 or 10 cc volume of combustion gas is taken using a combustion gas collection and handling system. The combustion gas collection and handling system achieves a low cost-per-analysis by reducing the amount of chemical reagents used for scrubbing and converting the nitrogen oxide combustion gas to nitrogen. A thermal conductivity (TC) cell is used for the detection of nitrogen in the combustion gas.

Sample Preparation

Samples must be a uniform consistency to produce suitable results. Refer to AOAC 990.03 and 992.23 for sampling and sample preparation information.

Accessories

502-186 Tin Foil Cup, 502-397 Large Tin Foil Cup, 502-338 Small Gel Cap, 502-382 Medium Gel Cap, 502-810 Large Gel Cap

Calibration Samples

502-092 EDTA, 502-642 Phenylalanine, or other suitable pure compound/reference material

Reference Methods

AOAC 990.03, AOAC 992.23

Protein Factor

Protein factor varies by sample matrix

Analysis Parameters

Combustion Temperature:	950°C
Afterburner Temperature:	850°C

Instrument Model and Configuration

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analytical gas compared to the constant thermal conductivity of the reference gas. The greater the difference between the thermal conductivity of the carrier gas and the analyte gas, the greater the sensitivity of the detector. The FP628 is available in models that support either the use of helium or argon as the instrument's carrier gas for the thermal conductivity cell.

When used as a carrier gas, helium provides the highest sensitivity, providing the best performance at the lower end of the nitrogen range. Helium models also offer the additional advantage of replacing the 10 cc aliquot loop with a 3 cc loop within the instrument's gas collection and handling system. The 10 cc aliquot loop optimizes the instrument for the lowest nitrogen range and best precision. The 3 cc aliquot loop extends reagent life expectancy by approximately three fold compared to the 10 cc aliquot loop, while providing the lowest cost-per-analysis with minimal impact on practical application performance (see Typical Results section).

Due to the recent history of low supply and general availability issues for helium gas, the argon model was developed to utilize argon as a carrier gas. Since the thermal conductivity difference between argon and nitrogen is not as great as the thermal conductivity difference between helium and nitrogen, the detector is inherently less sensitive with argon as a carrier gas. The argon model (10 cc aliquot only) has a similar practical application performance compared to the helium model, operating with equivalent instrument and method configurations (see Typical Results section).

Note: Changing carrier gas and aliquot loop size requires hardware changes within the instrument.

Method Selection

Both the helium and argon (10 cc aliquot only) models and aliquot loop size system configurations have the option of a High Precision method or High Throughput method. The High Precision method is optimized to deliver the best performance in terms of nitrogen results resulting in an analysis time of 4 minutes. The High Throughput method is optimized to deliver the fastest analysis time of 3.5 minutes (210 seconds) while maintaining instrument performance specifications and acceptable practical application performance (see Typical Results section).

Element Parameters - Helium Model

	High Precision (10 cc & 3 cc)	High Throughput (10 cc & 3 cc)
Analyze	Yes	Yes
Baseline Delay Time	4 seconds	4 seconds
Min. Analysis Time	40 seconds	40 seconds
Comparator Level	1.00	1.00
Endline Time	2 seconds	2 seconds
Conversion Factor	1	1
Significant Digits	5	5
TC Baseline Time	10 seconds	6 seconds

Element Parameters - Argon Model

	High Precision (10 cc)	High Throughput (10 cc)
Analyze	Yes	Yes
Baseline Delay Time	4 seconds	4 seconds
Min. Analysis Time	60 seconds	60 seconds
Comparator Level	1.00	1.00
Endline Time	2 seconds	2 seconds
Conversion Factor	1	1
Significant Digits	5	5
TC Baseline Time	10 seconds	6 seconds

Burn Profile

Burn Steps	*Time (seconds)*	*Furnace Flow*
1	90 seconds	High

Macro Ballast Parameters

	High Precision	High Throughput
Equilibrate Time	30 seconds	10 seconds
Not Filled Timeout	300 seconds	300 seconds
Aliquot Loop Fill Pressure Drop	200 mm Hg	200 mm Hg
Equilibrate Pressure Time	8 seconds	4 seconds

**Refer to FP628 Operator's Instruction Manual for Method Parameter definitions.*

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Determine blank.
   a. Enter 1.0000 g mass into Sample Login (F3) using Blank as the sample name.
   b. Select 10 replicates.
   c. Initiate the analysis sequence (F5).
   d. Set the blank using at least five results following the procedure outlined in the operator's instruction manual.

   Note: Blank precision for nitrogen should be <0.001%.
3. Calibrate/Drift Correct.
   a. Weigh ~0.25 g of EDTA calibration sample into a tin foil cup and seal.
   b. Enter mass and sample indentification into Sample Login (F3).
   c. Transfer sample to the appropriate position of the sample carousel.
   d. Repeat steps 3a through 3c a minimum of five times for each calibration/drift sample used.
   e. Initiate the analysis sequence (F5).
   f. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   g. Verify the calibration by analyzing ~0.1 g of phenylalanine or other suitable reference material.
4. Analyze Samples.
   a. Weigh 0.2 to 0.3 g of the unknown sample into a tin foil cup and seal.
   b. Enter mass and identification information into Sample Login (F3).
   c. Transfer sample to the appropriate position of the sample carousel.
   d. Initiate the analysis sequence (F5).
5. Atmospheric Blank.
   Some atmosphere will be trapped with the sample when it is encapsulated in the tin foil cup. Some atmosphere may also be present when using the Gel caps as well. This will cause biased nitrogen results at low nitrogen concentrations, therefore an atmospheric blank should be determined and entered using the following procedure: Analyze an inert material such as LECO 501-427 Com-Aid several times using similar weights of the Com-Aid to the weight of samples being analyzed. Enter the actual weight of the Com-Aid (Com-Aid should be baked-off in a muffle furnace at ~1000°C for 15 minutes, allowed to cool, and stored for up to 24 hours in a desiccator until used). The nitrogen value obtained is considered the atmospheric blank and can be automatically compensated using the FP628 software. Refer to the operator's instruction manual for details regarding the setting of the atmospheric blank.

Note: The FP628 can be calibrated using several replicates of a single mass (nominal 0.25 g) of EDTA utilizing a single standard calibration. The calibration can be verified by analyzing a pure compound that is different than the material used for calibration, such as phenylalanine (~0.1 g) or nicotinic acid (~0.1 g). Multi-point (fractional weight or multiple calibration samples) may also be used to calibrate if desired.

TYPICAL RESULTS - High Precision Method

	3 cc Helium			10 cc Helium			10 cc Argon		
	Mass(g)	*% N*	*% Protein*	*Mass(g)*	*% N*	*% Protein*	*Mass(g)*	*% N*	*% Protein*
Grain A	0.2487	6.275	39.22	0.2599	6.296	39.35	0.2518	6.267	39.17
	0.2525	6.285	39.28	0.2526	6.292	39.32	0.2526	6.219	38.87
	0.2475	6.222	38.88	0.2416	6.300	39.37	0.2514	6.221	38.88
	0.2517	6.252	39.08	0.2452	6.292	39.32	0.2593	6.231	38.95
	0.2496	6.258	39.12	0.2493	6.308	39.42	0.2483	6.254	39.09
	Avg =	***6.258***	***39.12***	***Avg =***	***6.298***	***39.36***	***Avg =***	***6.238***	***38.99***
	s =	***0.024***	***0.15***	***s =***	***0.007***	***0.04***	***s =***	***0.021***	***0.13***
Grain B	0.2437	4.754	29.71	0.2494	4.819	30.12	0.2434	4.795	29.97
	0.2561	4.771	29.82	0.2532	4.814	30.09	0.2478	4.812	30.07
	0.2470	4.755	29.72	0.2444	4.829	30.18	0.2521	4.792	29.95
	0.2494	4.758	29.74	0.2545	4.817	30.11	0.2568	4.785	29.91
	0.2478	4.786	29.91	0.2405	4.792	29.95	0.2433	4.788	29.92
	Avg =	***4.765***	***29.78***	***Avg =***	***4.814***	***30.09***	***Avg =***	***4.794***	***29.96***
	s =	***0.014***	***0.08***	***s =***	***0.014***	***0.09***	***s =***	***0.011***	***0.06***
Dry Pet Food A	0.2515	4.451	27.82	0.2566	4.416	27.60	0.2461	4.398	27.48
	0.2472	4.418	27.61	0.2429	4.398	27.49	0.2424	4.409	27.56
	0.2419	4.419	27.62	0.2560	4.420	27.62	0.2404	4.416	27.60
	0.2478	4.418	27.61	0.2487	4.408	27.55	0.2466	4.407	27.54
	0.2449	4.415	27.59	0.2545	4.414	27.58	0.2597	4.432	27.70
	Avg =	***4.424***	***27.65***	***Avg =***	***4.411***	***27.57***	***Avg =***	***4.412***	***27.58***
	s =	***0.015***	***0.10***	***s =***	***0.009***	***0.05***	***s =***	***0.013***	***0.08***
Dry Pet Food B	0.2440	5.732	35.83	0.2544	5.699	35.62	0.2457	5.661	35.38
	0.2540	5.690	35.56	0.2518	5.694	35.59	0.2540	5.685	35.53
	0.2527	5.657	35.36	0.2491	5.711	35.69	0.2543	5.700	35.62
	0.2440	5.676	35.47	0.2512	5.680	35.50	0.2465	5.667	35.42
	0.2441	5.701	35.63	0.2614	5.726	35.79	0.2504	5.720	35.75
	Avg =	***5.691***	***35.57***	***Avg =***	***5.702***	***35.64***	***Avg =***	***5.687***	***35.54***
	s =	***0.028***	***0.18***	***s =***	***0.017***	***0.11***	***s =***	***0.024***	***0.15***

TYPICAL RESULTS - High Throughput Method

	3 cc Helium			10 cc Helium			10 cc Argon		
	Mass(g)	*% N*	*% Protein*	*Mass(g)*	*% N*	*% Protein*	*Mass(g)*	*% N*	*% Protein*
Grain A	0.2428	6.308	39.42	0.2433	6.279	39.25	0.2533	6.230	38.94
	0.2502	6.249	39.06	0.2496	6.283	39.27	0.2462	6.273	39.21
	0.2556	6.257	39.11	0.2483	6.285	39.28	0.2481	6.235	38.97
	0.2543	6.271	39.19	0.2539	6.282	39.26	0.2491	6.237	38.98
	0.2473	6.273	39.21	0.2436	6.270	39.19	0.2512	6.255	39.09
	Avg =	***6.272***	***39.20***	***Avg =***	***6.280***	***39.25***	***Avg =***	***6.246***	***39.04***
	s =	***0.023***	***0.14***	***s =***	***0.006***	***0.04***	***s =***	***0.018***	***0.11***
Grain B	0.2460	4.790	29.94	0.2486	4.805	30.03	0.2546	4.744	29.65
	0.2478	4.799	29.99	0.2446	4.805	30.03	0.2486	4.790	29.94
	0.2493	4.840	30.25	0.2561	4.796	29.97	0.2480	4.788	29.92
	0.2536	4.817	30.11	0.2479	4.798	29.99	0.2526	4.759	29.74
	0.2536	4.795	29.97	0.2505	4.802	30.01	0.2437	4.795	29.97
	Avg =	***4.808***	***30.05***	***Avg =***	***4.801***	***30.01***	***Avg =***	***4.775***	***29.84***
	s =	***0.021***	***0.13***	***s =***	***0.004***	***0.03***	***s =***	***0.022***	***0.14***
Dry Pet Food A	0.2490	4.448	27.80	0.2551	4.460	27.87	0.2446	4.443	27.77
	0.2417	4.441	27.76	0.2418	4.449	27.81	0.2508	4.455	27.84
	0.2412	4.457	27.85	0.2449	4.441	27.76	0.241	4.379	27.37
	0.2416	4.449	27.81	0.2417	4.437	27.73	0.2512	4.433	27.71
	0.2498	4.426	27.66	0.2437	4.457	27.86	0.2556	4.437	27.73
	Avg =	***4.444***	***27.78***	***Avg =***	***4.449***	***27.81***	***Avg =***	***4.429***	***27.68***
	s =	***0.012***	***0.07***	***s =***	***0.010***	***0.06***	***s =***	***0.029***	***0.18***
Dry Pet Food B	0.2435	5.665	35.40	0.2500	5.690	35.56	0.2538	5.719	35.74
	0.2548	5.643	35.27	0.2439	5.701	35.63	0.2544	5.668	35.43
	0.2454	5.710	35.69	0.2563	5.718	35.74	0.2497	5.759	36.00
	0.2512	5.710	35.68	0.2540	5.717	35.73	0.2446	5.777	36.11
	0.2492	5.654	35.34	0.2465	5.736	35.85	0.2474	5.641	35.26
	Avg =	***5.676***	***35.48***	***Avg =***	***5.712***	***35.70***	***Avg =***	***5.713***	***35.71***
	s =	***0.031***	***0.20***	***s =***	***0.018***	***0.11***	***s =***	***0.058***	***0.36***

Note: A protein factor of 6.25 was used to calculate the % Protein. This protein factor was obtained from the United States Department of Agriculture, Circular No. 183. The choice of protein factor to be used for determining protein content in different materials is the subject of some debate. As a result, if being used for commerce, the value of this conversion factor should be part of the contractual agreement between buyer and seller.

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