Optimizing a Total Protein Combustion Instrument for Maximum Sample Throughput and Lowest Cost-per-Analysis

Dennis Lawrenz, Mason Marsh, Jeffery Gast, Fred Schultz | LECO Corporation, St. Joseph, MI

Introduction

Total protein in foods and feeds is calculated using the total measured nitrogen content in the sample and a multiplier specific to the sample matrix. Nitrogen determination is commonly performed by one of two major methods—a classical wet chemistry (Kjeldahl) technique or combustion instrument-based technique—with the combustion technique gaining popularity due to several advantages including shorter analysis times, ease of operation, and improved safety characteristics.

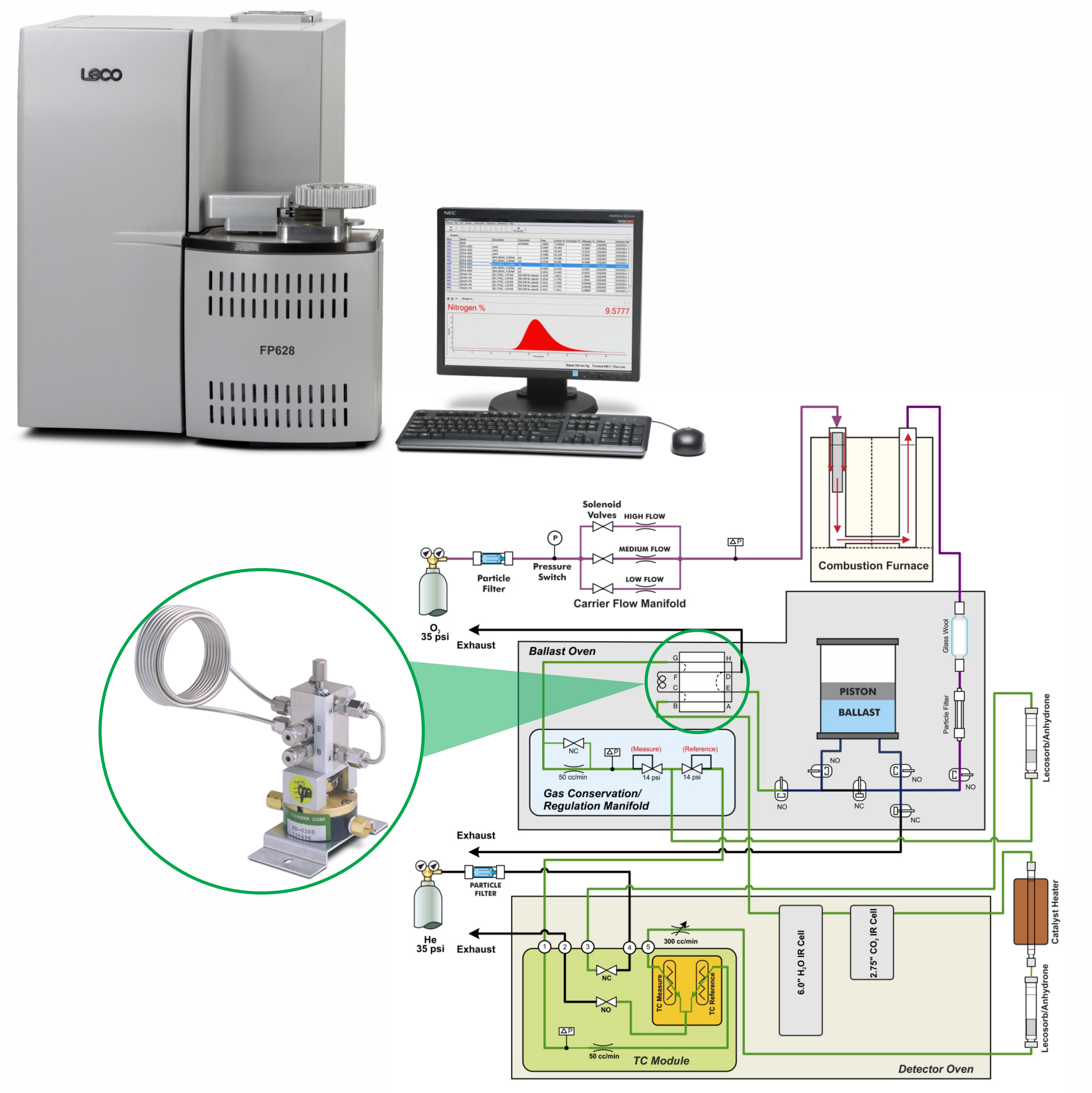
Total nitrogen combustion instruments use a high-temperature furnace with a pure oxygen environment to combust the sample. The nitrogen gases within the sample combustion gas are subsequently reduced to N_2 gas, and are detected using a thermal conductivity (TC) detector, with the excess oxygen and other sample combustion products being removed using multiple reagents within the instrument flow path. Helium or argon gas is typically used for the carrier gas within the instrument. Total nitrogen combustion instruments manufactured by LECO Corporation utilize a system which collects and equilibrates the combustion gas, and then samples a small aliquot of the equilibrated combustion gas for nitrogen measurement, thereby reducing the reagent demand and cost associated with treating this gas for nitrogen measurement.

LECO total nitrogen combustion instruments can be optimized for analysis time, resulting in the maximum throughput for the instrument using a few variables within the method parameters and the choice of carrier gas (helium or argon). The volume of the equilibrated combustion gas aliquot can be optimized for the lowest nitrogen detection range and best nitrogen precision while maximizing the reagent lifetime, resulting in the lowest cost-per-analysis.

This poster presents the optimization of a total nitrogen combustion instrument for maximum sample throughput by optimizing the instrument method parameters, and concurrently reducing the cost-per-analysis by optimizing the aliquot gas volume while maintaining the instrument's performance requirements. Data presented includes common food, feed, and reference materials analyzed with a LECO FP628 instrument using helium and argon gas as a TC carrier gas.

Methodology

Equipment | LECO FP628



FP628 Theory Of Operation

A pre-weighed and encapsulated sample is placed in the instrument's loader where the sample will be transferred to the purge chamber directly above the furnace, eliminating the atmospheric gases from the transfer process. The sample is then introduced to the primary furnace containing only pure oxygen, resulting in a rapid and complete combustion (oxidation) of the sample. Nitrogen present in the sample is oxidized to NO_x and swept with the sample combustion gas by the oxygen carrier from the furnace through a pre-cooler and thermoelectric cooler to remove the water vapor from the sample combustion gas. The combustion gas is then collected in a vessel known as a ballast for equilibration. The homogenized gases from the ballast are swept through an aliquot loop (3 or 10 cc volume), and then passed into a helium or argon carrier gas. The NO_x gases are passed through a reduction tube filled with copper to reduce the gases to N_2 and remove any excess oxygen present from the combustion process. The aliquot gas passes through Lecosorb and Anhydrone to remove CO_2 and the water generated during the CO_2 trapping process, and then into a thermal conductivity cell (TC) utilized to detect the N_2.

Optimization Parameters

The instrument's analysis time and resulting throughput is determined by the method parameters. The method parameters listed under High Precision below were optimized to deliver the best instrument performance for precise nitrogen results. The method parameters listed under High Throughput below were optimized to deliver the fastest analysis time and thus the highest throughput for the instrument while maintaining an acceptable practical performance for precision of nitrogen results. The instrument carrier gas configuration of helium or argon are listed separately as the minimum analysis time method parameter is dependent upon the carrier gas being used, and this parameter will affect the total instrument analysis time.

Method Parameters	Helium Carrier Gas		Argon Carrier Gas	
	High Precision	*High Throughput*	*High Precision*	*High Throughput*
Minimum Analysis Time (sec)	40	40	60	60
TC Baseline Time (sec)	10	6	10	6
Ballast Equilibrate Time (sec)	30	10	30	10
Aliquot Equilibrate Time (sec)	8	4	8	4
Total Analysis Time (min)	**4**	**3.5**	**4.5**	**4**
Throughput (samples/hr)	***15***	***17***	***13***	***15***

The instrument's reagent usage and resulting reagent costs are directly proportional to the volume of the combustion gas aliquot loop used in the instrument's system configuration. Instruments configured to utilize helium as a carrier not only have the highest sensitivity for the TC cell detector providing the best performance at the lower end of the nitrogen range, but also offer the ability to decrease the volume of the aliquot loop. Data and costs were calculated for both the standard 10 cc aliquot loop and a 3 cc aliquot loop for the helium carrier gas instrument configurations.

Results

The FP628 methods and instrument configurations were calibrated using the pure chemical reference material EDTA (LECO 502-092, lot 1061), and a single standard calibration with the mass group listed in Table 1 below. When using a nominal sample mass of ~0.25 g, the calibration covers a range of 9.56 to ~0.008% nitrogen (59.75 to 0.05% protein, using a 6.25 protein multiplier).

EDTA (LECO 502-092, lot 1061)	
Sample mass	~0.25 g
Certificate Value	9.56 ± 0.04% N
Replicate number	3
Standard Deviation	0.02
RSD (%)	0.2

Table 1. Nitrogen Calibration Data (High Throughput 10 cc Helium)

The calibration was verified using the pure chemical reference material phenylalanine (LECO 502-642, lot 1014). A ~0.10 g sample of phenylalanine was analyzed after the calibration and interspersed within the food and feed samples analysis sequence, serving as a calibration and stability check sample. The ~0.10 g sample of phenylalanine represents the equivalent of ~3.37% N (21.08% protein) in a sample with a nominal ~0.25 g sample mass.

Phenylalanine (LECO 502-642, lot 1014)	
Sample mass	~0.10 g
Certificate Value	8.43 ± 0.05% N
Replicate number	12
Average % N	8.42
Standard Deviation	0.03
RSD (%)	0.4
Recovery (%)	99.9

Table 2. Nitrogen Check Sample Data (High Throughput 10 cc Helium)

Sample Data

Sample suites for animal feed and human food were chosen for this study to demonstrate the analytical performance and application capability of the instrument methods and configurations to deliver results for total nitrogen/protein. The sample suites represent typical animal feed and human food materials that are routinely tested for, quality ranked on, and characterized for their nitrogen/protein content. Protein is determined by multiplying the nitrogen percent by a factor. This protein factor varies depending on the type of material being analyzed. The products analyzed have a factor of 6.25.

Two fodder and dry pet food samples were chosen for the animal feed samples. The fodder samples were composite feeds intended for animal husbandry. The dry pet food samples were commercially sourced dry pet food products intended for domesticated animals.

Feed Samples	Sample mass & replicate number		10 cc Helium		3 cc Helium		10 cc Argon	
			High Throughput	High Precision	High Throughput	High Precision	High Throughput	High Precision
			% N	% N	% N	% N	% N	% N
Fodder Sample A	~0.25 g n=5	Average	6.28	6.297	6.272	6.258	6.246	6.238
		Std Dev	0.006	0.007	0.023	0.024	0.018	0.021
		RSD (%)	0.10	0.11	0.37	0.38	0.29	0.34
Fodder Sample B	~0.25 g n=5	Average	4.801	4.814	4.808	4.765	4.775	4.794
		Std Dev	0.004	0.014	0.021	0.014	0.022	0.01
		RSD (%)	0.08	0.29	0.44	0.29	0.46	0.21
Dry Pet Food Sample A	~0.25 g n=5	Average	4.449	4.411	4.444	4.424	4.429	4.412
		Std Dev	0.01	0.008	0.031	0.015	0.029	0.013
		RSD (%)	0.22	0.18	0.70	0.34	0.65	0.29
Dry Pet Food Sample B	~0.25 g n=5	Average	5.712	5.702	5.676	5.691	5.713	5.687
		Std Dev	0.017	0.017	0.012	0.028	0.058	0.024
		RSD (%)	0.30	0.30	0.21	0.49	1.02	0.42

The four human food samples are flour samples representing a wheat, rye, rice, and corn matrix source. The flour samples were all LECO Reference Materials with certified values for nitrogen. The flour reference material certified nitrogen values provide an assessment of data accuracy in addition to precision.

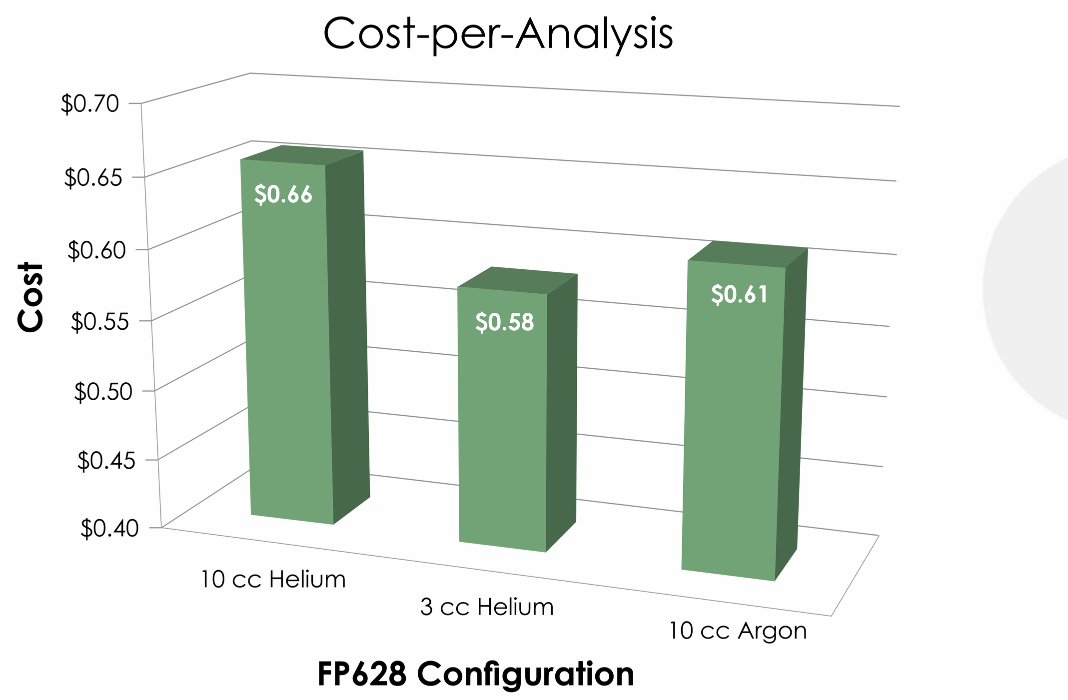
Flour Samples	Sample mass & replicate number		10 cc Helium		3 cc Helium		10 cc Argon	
			High Throughput	High Precision	High Throughput	High Precision	High Throughput	High Precision
			% N	% N	% N	% N	% N	% N
Wheat Flour Sample 502-274 lot 1015 Cert. Value 2.68 ±0.03% N	~0.25 g n=5	Average	2.673	2.671	2.665	2.667	2.680	2.670
		Std Dev	0.007	0.013	0.007	0.007	0.024	0.013
		RSD (%)	0.26	0.49	0.26	0.26	0.90	0.49
Rye Flour Sample 502-275 lot 1007 Cert. Value 1.74 ±0.06% N	~0.25 g n=5	Average	1.749	1.747	1.735	1.721	1.698	1.717
		Std Dev	0.005	0.008	0.008	0.005	0.016	0.029
		RSD (%)	0.29	0.46	0.46	0.29	0.94	1.69
Rice Flour Sample 502-278 lot 1013 Cert. Value 1.17 ±0.05% N	~0.25 g n=5	Average	1.197	1.199	1.197	1.179	1.159	1.154
		Std Dev	0.004	0.002	0.007	0.03	0.02	0.012
		RSD (%)	0.33	0.17	0.58	2.54	1.73	1.04
Corn Flour Sample 501-563 lot 1014 Cert. Value 1.21 ±0.02% N	~0.25 g n=5	Average	1.218	1.226	1.228	1.226	1.206	1.21
		Std Dev	0.009	0.006	0.019	0.008	0.019	0.014
		RSD (%)	0.74	0.49	1.55	0.65	1.58	1.16

Cost-per-Analysis

The cost-per-analysis of the FP628 is comprised of costs associated with compressed gases, reagents, and a few hardware parts that will need replacement based upon the usage of the instrument. The itemized list of gases, reagents, and parts with usage or longevity along with resulting costs are given in Table 3. The overall cost-per-analysis for the instrument can be reduced compared to the standard instrument configuration (10 cc aliquot loop) using helium carrier gas by changing the carrier gas to argon or reducing the aliquot loop volume. Utilizing argon as a carrier gas offers not only a lower cost option, but also has a relatively stable availability and price. Decreasing the volume of the aliquot loop from 10 cc to 3 cc not only offers a lower cost for reagents, but also provides a longer period of time between the required reagent maintenance intervals, thus increasing the instrument's uptime. The differences from the standard 10 cc helium configuration in reagent longevity and resulting costs are italicized in bold within Table 3.

Consumable	LECO Part#	Amount	Cost	Amount Used	10 cc Helium		3 cc Helium		10 cc Argon	
					# of Analysis	Cost/ Analysis	# of Analysis	Cost/ Analysis	# of Analysis	Cost / Analysis
Oxygen	N/A	9350 L	$200.00	10.4 L	900	$0.222	900	$0.222	900	$0.222
Helium	N/A	8000 L	$250.00	2 L	4000	$0.063	4000	$0.063	N/A	N/A
Argon	N/A	9300 L	$75.00	2 L	N/A	N/A	N/A	N/A	***4500***	***$0.017***
Copper Sticks	502-878	100 grams	$32.00	100 grams	750	$0.043	***2000***	***$0.016***	750	$0.043
Anhydrone	501-171-HAZ	454 grams	$40.70	8 grams	150	$0.005	***450***	***$0.002***	150	$0.005
Lecosorb	502-174-HAZ	500 grams	$98.30	17 grams	150	$0.022	***450***	***$0.007***	150	$0.022
Combustion Tube	619-065	1	$180.00	1	5000	$0.036	5000	$0.036	5000	$0.036
N-Catalyst	502-049	50 grams	$105.00	14 grams	750	$0.039	***2000***	***$0.015***	750	$0.039
Quartz Wool Strips	608-379	10 strips	$40.00	9	1500	$0.024	1500	$0.024	1500	$0.024
Glass Wool	501-081	454	$82.80	3	100	$0.005	100	$0.005	100	$0.005
Furnace reagent	501-609-HAZ	100 grams	$30.00	50 grams	3000	$0.005	3000	$0.005	3000	$0.005
Steel Wool	502-310	454	$16.80	4.5	150	$0.006	150	$0.006	150	$0.006
Copper Turnings	502-656	60 grams	$48.90	10 grams	750	$0.011	***2000***	***$0.004***	750	$0.011
Porous Crucible	614-961-110	10	$165.00	1	250	$0.066	250	$0.066	250	$0.066
C-flex tubing repl. Kit	625-602-470	1	$60.00	1	3000	$0.020	3000	$0.020	3000	$0.020
Small Tin Foil Cups	502-186-100	1000	$90.00	1	1	$0.090	1	$0.090	1	$0.090
				TOTAL Cost		**$0.66**		**$0.58**		**$0.61**

Table 3. FP628 Cost-per-Analysis



Conclusion

The objective of this work was to demonstrate the analytical performance and application capability of the instrument methods optimized to maximize throughput and instrument configurations optimized to have the lowest cost-per-analysis.

The helium configuration of the FP628 has a 13% higher throughput compared to the argon configuration of the instrument due to the method settings required for the difference in the carrier gas. The throughput of the helium instrument configuration can be increased by 13% (from 15 to 17 samples/hr), and the argon configuration increased by 15% (13 to 15 samples/hr) by optimizing the method parameters. The high throughput and high precision optimized methods for both the helium and argon instrument configurations analyzing the feed and food samples resulted in nitrogen data relative standard deviation (RSD) well below 2%. The highest RSD for the high precision method helium configuration instrument was 0.74%, with the high throughput argon configuration instrument having a 1.58% maximum RSD. There was no clear decrease or trend in precision when comparing the sample suite results from the high precision to high throughput method for either the helium or argon instrument configurations. The helium and argon instrument configurations using both high throughput and high precision methods had nitrogen results for all of the flour reference material samples that were within the certified values. The high throughput method provides a 13-15% greater throughput with minimal impact on practical analytical performance for the feed and food samples within this study.

The argon instrument configuration has a 7.5% lower cost-per analysis ($0.61) compared to the equivalent helium instrument configuration ($0.66). The argon instrument configuration provides a lower cost-per-analysis along with argon gas having additional benefits of a relatively stable availability and pricing compared to helium gas.

Reducing the aliquot loop size of the helium instrument configuration to 3 cc lowers the cost-per-analysis by 12% ($0.58), compared to the cost-per-analysis of the instrument configured with the standard aliquot loop of 10 cc ($0.66). The precision for the 3 cc instrument configuration is worse than the 10 cc instrument configuration for both the feed and food samples, but all of the 3 cc instrument data had an RSD well below 2%. All of the nitrogen data for the flour samples were within the certified value for the 10 cc and 3 cc helium instrument configurations. The 3 cc helium instrument configuration provides the lowest cost-per-analysis of $0.58 and extends the reagent maintenance intervals increasing the instrument's uptime with minimal impact on practical analytical performance for the feed and food samples within this study.

Delivering the Right Results