Application Note

Instrument: FP928

Determination of Nitrogen/Protein in Beer

LECO Corporation; Saint Joseph, Michigan USA

Introduction

Nitrogen determination in beer is utilized to calculate the protein concentration using a nitrogen protein conversion factor. The protein content of beer is not only an important criterion in evaluating the quality of beer but also is an important parameter utilized to monitor process quality during production. The protein contained in beer is primarily water-soluble proteins from the grains used in the malting and brewing process. Control and measurement of protein throughout the brewing process is important in order to ensure the survival, growth, and productivity of the yeast utilized to convert sugars to ethanol and carbon dioxide. The yeast depends on a variety of conditions, including the availability of amino groups derived from enzymatic hydrolysis of protein during the brewing process.

The LECO FP928 is a macro combustion nitrogen/protein determinator that utilizes a pure oxygen environment in a high-temperature horizontal ceramic combustion furnace, using ceramic combustion boats designed to handle macro sample masses (~1 g). A thermoelectric cooler removes moisture from the combustion gases before they are collected in a ballast. The gases equilibrate and mix in the ballast before a representative aliquot (3 cm^3 or 10 cm^3 volume) of the gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot of gas is carried through a heated reduction tube filled with copper to convert nitrogen oxide combustion gas species (NO_x) to nitrogen (N_2). The aliquot gas is then carried to a thermal conductivity cell (TC) for the detection of nitrogen (N_2).

Instrument Model and Configuration

Thermal conductivity detectors work by detecting changes in the thermal conductivity of the analyte gas compared to a reference/carrier gas. The greater the difference between the thermal conductivity of the carrier gas and the analyte gas, the greater sensitivity of the detector. The FP928 supports either the use of helium or argon as the instrument's carrier gas. When used as a carrier gas, helium provides the highest sensitivity and the best performance at the lower limit of the nitrogen range. The thermal conductivity difference between argon and nitrogen is not as great as the thermal conductivity difference between helium and nitrogen, therefore the detector is inherently less sensitive when using argon as a carrier gas.

The LECO FP928 offers the additional advantage of utilizing either a 10 cm^3 aliquot loop or a 3 cm^3 aliquot loop within the instrument's gas collection and handling system. The 10 cm^3 aliquot loop optimizes the system for the lowest nitrogen range and provides the best precision. The 3 cm^3 aliquot loop extends reagent life expectancy by approximately three-fold when compared to the 10 cm^3 aliquot loop, while providing the lowest cost-per-analysis.

Note: When changing carrier gas type, the flow needs to be adjusted following instructions provided in the FP928 Operator's Instruction Manual. The aliquot loop size is changed by selecting the desired aliquot loop size in the software's Method Parameters.

Sample Preparation

Beer samples should be prepared according to official AOAC Method 920.49: Beer - Preparation of Sample. The AOAC method states that beer should be degassed at room temperature. Reference materials should be prepared as directed by the certificate prior to analysis. Glycine solutions should be prepared using the procedure found on the last page of this document.

Accessories

528-203 Ceramic Combustion Boats* with 502-343 Nickel Boat Liners or 625-505-430 Nickel Boats, and disposable pipettes.

**For optimal precision, ceramic combustion boats should be baked in a muffle furnace at 1,000 °C for a minimum of 40 minutes. Once the ceramic combustion boats have cooled, they should be transferred to a desiccator for storage. If the ceramic combustion boats are not used within twenty-four hours, they should be re-baked. After baking, handle ceramic combustion boats with clean tongs only; do not use fingers.*

Reference Materials

Calibration should be performed using glycine solutions prepared using the procedure found on the last page of this document. Verification can be performed using appropriate concentrations of a glycine solution and/or an ammonium solution.

*Method Parameters***

Gas Type	Helium or Argon
Furnace Temperature	1100 °C
Dehydration Time	0 s
Nominal Mass	1.0000 g
Purge Cycles	3
Ballast Equilibrate Time	10 s
Ballast Not Filled Timeout	300 s
Aliquot Loop Fill Pressure Drop	200 mm Hg
Aliquot Loop Equilibrate Time	4 s
Dose Loop Size	10 cm^3 or 3 cm^3

Element Parameters**

Parameter	Helium 10 cm^3 & 3 cm^3 Nitrogen	Argon 10 cm^3 and 3 cm^3 Nitrogen
Integration Delay	0 s	9 s
Starting Baseline	10 s	10 s
Post Baseline Delay	20 s	16 s
Use Comparator	No	No
Integration Time	50 s	70 s
Use Endline	Yes	Yes
Endline Delay	30 s	30 s
Ending Baseline	5 s	5 s
Use Profile Blank	--	Yes

***Refer to FP928 Operator's Instruction Manual for Parameter definitions.*

Burn Profile

Burn Step	Lance Flow	Furnace Flow	Time
1	No	Yes	5 s
2	Yes	Yes	35 s
3	Yes	No	End

Procedure

1. Prepare instrument for operation as outlined in the operator's instruction manual.
2. Condition the System.
   a. Select five or more Blank replicates in the Login screen (combustion boat is not required).
   b. Initiate the analysis sequence.
3. Determine Blank.
   a. Select five or more Blank replicates in the Login screen.
   b. Place 528-203 Ceramic Combustion Boats lined with 502-343 Nickel Boat Liners or 625-505-430 Nickel Boats in the appropriate positions in the autoloader.
   c. Initiate the analysis sequence.
   d. Set the blank following the procedure outlined in the operator's instruction manual.

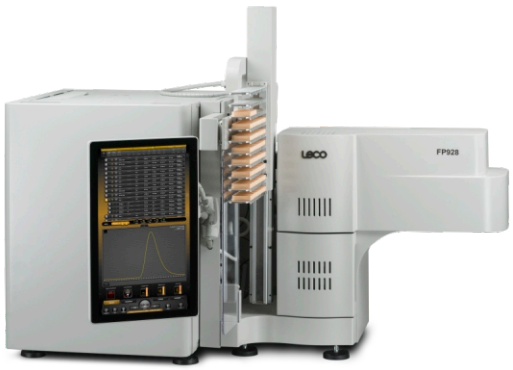
   Note: The standard deviation of the last five blanks should be less than or equal to 0.001% (10 ppm) when utilizing helium as a carrier gas, and less than or equal to 0.005% (50 ppm) when utilizing argon as a carrier gas. Additional blanks beyond the recommended five may be required in order to achieve the recommended precision.
4. Calibrate/Drift Correct.
   a. Select the desired number of calibration/drift replicates in the Login screen (minimum of five).
   b. Using a pipette, weigh ~1.0 g of an appropriate concentration of glycine solution into a 528-203 Ceramic Combustion Boat lined with a 502-343 Nickel Boat Liner or a 625-505-430 Nickel Boat.
   c. Enter sample mass and identification into the Login screen.
   d. Transfer the combustion boat containing the glycine solution to the appropriate position in the autoloader.
   e. Perform steps 4b through 4d a minimum of five times for each calibration/drift solution used.
   f. Initiate the analysis sequence.
   g. Calibrate or Drift Correct the instrument following the procedure outlined in the operator's instruction manual.
   h. Verify the calibration by analyzing ~1.0 g of a different concentration of glycine solution and confirm that the results are within the acceptable tolerance range.
5. Analyze Samples.
   a. Select the desired number of sample replicates in the Login screen.
   b. Using a pipette, weigh ~1.0 g of the sample into a 528-203 Ceramic Combustion Boat lined with a 502-343 Nickel Boat Liner or a 625-505-430 Nickel Boat.
   c. Enter sample mass and identification information into the Login screen.
   d. Transfer the combustion boat containing the sample to the appropriate position in the autoloader.
   e. Perform steps 5b through 5d for each sample to be analyzed.
   f. Initiate the analysis sequence.

TYPICAL RESULTS

Data was generated utilizing a linear, force through origin calibration using a 0.2% N glycine solution. The calibration was verified using 502-602 Ammonium Solution (0.1% N), 502-601 Ammonium solution (0.01% N), a 0.1% N glycine solution, and a 0.05% N glycine solution. All samples were weighed and analyzed at ~1.0 g. A protein factor of 6.25† was used for all samples to calculate the protein content.

	10 cm^3 Helium			3 cm^3 Helium			10 cm^3 Argon			3 cm^3 Argon		
	Mass (g)	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*	*Mass (g)*	*% N*	*% Protein*
Porter	1.0012	0.089	0.558	1.0387	0.090	0.564	1.0082	0.090	0.565	1.0188	0.096	0.599
	1.0035	0.088	0.551	1.0318	0.091	0.567	1.0073	0.087	0.544	1.0029	0.086	0.538
	1.0092	0.087	0.543	1.0238	0.089	0.554	1.0084	0.086	0.538	1.0125	0.092	0.572
	1.0322	0.089	0.555	1.0457	0.087	0.546	1.0208	0.087	0.544	1.0220	0.100	0.626
	1.0145	0.088	0.551	1.0044	0.088	0.552	1.0032	0.088	0.551	1.0065	0.081	0.506
	Avg =	***0.088***	***0.552***	***Avg =***	***0.089***	***0.557***	***Avg =***	***0.088***	***0.548***	***Avg =***	***0.091***	***0.568***
	s =	***0.001***	***0.005***	***s =***	***0.001***	***0.009***	***s =***	***0.002***	***0.010***	***s =***	***0.008***	***0.048***
American	1.0418	0.111	0.697	1.0079	0.110	0.690	1.0195	0.109	0.681	1.0361	0.097	0.607
IPA	1.0290	0.110	0.687	1.0107	0.110	0.687	1.0105	0.107	0.668	1.0021	0.103	0.643
	1.0125	0.111	0.691	1.0021	0.107	0.666	1.0117	0.104	0.648	1.0305	0.109	0.681
	1.0101	0.110	0.688	1.0189	0.107	0.668	1.0041	0.109	0.683	1.0246	0.096	0.602
	1.0278	0.111	0.691	1.0165	0.108	0.674	1.0348	0.112	0.700	1.0112	0.111	0.692
	Avg =	***0.111***	***0.691***	***Avg =***	***0.108***	***0.677***	***Avg =***	***0.108***	***0.676***	***Avg =***	***0.103***	***0.645***
	s =	***0.001***	***0.004***	***s =***	***0.002***	***0.011***	***s =***	***0.003***	***0.019***	***s =***	***0.007***	***0.041***
Pilsner	1.0385	0.032	0.199	1.0144	0.031	0.195	1.0098	0.031	0.191	1.0580	0.033	0.208
	1.0054	0.032	0.201	1.0088	0.032	0.199	1.0380	0.032	0.201	1.0251	0.029	0.179
	1.0165	0.031	0.196	1.0167	0.030	0.184	1.0101	0.031	0.191	1.0408	0.024	0.148
	1.0141	0.031	0.194	1.0084	0.031	0.196	1.0233	0.031	0.192	1.0281	0.036	0.223
	1.0156	0.032	0.197	1.0144	0.029	0.181	1.0338	0.032	0.198	1.0156	0.031	0.191
	Avg =	***0.032***	***0.197***	***Avg =***	***0.031***	***0.191***	***Avg =***	***0.031***	***0.195***	***Avg =***	***0.030***	***0.190***
	s =	***< 0.001***	***0.003***	***s =***	***0.001***	***0.008***	***s =***	***0.001***	***0.005***	***s =***	***0.005***	***0.029***

†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.



LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496
info@leco.com • www.leco.com | ISO-9001:2015 Q-994 | *LECO is a registered trademark of LECO Corporation.*

GLYCINE SOLUTION PREPARATION

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

$$G = \frac{C}{(0.99^{\dagger\dagger} * 0.18658)}$$

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

Example for 1% solution:

$$G = \frac{1}{(0.99^{\dagger\dagger} * 0.18658)} = 5.414$$

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 mass.
4. Add distilled water until the total mass equals 100 g, then record the mass (W).
5. Seal the flask and mix the contents.
6. To figure the exact concentration:

$$\% \text{ Nitrogen} = \frac{G\,(18.658 * 0.99^{\dagger\dagger})}{W}$$

where: G = mass in grams of glycine recorded in step 3
W = mass in grams of water and glycine powder recorded in step 4

7. If the distilled water is not pure, determining the nitrogen concentration may be necessary.
   a. 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%

$$\frac{1.672(18.471)}{(99.824)} + 0.004 = 0.313\%\ \text{N}$$

QUICK REFERENCE CONCENTRATION TABLE

Nitrogen Concentration	Grams of Glycine[††]
0.10%	0.541
0.30%	1.624
0.50%	2.707
0.75%	4.060
1.00%	5.414

[††]*Assuming 99.0% purity of glycine powder.*