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Purpose

The LECO Protein/Nitrogen determinators, FP-528 (AAS000569 and AAS000715) must be calibrated before being used for analyses. LECO Corporation provides methods for these calibrations which are found in the instruction manuals for the instruments. Following calibration, both instruments must pass AOAC accuracy and precision tests. The methods for these tests are *AOAC Official Method 993.13* for nitrogen analyses and *AOAC Official Method 990.03* for protein analyses. These calibration and accuracy procedures are outlined in this supplement to the primary analysis SOPs 11002 and 21012.

Scope / Field of Application

The procedures detailed below are specific to combustion analyses using the LECO FP-528 instrument. Recalibration should be performed only when necessary, such as after a major change to the instrument. Accuracy determinations should be performed immediately after recalibration or significant instrument repairs.

Responsibilities

Chemists – perform weighing of reagents, calibration and verification of instruments, and documentation of calibrations and verifications.

Managers - assure SOP procedures and quality assurance procedures are followed.

Materials Required

Use reagent grade chemicals unless otherwise specified.
Refer to appropriate MSDS for chemical hazards.
Refer to ICN database for current manufacturer and product number.
Except where indicated, dispose of solutions by flushing down the drain with copious amounts of water.

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REAGENTS

Alumina Oxide Pellets, Al₂O₃

Used in the combustion tube to aid in filtering acidic and halogenated contaminants from the oxygen side sample flow.

Ammonium Nitrate, (NH₄)(NO₃) granular, 99.999% pure

Used to verify accuracy of nitrogen analyses, and as a secondary working control for nitrogen analyses. It should be dried at 105° C for 2 hours and cooled to room temperature in a desiccator prior to being used. It should not be used again without being redried if its time in the desiccator exceeds 24 hours.

Ammonium Sulfate, (NH₄)₂SO₄ granular, 99.999% pure

Used to verify accuracy of nitrogen analyses. It should be dried at 105° C for 2 hours and cooled to room temperature in a desiccator prior to being used. It should not be used again without being redried if its time in the desiccator exceeds 24 hours.

Calcium Oxide/Kaolin (50% each), CaO/Al₂Si₂O₅(OH)₄, "Furnace Reagent^R"

Used in the combustion tube to aid in filtering acidic and halogenated contaminants from the oxygen side sample flow. CaO also reacts with H₂O and CO₂.

CO₂ Absorbent (Sodium hydroxide on a non-fibrous silicate carrier), "LECOsorb^R"

Use in both the aliquot reagent tube and the incoming helium reagent tube to remove carbon dioxide from the gas stream.

DISPOSAL: Save in a labeled waste bottle to be tagged and collected by Environmental Health and Safety personnel.

Copper, Cu, reduced, sticks and turnings

Reduced copper sticks are used in the reduction reagent tube to remove free oxygen and sulfur from the gas stream, and to reduce NO_x to N₂. Copper turnings are used to pack the nitrogen catalyst and copper sticks within the reduction reagent tube.

EDTA, Ethylenediaminetetraacetic acid, C₁₀H₁₆N₂O₈, powder, > 99.5% pure

Used for calibration, drift correction of protein and nitrogen analyses, and as a working control for nitrogen analyses. Do not dry.

Iron/Steel wool N°2, untreated

Used as an oxidation and filtering agent in the furnace filter tube.

L-Lysine monohydrochloride, C₆H₁₄N₂O₂HCl, powder, > 99.5% pure

Used to verify accuracy of protein analyses and also as a working control for protein analyses. Do not dry.

Magnesium Oxide Pellets, MgO, "Magnesium Oxide Furnace Reagent^R"

Used in the combustion tube to aid in filtering acidic and halogenated contaminants from the oxygen side sample flow.

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Magnesium Perchlorate, Cl_2MgO_8 , saturated with CO_2 , 10/16 mesh rough grains, "Anhydrone^R"
Used as a powerful desiccant in the aliquot absorption and helium reagent tubes to remove residual water from the helium gas streams.

DISPOSAL: Save in a labeled waste bottle to be tagged and collected by Environmental Health and Safety personnel.

Nicotinic Acid, $\text{C}_6\text{H}_5\text{NO}_2$ powder, > 99.5% pure
Used to verify accuracy of protein analyses. It should be dried at 105° C for 2 hours and cooled to room temperature in a desiccator prior to being used. It should not be used again without being redried if its time in the desiccator exceeds 24 hours.

Nitrogen catalyst (alumina with platinum on a clay bead-pellet)
Used in the reduction reagent tube to reduce NO_2 to N_2 , and to convert hydrogen and oxygen to water, carbon and oxygen to CO_2 , and halogenated compounds (in the form of $(\text{CH})_x$) to water and CO_2 .

DISPOSAL: Place in wastebasket

Sucrose, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ granular, GR grade
Used to aid combustion of nitrogen standards used to verify accuracy for fertilizer analyses.
Grind the sucrose with a mortar and pestle before using.

EQUIPMENT

Analytical Balance, readable to 0.0001 g

Combustion Unit, LECO Corporation Nitrogen Determinator, FP-528

Oven, capable of maintaining $100 \pm 5^\circ\text{C}$

Printer

INTERFERENCES AND TROUBLESHOOTING

Systemic errors

Running the unit after routine maintenance without drift correction. Opening up either system (O_2 or He) introduces atmospheric air into the system. New components or reagents will contribute to changing the system nitrogen levels, which in turn affect the reported sample results.

Exceeding the counter limits for crucibles/aliquot reagent tubes/reduction tubes by more than 20%.

Overfilling the crucible may affect the furnace temperature, and could cause loose ash particles and soot to prematurely clog the combustion tube and the gas line leading from the furnace.

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Depleting the absorption reagents causes CO₂ and H₂O to not be scrubbed effectively from the gas sample. These have different thermal conductivity constants than nitrogen or helium, and cause the TC cell measurements to give false readings.

Depleting the copper within the reduction tube causes nitrogen oxides (NO_x's) to not be reduced effectively to N₂. The TC cell then reads an abnormally low or erratic level of nitrogen from sample to sample, and reports abnormally low or erratic results.

Inputting a false value for nitrogen/protein content for the calibration standard. If the value input for the calibration standard is significantly different from the actual value within the standard, then reported results for samples and controls will be abnormally high/low. Always check the certificate of analysis of the calibration standard for the true analyzed nitrogen/protein value of the lot being used to calibrate the unit. This is especially true if changing sources, because different manufacturing processes/sources may contribute different impurity levels, including nitrogen to the calibration, affecting the overall nitrogen content of the standard.

Inputting an incorrect atmospheric blank value. This will causes the unit to incorrectly compensate for nitrogen contribution from air in the sample, leading to inaccurate results.

Improper maintenance of the aliquot dosing value and ballast tank. Accumulation of soot and other deposits within these two components causes precision to be poor.

If the ballast filter count is high, even if the counter limit has not been reached, and the verification samples are running consistently low, changing the ballast filter may be necessary.

A high volume of liquid fertilizers can deplete the combustion tube reagents before the counter limits are reached. Care should be taken to weigh the correct amount so that these reagents are not prematurely depleted. If the liquid fertilizer control is running low, replace the combustion tube.

Inadequate drying of hygroscopic standards can lead to low results. Standards should be redried as necessary and stored in a desiccator.

Random Errors

Significant deviations from the weight ranges given for a sample. Too little or too much sample may give inaccurate results.

Loss of sample after weighing, due to improper folding of the tin foil cup/capsule or holes/tears in the foil, causes a loss of accuracy in reported results.

Loss of part of the foil cup-tin capsule after taring. Twisting off the edges of the foil cup or the capsule results in weighing errors.

Failure to add sucrose to liquid samples may cause inaccurate results.

Procedure

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CALIBRATION SUMMARY

The instrument is calibrated according to instructions for instrument calibration in the FP-528 user manual (pages 5-64 to 5-65, 5-92 to 5-94, and 7-11 to 7-12). The FP-528 has been calibrated in the past with multiple standards. The current practice is to use a single standard (EDTA) at several weights to create a linear curve. The analyst should choose the curve type and weighting that provides the best fit.

ACCURACY DETERMINATION SUMMARY

The instrument is approved for use after meeting or exceeding performance requirements according to *AOAC Official methods 990.03 and 993.13*, for protein and nitrogen respectively. The furnace temperature must be at least 950°C. For nitrogen analyses, accuracy and precision are demonstrated by performing 10 successive determinations of ammonium sulfate and 10 successive determinations of ammonium nitrate. The means must be within ± 0.20 units of the respective theoretical value, with standard deviations of $\leq 0.10\% \text{ N}$ for ammonium sulfate and $\leq 0.20\% \text{ N}$ for ammonium nitrate. For protein analyses, accuracy and precision are demonstrated by performing 10 successive determinations of nicotinic acid and 10 successive determinations of Lysine•HCl. The means must be within ± 0.15 units of the respective theoretical value, with standard deviations of $\leq 0.15\% \text{ N}$.

Weighing Calibration and Verification Standards

Protein Weighing

Place a tin foil cup into the cup holder; tare the assembly.

Weigh the appropriate amount of reagent material into the tin foil cup according to instructions outlined in the following determination section.

Carefully lift the foil cup out of the holder and gather the corners together. Pinch together the top and twist/fold the foil over to carefully roll/twist/press the sample to press out trapped air. Wrap the sample as tight as possible until it takes the shape of a rod or teardrop. Visually inspect the sample to ensure that foil is intact.

Place the sample back into the cup holder. It should fit easily within the opening. If it does not fit, continue to gently shape it until it does fit. Allow the balance to stabilize. Record the weight.

Nitrogen Weighing

Place a tin foil cup into the cup holder; tare the assembly.

Weigh the appropriate amount of reagent material into the tin foil cup according to instructions outlined in the following determination section. Allow the balance to stabilize. Record the weight.

Add enough sucrose to bring the final weight to $\sim 0.34\text{XX}-0.36\text{XXg}$

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NOTE: To obtain more precise results for ammonium nitrate, it is important that sucrose be above and below the reagent. Weigh approximately 0.1 g of sucrose first into the tin cup. The instrument should then be tared and the ammonium nitrate weighed, followed by more sucrose. The balance should read **~0.24XX-0.26XXg**. The total weight of the sample will actually be **~0.34XX-0.36XXg**.

Carefully lift the foil cup out of the holder and gather the corners together. Pinch together the top and twist/fold the foil over to carefully roll/twist/press the sample to press out trapped air. Wrap the sample as tight as possible until it takes the shape of a rod or teardrop. Visually inspect the sample to ensure that sample has not escaped, or that the foil has torn.

Place the wrapped sample back into the cup holder. It should fit easily within the opening. If it does not fit, continue to gently shape it until it does fit.

DETERMINATION

Preparation of Instrument for Calibration or Accuracy Determination

Perform any necessary maintenance.

Check the ambient monitor to ensure all parameters are within range. This is found under the diagnostic menu. The range limits can be found on page 4-18 of the FP-528 User Manual. The nominal value for the Combustion Furnace Temperature is $950 \pm 25^{\circ}\text{C}$.

Perform the oxygen leak check.

Perform the helium leak check.

If all parameters are within limits and the leak checks pass, the instrument is ready for analysis.

Instrument Calibration (Single Standard)

Prior to performing blanking steps, samples to be analyzed should be already weighed out or in the process of being weighed. Once blanking is completed, the instrument should not be idle for longer than a couple of minutes or it can drift from the calibration.

Under the sample menu, login 10 to 20 blanks.

Press Analyze to begin the analysis.

When the analysis is finished, highlight the last five blanks. If the standard deviation is less than **0.0020 %N**, set the blank by going to the configuration menu. Otherwise, continue blanking until the specified standard deviation is attained.

Weigh **in triplicate** EDTA in the amounts shown (unit is g):

0.03XX, 0.05XX, 0.10XX, 0.15XX, 0.20XX, 0.25XX, 0.30XX, 0.35XX, 0.40XX, 0.45XX, and 0.50XX

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As soon as the blank correction has been set, under the Sample menu, login each of the 33 calibration standards.

Load calibration standards onto autosampler.

Press Analyze to begin the analysis.

When the analysis is complete, the data will be used to set the calibration. In the spreadsheet, select the analyzed standards for calibration by highlighting them.

From the Configuration menu, click calibrations. The calibrations dialog box will appear.

Select "Linear" for the curve type and "Normal" for the weighting. Click "OK"

View the calibration curve. The curve should intersect every calibration point. If not, other combinations of curve types and weighting options may be tried (it is recommended that only "linear" and "linear, fixed at origin" be used as curve options and that only "normal" and "1/certified" be chosen as weighting options). Outlier data points may be removed by double-clicking the data-point. If the curve cannot be made to intersect all the data points, then the parameters which yield the curve with the lowest RMS error should be chosen.

When the "Curve" and "Weighting" options have been chosen, click "Print" to print a copy of the calculated calibration.

Click "OK" to enter the new calibration. The new calibration equation and the previous calibration equation will be displayed. The instrument is now calibrated for protein and nitrogen analyses.

Nitrogen Accuracy Determination

Prior to performing blanking steps, samples to be analyzed should be already weighed out or in the process of being weighed. Once blanking is completed, the instrument should not be idle for longer than a couple of minutes or it can drift from the calibration.

Under the sample menu, login 10 to 20 blanks.

Press Analyze to begin the analysis.

When the analysis is finished, highlight the last five blanks. If the standard deviation is less than **0.0020 %N**, set the blank by going to the configuration menu. Otherwise, continue blanking until the specified standard deviation is attained.

Drift correction should be performed for nitrogen accuracy determinations. It is not required before calibration. Before blanking is completed, weigh five **0.35xx g** EDTA samples for nitrogen analyses. No sucrose is required.

Under the sample menu, login the five EDTA drift correction standards for nitrogen.

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When the analysis is completed, highlight the last four EDTA's. If the RSD is $\leq 0.35\%N$, set the drift by going to the configuration menu. The EDTA drift correction standards must be within the acceptable limits before the accuracy tests can be performed.

Prior to or during blanking, weigh ten replicates of ammonium sulfate, ten replicates of ammonium nitrate at **0.05xx g**. Make sure than these reagents have been recently dried.

Under the "Sample" menu, log in each of the ten ammonium sulfate standards and ten ammonium nitrate standards.

Load standards onto the autosampler.

Press "Analyze" to begin the analysis.

When the analysis is complete, the data will be used to verify the accuracy and precision of the instrument. In the spreadsheet, first select the analyzed ammonium sulfate standards by highlighting them.

The average and standard deviation values for the ten analyses can be seen in the bottom-right corner of the screen. If the average is **21.20 \pm 0.20%N**, and the standard deviation is $\leq 0.10\%N$, then the instrument has passed the ammonium sulfate accuracy test. Otherwise, adjustments must be made and the test process repeated.

If the instrument passes for ammonium sulfate, then, with the data still highlighted, select "Print" to print the data. Choose the "Tabular Report" options so that the statistical data will be printed for documentation.

Highlight ammonium nitrate standards. The average and standard deviation values for the ten analyses can be seen in the bottom-right corner of the screen. If the average is **35.00 \pm 0.20%N**, and the standard deviation is $\leq 0.20\%N$, then the instrument has passed the ammonium nitrate accuracy test. Otherwise, adjustments must be made and the test process repeated.

If the instrument passes for ammonium nitrate, then, with the data still highlighted, select "Print" to print the data. Choose the "Tabular Report" options so that the statistical data will be printed for documentation

If the instrument passes all nitrogen accuracy tests, it may now be used to analyze fertilizer samples.

Protein Accuracy Determination

Prior to performing blanking steps, samples to be analyzed should be already weighed out or in the process of being weighed. Once blanking is completed, the instrument should not be idle for longer than a couple of minutes or it can drift from the calibration.

Under the sample menu, login 10 to 20 blanks.

Press Analyze to begin the analysis.

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When the analysis is finished, highlight the last five blanks. If the standard deviation is less than **0.0020 %N**, set the blank by going to the configuration menu. Otherwise, continue blanking until the specified standard deviation is attained.

Drift correction should be performed for protein accuracy determinations. It is not required before calibration. Before blanking is completed, weigh five **0.20xx g** EDTA samples for protein analyses. No sucrose is required.

Under the sample menu, login the five EDTA drift correction standards for protein.

When the analysis is completed, highlight the last four EDTA's. If the RSD is $\leq 0.35\%N$, set the drift by going to the configuration menu. The EDTA drift correction standards must be within the acceptable limits before the accuracy tests can be performed.

Prior to or during blanking, weigh ten replicates of nicotinic acid, and ten replicates of lysine•HCl at **0.20xx g**. Make sure that nicotinic acid has been recently dried.

Under the "Sample" menu, log in each of the ten nicotinic acid standards and ten lysine standards.

Load standards onto the autosampler.

Press "Analyze" to begin the analysis.

When the analysis is complete, the data will be used to verify the accuracy and precision of the instrument. In the spreadsheet, first select the analyzed nicotinic acid standards by highlighting them.

The average and standard deviation values for the ten analyses can be seen in the bottom-right corner of the screen. If the average is **11.38 ± 0.15%N**, and the standard deviation is $\leq 0.15\%N$, then the instrument has passed the nicotinic acid accuracy test. Otherwise, adjustments must be made and the test process repeated.

If the instrument passes for nicotinic acid, then, with the data still highlighted, select "Print" to print the data. Choose the "Tabular Report" options so that the statistical data will be printed for documentation.

Highlight the analyzed lysine standards. The average and standard deviation values for the ten analyses can be seen in the bottom-right corner of the screen. If the average is **15.34 ± 0.15%N**, and the standard deviation is $\leq 0.15\%N$, then the instrument has passed the ammonium nitrate accuracy test. Otherwise, adjustments must be made and the test process repeated.

If the instrument passes for lysine, then, with the data still highlighted, select "Print" to print the data. Choose the "Tabular Report" options so that the statistical data will be printed for documentation.

If the instrument passes all protein accuracy tests, it may now be used to analyze feed samples.

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Documentation

The following Quality Records shall be generated and managed:

Required Record	Custodian
ICN database	QA Manager
Laboratory Notebook	Chemist/QA Manager
Completed Worksheets/Printouts	Chemist/QA Manager
Equipment Logs and Calibration Certificates	Chemist/QA Manager & Production Manager

Reference Procedures

Protein in Feeds by Combustion, OTSC SOP 11002, March 2012
Nitrogen in Fertilizers by Combustion, OTSC SOP 21012 March 2012
 AOAC *Official Methods of Analysis*, 16th edition, Method 990.03
 AOAC *Official Methods of Analysis*, 16th edition, Method 993.13

References

FP-528 DSP/Windows Instruction Manual, 2003, LECO Corporation

Revision History

New Protocol CKM July 2014

Revision 2 – Format change. Removed instruction to perform accuracy check every 6 months.
 SMW & CKM February 1, 2017