Optimizing a Total Protein Combustion Instrument for Lowest Cost-Per-Analysis

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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 a 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 an oxygen environment to combust the sample. The nitrogen gases within the sample combustion gas are subsequently reduced to N_2 gas, and detected using a thermo-conductivity (TC) detector using an inert carrier gas, with the excess oxygen and other sample combustion products being removed using multiple reagents within the instrument flow path. Total nitrogen combustion instruments manufactured by LECO Corporation use only pure oxygen within the furnace, and utilize a system collecting and equilibrating the combustion gas, then sampling 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 configured to utilize helium or argon as the TC carrier gas. The TC carrier gas used in the instrument is not only a factor in the cost-per-analysis (CPA) of the instrument, but also provides different options for the optimization of the instrument for the lowest CPA. Instruments configured for helium TC carrier gas can reduce the aliquot gas volume due to the high sensitivity of the TC detector when using helium as a carrier gas. Instruments configured for argon TC carrier gas can utilize lower grades of oxygen gas due to the lower sensitivity of the TC detector when using argon as a carrier gas.

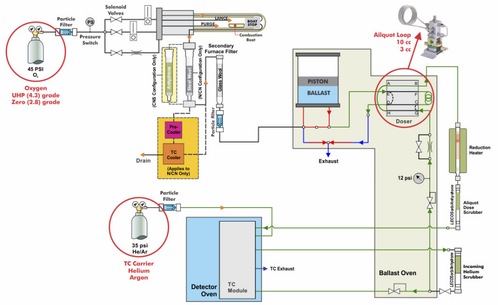
This poster presentation will cover the optimization of a total nitrogen combustion instrument for lowest cost-per-analysis (CPA) by optimizing the aliquot gas volume, TC carrier gas, and grade of oxygen gas utilized in the combustion furnace. Data will be presented that includes common foods, feeds, and reference materials analyzed with a TruMac® N instrument.

Methodology

Equipment

LECO TruMac N

TruMac N Flow Diagram



TruMac N Theory of Operation

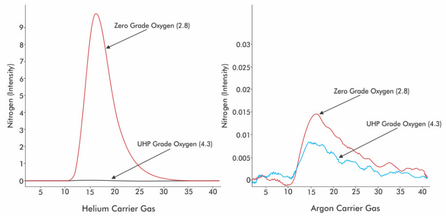
A pre-weighed macro sample is placed into a large ceramic boat and loaded into the purge chamber located in the front of the horizontal, ceramic, high-temperature furnace. After the entrained atmospheric gas is purged from the sample, the ceramic boat is introduced into the furnace regulated at a temperature of 1100 °C to 1250 °C. Complete oxidation of the macro sample is ensured by a pure oxygen environment within the furnace. The ceramic boat and all ash from the sample are removed from the furnace at the end of combustion. Nitrogen present in the sample is oxidized to NO, and swept with the sample combustion gas by the oxygen gas 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 combustion gas from the ballast is collected in the aliquot loop (3 or 10 cc volume), and then passed into a helium or argon carrier gas. The NO, 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 then passes through LECOSORB and Anhydrone to remove CO_2 and the water generated during the CO_2 trapping process, and then onto a thermal conductivity cell (TC) utilized to detect the N_2 .

Optimization Parameters

The instrument's cost per analysis (CPA) is primarily composed of the cost of reagents, the required gas for combustion, and the TC carrier gas. The reagent usage and resulting reagent costs are directly proportional to the volume of the aliquot loop volume used in the instrument's system configuration. Instruments configured to utilize helium as a TC carrier gas have the highest sensitivity for the TC detector and offer the ability to decrease the volume of the aliquot loop from the standard 10 cc to an optional 3 cc volume.

Instruments configured to utilize argon as a TC carrier gas have a lower sensitivity for the TC detector and offer the ability to utilize a lower grade of oxygen combustion gas. Ultra High Purity (UHP) grade oxygen with a purity of 99.993% (4.3) is required for instruments using helium as a TC carrier gas. Zero grade oxygen with a purity of 99.8% (2.8) can be utilized for instruments using argon as a TC carrier gas. Argon gas is one of the impurities present in the lower grade oxygen gas. The argon present in the lower grade oxygen will not affect the TC cell detector when using argon as a carrier gas. The combination of the lower sensitivity and the detector being blind to one of the impurities present in the lower grade oxygen enable the use of zero grade (2.8) oxygen for the combustion gas. Data will be presented that includes common foods, feeds, and reference materials analyzed with a TruMac N instrument.

Thermal Conductivity (TC) Detector Blank Response



The following instrument configurations were optimized for lowest CPA and tested.

TruMac N Instrument Configurations				
TC Carrier Gas	Helium	Helium	Argon	Argon
Aliquot Loop volume	10 cc	3 cc	10 cc	10 cc
Oxygen Grade	UHP (4.3)	UHP (4.3)	UHP (4.3)	Zero (2.8)

All instrument configurations were optimized for the lowest CPA while maintaining an acceptable practical performance for precision of nitrogen results. Qualification and performance data using reference materials, and food and feed samples were collected for all instrument configurations. The resulting CPA were also calculated for all of the instrument configurations.

Results

Calibration

All TruMac N 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 the table below. When using a nominal sample mass of ~0.75 g, the calibration covers a range of 9.56 to ~0.003% nitrogen (59.75 to ~0.02% protein, using a 6.25 protein multiplier) for a helium TC carrier gas instrument, and 9.56 to ~0.008% nitrogen (59.75 to ~0.05% protein, using a 6.25 protein multiplier) for an argon TC carrier gas instrument.

Nitrogen Calibration Data .

EDTA (LECO 502-092, Lot 1061) Cert. Value 9.56 ± 0.04 % N	TruMac N Instrument Configuration			
	Helium, carrier gas		Argon, carrier gas	
	10 cc aliquot	3 cc aliquot	10 cc aliquot	10 cc aliquot
	UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	Zero (2.8) O_2 gas
Calibration Mass	~0.75 g	~0.75 g	~0.75 g	~0.75 g
Replicate Number	7	7	7	7
Standard Deviation	0.010	0.012	0.011	0.010
RSD (%)	0.10	0.13	0.12	0.10

A qualification protocol was performed for all of the TruMac N instrument configurations verifying the calibration and instrument performance using reference materials with nitrogen ranging from 11.37% (Nicotinic Acid, LECO 502-050) to 0.01% (Ammonium solution, LECO 502-601).

Instrument Qualification Data

Reference Material	Sample Mass, Replicate number		TruMac N Instrument Configuration			
			Helium, carrier gas		Argon, carrier gas	
			10 cc aliquot	3 cc aliquot	10 cc aliquot	10 cc aliquot
			UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	Zero (2.8) O_2 gas
Nicotinic Acid (LECO 502-050, Lot 1011) Cert. Value 11.37 ± 0.05 % N	~0.30 g, n=5	Average N %	11.402	11.389	11.366	11.377
		Standard Deviation	0.005	0.012	0.025	0.010
		RSD (%)	0.04	0.11	0.22	0.08
Ammonium Solution (LECO 502-602, Lot CP-5042) Cert. Value 0.1002 % N	~1 g, n=5	Average N %	0.0985	0.1021	0.0994	0.1020
		Standard Deviation	0.0014	0.0022	0.0016	0.0034
		RSD (%)	1.42	2.16	1.63	3.33
Ammonium Solution (LECO 502-602, Lot CP-4621) Cert. Value 0.0100 % N	~1 g, n=5	Average N %	0.0107	0.0100	0.0102	0.0110
		Standard Deviation	0.0006	0.0008	0.0010	0.0013
		RSD (%)	5.61	7.50	9.30	11.87

All TruMac N instrument configurations produced average nitrogen results that were within the expected certificate values for all of the reference materials in the qualification test. The helium TC carrier gas TruMac N configurations had the best precision over the entire range of the qualification test with the helium, 10 cc loop configuration having a 5.6% relative standard deviation (RSD), and the helium, 3 cc loop configuration having a 7.5% RSD for the 0.01% N standard. The argon TruMac N instrument held relatively good precision for the 11.4% and 0.1% nitrogen reference materials with the UHP (4.3) oxygen configuration having the better precision of 1.6% RSD on the 0.1% nitrogen standard versus the zero grade (2.8) oxygen configuration (3.3% RSD). Both of the argon TC carrier gas configurations had RSD values >9% for the 0.01% nitrogen standard.

The instrument calibration and stability was verified using the pure chemical reference material nicotinic acid (LECO 502-050). A ~0.30 g sample of the nicotinic acid was analyzed after the calibration and interspersed within the food and feed samples analysis sequence serving as a calibration and stability check sample. The ~0.30 g sample of nicotinic acid represents the equivalent of ~3.41% N (21.31% protein) in a sample analyzed with a nominal ~1 g sample mass.

Reference Material	Sample Mass, Replicate number		TruMac N Instrument Configuration			
			Helium, carrier gas		Argon, carrier gas	
			10 cc aliquot	3 cc aliquot	10 cc aliquot	10 cc aliquot
			UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	Zero (2.8) O_2 gas
Nicotinic Acid (LECO 502-050, Lot 1011) Cert. Value 11.37 ± 0.05 % N	~0.30 g n=20	Average N %	11.40	011.388	11.374	11.369
		Standard Deviation	0.012	0.010	0.012	0.012
		RSD (%)	0.11	0.09	0.11	0.11

All TruMac N configurations had excellent calibration stability and precision over the entire analysis batch sequence, with the calculated RSD being <0.12% for the all the nicotinic acid verification reference material.

Sample Data

Sample suites for feed and food were chosen for this study to demonstrate the analytical performance and application capability of the instrument configurations to deliver results for total nitrogen/protein. The sample suites represent typical feed and food materials that are routinely tested for, quality ranked on, and characterized by their nitrogen/protein content.

A distillers grain, flax seed, and two dry pet food samples were chosen for the feed samples. The nitrogen range of the feed samples were ~3.2 to ~4.4%.

Feed Samples	Sample Mass, Replicate number		TruMac N instrument configuration			
			Helium, carrier gas		Argon, carrier gas	
			10 cc aliquot	3 cc aliquot	10 cc aliquot	10 cc aliquot
			UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	Zero (2.8) O_2 gas
Distillers Grain	~1 g n=5	Average N %	4.36	4.40	4.35	4.34
		Standard Deviation	0.012	0.023	0.014	0.040
		RSD (%)	0.28	0.52	0.32	0.92
Flax Seed	~1 g n=5	Average N %	3.62	3.62	3.60	3.60
		Standard Deviation	0.005	0.008	0.002	0.005
		RSD (%)	0.14	0.22	0.06	0.14
Dry Pet Food Sample A	~1 g n=5	Average N %	3.17	3.17	3.17	3.19
		Standard Deviation	0.025	0.024	0.020	0.009
		RSD (%)	0.79	0.76	0.63	0.28
Dry Pet Food Sample B	~1 g n=5	Average N %	3.51	3.49	3.49	3.48
		Standard Deviation	0.021	0.028	0.024	0.003
		RSD (%)	0.60	0.80	0.69	0.09

The four food samples are a corn starch and three flour samples representing a wheat, rye, and rice matrix source. The flour samples were all LECO reference materials with certified values for nitrogen. The flour reference material's certified nitrogen values provides an assessment of data accuracy in addition to precision. The nitrogen range of the food samples was ~0.02 to ~2.67%.

Food Samples	Sample Mass, Replicate number		TruMac N Instrument Configuration			
			Helium, carrier gas		Argon, carrier gas	
			10 cc aliquot	3 cc aliquot	10 cc aliquot	10 cc aliquot
			UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	UHP (4.3) O_2 gas	Zero (2.8) O_2 gas
Corn Starch	~2 g n=5	Average N %	0.02078	0.02138	0.02146	0.02266
		Standard Deviation	0.00045	0.00073	0.00061	0.00026
		RSD (%)	2.17	3.41	2.84	1.15
Rye Flour 502-275 lot 1007 Cert. Value 1.74 ± 0.06	~1 g n=5	Average N %	1.741	1.748	1.737	1.726
		Standard Deviation	0.0016	0.0028	0.0041	0.0013
		RSD (%)	0.09	0.16	0.24	0.08
Wheat Flour 502-274 lot 1015 Cert. Value 2.68 ± 0.03	~1 g n=5	Average N %	2.659	2.672	2.660	2.659
		Standard Deviation	0.0091	0.0210	0.0119	0.0048
		RSD (%)	0.34	0.79	0.45	0.18
Rice Flour 502-278 lot 1014 Cert. Value 1.13 ± 0.03	~1 g n=5	Average N %	1.138	1.153	1.142	1.139
		Standard Deviation	0.0066	0.0011	0.0025	0.0031
		RSD (%)	0.58	0.10	0.22	0.27

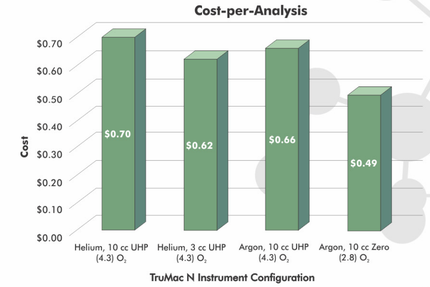
The average nitrogen results produced by all of the TruMac N configurations were in agreement for all of the food and feed samples. The average nitrogen results for the rye, wheat, and rice flour reference material samples were all within the certificate values for all of the TruMac N configurations. All TruMac N configuration nitrogen results for the food and feed samples produced RSD < 0.95% with the exception of the corn starch sample. The corn starch sample had a much lower nitrogen concentration (0.02% N) compared to the other food and feed samples resulting in higher RSD, but all TruMac N instrument configurations had acceptable precision results for the corn starch sample with the lowest RSD being 1.2% and the highest RSD being 3.4%. The TruMac N instrument configurations had no clear trend in precision performance for the food and feed samples when comparing the instrument configurations.

Cost-Per-Analysis (CPA)

The cost-per-analysis (CPA) of the TruMac 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 the table. The overall CPA for the instrument can be reduced compared to the standard instrument configuration using helium TC carrier gas with a 10 cc aliquot loop by reducing the aliquot loop volume or changing the carrier gas to argon. Utilizing argon as a carrier gas not only lowers the CPA, but also allows the option to use a less costly oxygen grade (zero grade, 2.8) for the combustion gas further reducing the overall CPA. Decreasing the volume of the aliquot loop from 10 cc to 3 cc offers a lower cost for reagents, and also provides a longer period of time between the required reagent's maintenance intervals, thus increasing the instrument uptime. The differences from the standard 10 cc helium configuration in reagent longevity and resulting costs are italicized in bold face within the table below.

Consumable	LECO Part#	Amount	Cost	Amount Used	N Instrument Configuration TruMac							
					Helium, carrier gas				Argon, carrier gas			
					10 cc aliquot, UHP (4.3) O_2		3 cc aliquot, UHP (4.3) O_2		10 cc aliquot, UHP (4.3) O_2		10 cc aliquot, Zero (2.8) O_2	
					# of Analysis	Cost / Analysis	# of Analysis	Cost / Analysis	# of Analysis	Cost / Analysis	# of Analysis	Cost / Analysis
Oxygen (UHP, 4.3)	N/A	9350 L	$200.00	15 L	600	$0.333	600	$0.333	600	$0.33	N/A	N/A
Oxygen (Zero, 2.8)	N/A	9350 L	$100.00	15 L	N/A	N/A	N/A	N/A	N/A	N/A	600	$0.167
Helium	N/A	8000 L	$250.00	2 L	4000	$0.063	4000	$0.063	N/A	N/A	N/A	N/A
Argon	N/A	9300 L	$75.00	2 L	N/A	N/A	N/A	N/A	4500	$0.02	4500	$0.02
Air (Pneumatic)	N/A	8000 L	$25.00	9 L	850	$0.029	850	$0.029	850	0.029	850	0.029
Combustion Tube outer	625-601-554	1	$145.00	1	6000	$0.024	6000	$0.024	6000	$0.024	6000	$0.024
Combustion Tube inner	625-601-555	1	$115.00	1	6000	$0.019	6000	$0.019	6000	$0.019	6000	$0.019
Combustion Lance	625-602-187	1	$45.00	1	6000	$0.008	6000	$0.008	6000	$0.008	6000	$0.008
Ceramic Boat stop	606-308	1	$65.00	1	6000	$0.011	6000	$0.011	6000	$0.011	6000	$0.011
Glass Wool	763-265	227 grams	$45.80	3 grams	100	$0.006	100	$0.006	100	$0.006	100	$0.006
Steel Wool	502-210	454 grams	$17.10	4.5 grams	100	$0.002	100	$0.002	100	$0.002	100	$0.002
Anhydrone	501-171-MAZ	454 grams	$41.50	8 grams	150	$0.005	***450***	***$0.002***	150	$0.005	150	$0.005
Lecosorb	502-174-MAZ	500 grams	$100.00	17 grams	150	$0.023	***450***	***$0.008***	150	$0.023	150	$0.023
Copper Sticks	502-878	100 grams	$32.60	100 grams	750	$0.043	***2000***	***$0.016***	750	$0.043	750	$0.043
Copper Turnings	502-656	60 grams	$49.90	10 grams	750	$0.011	***2000***	***$0.004***	750	$0.011	750	$0.011
N Catalyst	502-049	50 grams	$107.00	14 grams	750	$0.040	***2000***	***$0.015***	750	$0.040	750	$0.040
Ceramic Boats	528-203-250	250	$142.00	1	10	$0.057	10	$0.057	10	$0.057	10	$0.057
Cup Seal	625-602-319	1	$12.00	2	3000	$0.008	3000	$0.008	3000	$0.008	3000	$0.008
C-Flex Tubing Replacement Kit	625-602-470	1	$60.00	1	3000	$0.020	3000	$0.020	3000	$0.020	3000	$0.020
				Total Cost		$0.70		$0.62		$0.66		$0.49

Reducing the aliquot loop size to 3 cc for the helium TC carrier gas configurations has an 11% savings ($0.08) compared to the 10 cc loop instrument using helium carrier gas. Using argon TC carrier gas with a 10 cc aliquot loop has a 6% savings ($0.04) compared the 10 cc loop instrument using helium carrier gas. The instrument configured for argon TC carrier gas using zero grade (2.8) oxygen has the greatest savings of 30% ($0.21) compared to the 10 cc loop instrument using helium carrier gas.



Conclusion

The objective of this work was to demonstrate the analytical performance and application capability of the TruMac N instrument configurations that have been optimized to have the lowest cost-per-analysis (CPA).

- All of the TruMac N instrument configurations produced acceptable results in a qualification test using pure chemical reference materials ranging from 11% to 0.1% nitrogen.
- TruMac N instruments using helium TC carrier gas had the best precision performance for the qualification test with a range extending down to the 0.01% nitrogen.
- All of the TruMac N configurations average nitrogen results were in agreement for all of the food and feed samples.
- The LECO flour reference material samples (rye, wheat, and rice) tested within the food sample suite were all within the certificate values for all TruMac N configurations.
- All TruMac N configuration nitrogen results for the food and feed samples produced acceptable precision and had no clear trend in precision performance when comparing the instrument configurations.
- The TruMac N instrument configured for helium TC gas and a 3 cc aliquot loop provided a CPA savings of 11% ($0.08), but also had the advantage of maintaining similar precision performance for reference materials as low as 0.01% nitrogen, showed equivalent performance for the food and feed samples, and decreased some of the reagent maintenance intervals of the instrument.
- The TruMac N instrument configured for argon TC gas provided a modest CPA savings of 6% ($0.04).
- The TruMac N instrument configured for argon TC gas and using zero grade (2.8) oxygen resulted in the greatest CPA savings of 30% ($0.21) compared to the 10 cc loop instrument using helium and UHP grade (4.3) oxygen, and produced acceptable results in a qualification test down to 0.1% nitrogen.

Delivering the Right Results