METHOD VALIDATION REPORT

Secondary (Lab) Standard Validation for the Analysis of $\delta^{15}N$ Using the Costech Elemental Analyzer and IRMS

Date: May 19, 2010

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SUMMARY

International (Primary) Standards (IAEA Reference Material) Primary Standard Absolute Values	USGS40, L-Glutamic acidUSGS41, L-Glutamic acidIAEA-N-2, Ammonium Sulfate $(NH_4)_2SO_4$ IAEA-NO-3, Potassium Nitrate (KNO_3) Primary Standard $\delta^{15}N\%_{0airN2}$ USGS40-4.52						
	USGS40			+47.57			
	IAEA-N-2			+20.3			
	IAEA-NO-3			+4.7			
Primary Standard Experimental Values	<u>Primary</u> <u>Standard</u>	$\delta^{15}N\%_{airN2}$	<u>S.D.</u>	<u>%CV</u>	<u>%Acc</u>	<u>n</u>	
and Statistics	USGS40	-4.67	0.07	1.50	103.32	2 9	
	USGS41	+47.48	0.18	0.38	99.81	9	
	IAEA-N-2	+20.62	0.15	0.73	101.58	3 9	
	IAEA-NO-3*	+3.37	0.24	7.12	71.70	15	
	*Standard not used due to inconsistent readback values and sta					s	
Secondary (Laboratory) Standards	 Caffeine: Lot#1337874; Fluka > 99.0%(HPLC) CBG: Cabbage leaf, received from Cornell University, July 2009, origin unknown. CBT: Trout tissue, received from Cornell University, July 2009, origin unknown. Mink: Mink tissue, received from Cornell University, July 2009 origin unknown. Urea: Lot#1280597; Fluka 51456; Assay >/= 99.5% 						
Lab (Secondary)	Secondary Standard	δ^{15} N‰ _{airN2}	<u>S.</u>	<u>D.</u>	<u>%CV</u>	<u>n</u>	
Standard Experimentally Determined δ ¹⁵ N	Caffeine	-2.87		10	3.48	18	
	CBG**	9.76		16	1.64	18	
Values and Statistics	CBT Mink	+17.63 +11.55		06 11	0.34 0.95	18 18	
	Urea***	-0.44			38.64	18	
	0.00	-0.44	0.	-1	00.01	10	

Standard Target	Standard	Target Weight (mg)	~ m/z 28 Intensity
Weights and		funget (fing)	(mV)
Approximate m/z 28	USGS 40	0.7 – 0.9	3500
Intensities	USGS 41	0.7 – 0.9	3500
	IAEA-N-2	0.4 - 0.6	3500
	IAEA-NO-3	0.7 - 0.9	3500
	Caffeine	0.3 – 0.5	3500
	CBG*	2.5 - 3.5	3500
	CBT	0.9 – 1.1	3500
	Mink	0.8 - 1.0	3500
	Urea**	0.15 – 0.3	3500

** CBG is not recommended due to the weight of sample needed to provide an adequate nitrogen signal (~ 3.0 mg). Sample weights of greater than 2.5 - 3.0 mg can potentially jam the 100 sample autosampler jeopardizing the entire analysis sequence.

*** Urea is not recommended due to its large %CV value of 38.64.

SIGNATURE PAGE

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Date

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1. INTRODUCTION

This report describes the qualification/validation process for δ^{15} N Secondary (Lab) Standards using the Costech Elemental Analyzer (combustion) Isotope Ratio Mass Spectrometry technique. Various samples were analyzed to be evaluated as possible Secondary (Lab) standards. Three international (primary) standards were included in the analyses, they were USGS40, USGS41 and IAEA-N-2. A fourth international standard, IAEA-NO-3 (KNO₃) was also analyzed. Due to considerable inconsistencies in its readback data and statistics it was not used in the determination of the secondary standard values. The goal of the analysis was to identify the laboratory standards which provided acceptable experimental precision and encompassed the δ^{15} N ranges expected for samples submitted for analysis. The Lab Standards (without asterisks) identified in the Summary section of this report fulfilled these requirements.

2. EXPERIMENTAL

2.1. CHEMICALS AND MATERIALS

Five samples were chosen for this secondary (Lab) standard determination validation, as well as the four international (or primary) standards. The five laboratory standard candidates were as follows:

- 1. Caffeine: Lot#1337874; Fluka > 99.0%(HPLC).
- 2. CBG: Cabbage leaf, received from Cornell University, July 2009, origin unknown.
- 3. CBT: Trout tissue, received from Cornell University, July 2009, origin unknown.
- 4. Mink: Mink tissue, received from Cornell University, July 2009 origin unknown.
- 5. Urea: Lot#1280597; Fluka 51456; Assay >/= 99.5%.

The four international standards were as follows:

- 1. USGS40, L-Glutamic acid (Certificate of Analysis $\delta 15N = -4.52\%$)
- 2. USGS41, L-Glutamic acid (Certificate of Analysis $\delta 15N = +47.57\%$)
- 3. IAEA-N-2, Ammonium Sulfate (IAEA Website $\delta 15N = +20.3\%$)
- 4. IAEA-NO-3, Potassium Nitrate* (IAEA Website $\delta 15N = +4.7\%$)

* Due to poor readback values, % Accuracy, and %CV statistics, IAEA-NO-3 was not used in the determination of secondary laboratory values in this report.

Other materials were as follows:

Column: 4 meter NC packed column (Costech)

Column Temperature: 60°C

Helium Gas: Grade 5.0, 50 psi tank gauge, 15 psi EA gauge, 15 psi Conflo III gauge Helium Flow rate: ~ 85mL/min.

Oxygen: Research Grade, Airgas P/N: OX-R300, 36 psi gauge, 19-20 psi EA gauge Nitrogen: Grade 5.0, reference gas 2 on Conflo III, 55 psi gauge.

Reaction Tube: packed with 11cm of Chromium oxide catalyst (Costech P/N 011001), 6 cm of Silvered Cobaltous oxide (P/N 011007), separated by 1 cm of quartz wool (P/N 021035).

Combustion tube (insert) packed with ~ 0.25" quartz wool and ~ 0.25" of Chromium Oxide catalyst. Reduction tube packed with ~ 45 cm of Copper wire, reduced 0.7mm (P/N 011013), with 4 cm of quartz wool packed in the bottom and 1 cm of quartz wool packed in the top. Moisture trap packed with Magnesium perchlorate (P/N 021022) and a few quartz turnings (P/N 021025), pack each end of the moisture trap with ~ 5 mm of quartz wool. Sample Tins: Costech 3.5mm x 5mm pressed tin capsules (P/N 041074).

2.2. INSTRUMENTATION (IRMS, ELEMENTAL ANALYZER, AND CONFLO III)

The IRMS instrument is a Thermo Scientific Delta V Advantage along with a Costech 4010 Elemental Analyzer, Costech Zero Blank auto-sampler (50 or 100 sample turret), and Finnigan Conflo III. Samples were weighed on a Sartorious Model CP2P, S/N 19502516 micro-balance (calibrated annually and checked daily).

 IRMS Data Acquisition System: Isodat 2.5 Gas Isotope Ratio MS Software Acquisition - Used for running the analysis (acquiring data).
 Workspace – Used for analysis setup, methods and sequence development, and data review. Instrument Control – Used to monitor and control various aspects of the instrument.

2.3. ANALYSIS PROCEDURE, SAMPLE PREPARATION AND INSTRUMENT CONDITIONS

Analysis Procedure

Four analysis days (three Primary standards to Secondary standard evaluations and one Secondary to Primary standard evaluation) were performed during the course of the validation. The first three analysis days consisted of 55 samples for each run, the final Secondary to Primary analysis consisted of a 56 sample run. Two method files were created for this validation. The first method file N2_CO2_60C_DIL_USGS40_012610.met was used for the three primary to secondary standard analyses. This file identified Primary standard USGS 40 as the reference for Wt% calculations (if required). Method file N2_CO2_60C_DIL_MINK_022510.met was created and used for the Secondary to Primary analysis. This file identified the Secondary standard MINK as the reference for Wt% calculations (if required). Both of these method files utilized the diluter during the CO₂ peak elution.

Sample Preparation

Samples were weighed into small sample tins (3.5mm x 5mm) using the suggested target weights identified in the summary. The various sample target weights were selected to give an m/z 28 signal in the 3500 mV range. The procedure for weighing and preparing the samples is described in the Sample Weighing SOP. The sequence consisted of 55 analyses which included 4 primary standards analyzed five times each, 5 secondary standard candidates analyzed six times each, 3 tin blanks, and 2 double blanks (no sample or tin). This same sequence was performed on three different days. After the three sequences (three validation days) a fourth sequence (fourth validation day) was performed using the secondary standard candidates to back calculate the primary standard values for accuracy verification. The files can be found in ISODAT Workspace, Gas Configuration = Conflo, in the Sequence tab (see Figure 7 for an example of a sequence file).

- 1. N2_021710_Val_1.seq
- 2. N2_021810_Val_2.seq

- 3. N2_022210_Val_3.seq
- 4. N2_022510_Sec_Prim.seq

The method files associated with these sequence files are identified as:

- 1. N2_CO2_60C_Dil_USGS40_012610.met (see Figures 3 6)
- 2. N2_CO2_60C_Dil_MINK_022510.met

The samples were loaded into the 100 sample auto-sampler using the procedure outlined in the Loading the auto-sampler SOP.

The following was then performed:

- Open Instrument Control software, check and record the MS pressure.
- Open the EA inlet valve on the IRMS.
- Wait a few minutes for the pressure to stabilize, and record the pressure.
- Turn on the filament.
- Monitor m/z 18 (H₂O) on cup 3. (The m/z 18 signal should drop below 1000 mV within 1 2 hours of turning on the filament.)
- With the m/z 18 signal below ~1000 mV, perform an autofocus for N₂ using Autofocus_N2_(Date) file in Instrument Control. Turn on the N₂ reference gas.
- Typically use the following parameters in the Autofocus dialog box (see Figure 2):
 - Measuring Channel: 2
 - Integration Time: 0.100(s)
 - Minimum Step Width: 1
 - Maximum Step Width: 10
 - Minimum delay time(ms): 50
 - Maximum delay time(ms): 500
 - Maximum iterations: 3
 - Simulated Poti Turns: 2
 - o Accelerating Voltage: unchecked
 - Electron Energy: unchecked
 - Emission: unchecked
 - Trap: unchecked
 - X-Deflection: Checked
 - Focus Voltage: Checked
 - Extraction Voltage: unchecked
 - Y-Defl Voltage: Checked
 - Focus Symmetry: Checked
 - Extraction Symmetry: Checked
 - Y-Defl Symmetry: Checked
- Repeat the autofocus until there is no further N_2 signal improvement.
- Select *Pass to Gasconfiguration* in the Focus Delta administrative panel.

- Perform on-off (N2_On-Off.met) and linearity (N2_On-Off.met) system suitability using N₂ as the reference gas. $\delta^{15}N$ On-Off: std.dev. < 0.05‰, $\delta^{15}N$ Linearity: regression slope std. dev. < 0.06‰ with increasing N₂ pressure (see Figures 8 and 9).
- Adjust the N₂ reference gas to give a reference peak (m/z 28, cup 2) signal of between 3000 and 4000 mV (m/z 29 ~ 2000 2500 mV). Close Instrument Control, open Isodat Acquisition.
- Verify that Isodat Acquisition, and Isodat Workspace programs are open (and Instrument Control is closed). Note: To minimize potential computer issues, it is recommended to reset the computer before starting any extended analysis sequence.
- In Acquisition, check and record mass spectrometer pressure, the CO₂, N₂, m/z 18 (cup 3), m/z 32 (cup 3), and m/z 40 (cup 3) intensities.
- Verify system readiness for analysis, e.g., Helium tank pressures, packed column temperature, REMOTE switch on Costech on, etc.
- Verify that the correct sequence has been selected and double check the information.
- When all is correct, click "Start".
- Identify the folder in which the data files are to be stored (typically use N2 followed by an underscore and then the analysis date).
- Next choose how to identify the data files.
- Un-check the "Auto Enum" button.
- Start the analysis by checking the "OK".
- Observe the first few samples for correct Auto-sampler operation, peak acquisition, and timing.
- Completed files can be reviewed in Isodat Workspace...\Results\filename. (See Figures 10 12 for example chromatograms of a blank, a Primary standard, and a Lab standard).
- When the analysis is complete, review the files in Workspace to verify all samples were properly acquired and analyzed. (It is useful to record any anomalous findings or notes on the analysis worksheet.)
- Print the data files in Workspace.
- Re-process the data files using the export file N2_and_CO2_wt%_052308.wke, this will put the data into EXCEL format (Figure 13).
- Transfer the re-processed data via an appropriate technique to another computer for statistical analysis.
 - First copy the data into a new worksheet.
 - Clean up the spreadsheet, set significant figures, alignments, headings, etc, to make the spreadsheet easier to handle and interpret.
 - Sort on "Peak No." to separate out the reference peaks.
 - Cut and paste the reference peak data into a new worksheet.
 - \circ $\;$ After the reference peaks have been removed, sort on the sample ID.
 - $\circ~$ Create a calibration curve for $\delta^{15}N\%$ using the primary standards, plot the known values vs. the IRMS determined values.
 - $\circ~$ Plot the trend line, the equation of the trend line is the regression formula used to determine the corrected $\delta^{15}N$ ‰ values.

 $\circ~$ Perform statistical analysis (mean, standard deviation, accuracy, and %CV) on all average $\delta^{15}N$ ‰ values determined for each sample. This is the intra-statistical analysis.

Instrument Conditions

Elemental Analyzer

- Packed Column Temperature 60°C
- Packed Column Flow Rate 1.0 1.5 mL/min.
- He Pressure (at tank) 50 psi
- He pressure (at EA) 13 15 psi (flow rate ~ 85 mL/min.)
- O2 pressure (at tank) ~ 36psi
- O2 pressure (at EA) ~ 15psi
- O2 injection micro setting
- N_2 pressure (at tank) ~ 35 psi
 - (at Conflo) adjust to 3 5 volts m/z 28 signal in cup 2 (25 35 psi)

IRMS

- Tune File e.g.: autofocus_N2_(Date of last tune)
- High Vacuum (MS Valve open) ~ 5.5e-7 mB
- High Vacuum (MS Valve closed) ~9.0e-8 mB
- Instrument configuration Conflo
- N₂ reference peak intensity (m/z 28 cup 2) ~ 3500 mV
- Methods Validation N2_CO2_60C_Dil_USGS40_012610.met Sec to primary - N2_CO2_60C_Dil_MINK_022510.met

2.4. δ^{15} N STANDARD VALIDATION DATA

The Excel files used for this validation can be found on the Hamilton College network, the path is as follows: Campus on ESS P:\Instrumentation\Geosciences\Data\Thermo_IRMS\EA\ Validation\Nitrogen\(file names). The file names and contents are listed below:

1 N2 Vol 1 021710 -less Vol det en des 1 results

- 1. N2_Val_1_021710.xlsx Validation day 1 results
- 2. N2_Val_2_021810.xlsx Validation day 2 results
- 3. N2_Val_3_022210.xlsx Validation day 3 results
- 4. N2_022510_Sec_Prim.xlsx Validation day 4 results, experimentally determined values for Secondary standards used to determine Primary standard values

Table 1:

Summary Statistics for Day 1 Validation - Primary Standards

Primary Standards Statistics	
<u>USGS 40</u>	δ ¹⁵ N ‰
Average	-4.79
Std. Deviation	0.15
%CV	3.13
%Acc	105.97
n	3
Known δ ¹⁵ N _{air N2}	-4.52
<u>USGS 41</u>	δ ¹⁵ N ‰
Average	47.57
Std. Deviation	0.09
%CV	0.19
%Acc	100.00
n	3
Known δ ¹⁵ N _{air N2}	+47.57
IAEA-N-2	δ^{15} N ‰
Average	20.70
Std. Deviation	0.22
%CV	1.06
%Acc	101.97
n	3
Known δ ¹⁵ N _{air N2}	+20.3
KNO3	δ ¹⁵ N ‰
Average	3.50
Std. Deviation	0.27
%CV	7.71
%Acc	74.46
n	5
Known δ ¹⁵ N _{air N2}	+4.7

File Name: N2_Val_1_021710.xlxs

Note: %CV = Coefficient of Variation

%Acc = Accuracy

Table 2:

Summary Statistics for Day 1 Validation – Secondary Standards

File Name: N2_Val_1_021710.xlxs

Secondary Standards Statistics	
Caffeine	δ ¹⁵ N ‰
Average	-2.91
Std. Deviation	0.09
%CV	3.09
	6
n	0
CBG (cabbage)	δ ¹⁵ N ‰
Average	10.01
Std. Deviation	0.20
%CV	2.00
n	6
CBT (trout)	δ ¹⁵ N ‰
Average	17.77
Std. Deviation	0.11
%CV	0.62
n	6
Mink	δ^{15} N ‰
Average	11.62
Std. Deviation	0.14
%CV	1.20
n	6
<u>Urea</u>	δ^{15} N ‰
average	-0.46
Std. Deviation	0.11
%CV	23.91
n	6

Note: %CV = Coefficient of Variation

Table 3:

Summary Statistics for Day 2 Validation - Primary Standards

Primary Standards Statistics	
<u>USGS 40</u>	δ ¹⁵ N ‰
Average	-4.66
Std. Deviation	0.03
%CV	0.64
%Acc	103.10
n	3
Known δ ¹⁵ N _{air N2}	-4.52
<u>USGS 41</u>	δ ¹⁵ N ‰
Average	47.45
Std. Deviation	0.16
%CV	0.34
%Acc	99.75
n	3
Known δ ¹⁵ N _{air N2}	+47.57
IAEA-N-2	δ ¹⁵ N ‰
Average	20.58
Std. Deviation	0.14
%CV	0.68
%Acc	101.38
n	3
Known δ ¹⁵ N _{air N2}	+20.3
KNO3	δ ¹⁵ N ‰
Average	3.35
Std. Deviation	0.26
%CV	7.76
%Acc	71.28
n	5
Known δ ¹⁵ N _{air N2}	+4.7

File Name: N2_Val_2_021810.xlxs

Note: %CV = Coefficient of Variation

%Acc = Accuracy

Table 4:

Summary Statistics for Day 2 Validation – Secondary Standards

File Name: N2_Val_2_021810.xlxs

Secondary Standards Statistics	
Caffeine	δ ¹⁵ N ‰
Average	-2.93
Std. Deviation	0.08
%CV	2.73
n	6
CBG (cabbage)	δ^{15} N ‰
Average	9.61
Std. Deviation	0.11
%CV	1.14
n	6
CBT (trout)	δ ¹⁵ N ‰
	0 1 700
Average	17.62
Std. Deviation	0.04
%CV	0.23
n	6
Mink	δ ¹⁵ N ‰
Average	11.52
Std. Deviation	0.05
%CV	0.43
n	6
Urea	δ^{15} N ‰
average	-0.56
Std. Deviation	0.27
%CV	48.21
n	6

Note: %CV = Coefficient of Variation

Table 5:

Summary Statistics for Day 3 Validation - Primary Standards

Primary Standards Statistics	
<u>USGS 40</u>	δ ¹⁵ N ‰
Average	-4.56
Std. Deviation	0.04
%CV	0.88
%Acc	100.88
n	3
Known δ ¹⁵ N _{air N2}	-4.52
<u>USGS 41</u>	δ ¹⁵ N ‰
Average	47.43
Std. Deviation	0.28
%CV	0.59
%Acc	99.71
n	3
Known δ ¹⁵ N _{air N2}	+47.57
IAEA-N-2	δ ¹⁵ N ‰
Average	20.58
Std. Deviation	0.09
%CV	0.44
%Acc	101.38
n	3
Known δ ¹⁵ N _{air N2}	+20.3
KNO3	δ ¹⁵ N ‰
Average	3.26
Std. Deviation	0.19
%CV	5.83
%Acc	69.36
n	5
Known δ ¹⁵ N _{air N2}	+4.7

File Name: N2_Val_3_022210.xlxs

Note: %CV = Coefficient of Variation

%Acc = Accuracy

Table 6:

Summary Statistics for Day 3 Validation – Secondary Standards

File Name: N2_Val_3_022210.xlxs

Std. Deviation 0.19 %CV 1.97 n 6 CBT (trout) $\delta^{15}N \%$ Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink $\delta^{15}N \%$ Average 11.51 Std. Deviation 0.13 %CV 0.13 %CV 1.13 n 6 Urea $\delta^{15}N \%$ average -0.31	Secondary Standards Statistics	
Std. Deviation 0.14 %CV 5.05 n 6 CBG (cabbage) $\delta^{15}N \%_0$ Average 9.66 Std. Deviation 0.19 %CV 1.97 n 6 CBT (trout) $\delta^{15}N \%_0$ Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink $\delta^{15}N \%_0$ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea $\delta^{15}N \%_0$	Caffeine	δ ¹⁵ N ‰
Std. Deviation 0.14 %CV 5.05 n 6 CBG (cabbage) $\delta^{15}N \%_0$ Average 9.66 Std. Deviation 0.19 %CV 1.97 n 6 CBT (trout) $\delta^{15}N \%_0$ Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink $\delta^{15}N \%_0$ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea $\delta^{15}N \%_0$		
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Std. Deviation 0.19 %CV 1.97 n 6 CBT (trout) $\delta^{15}N \%_0$ Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink $\delta^{15}N \%_0$ Average 11.51 Std. Deviation 0.13 %CV 0.13 %CV 1.13 n 6 Urea $\delta^{15}N \%_0$ average -0.31	CBG (cabbage)	δ ¹⁵ N ‰
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n 6 CBT (trout) δ ¹⁵ N ‰ Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink δ ¹⁵ N ‰ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	Std. Deviation	0.19
CBT (trout) δ ¹⁵ N ‰ Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink δ ¹⁵ N ‰ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	%CV	1.97
Average 17.50 Std. Deviation 0.04 %CV 0.23 n 6 Mink δ ¹⁵ N ‰ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	n	6
Std. Deviation 0.04 %CV 0.23 n 6 Mink δ ¹⁵ N ‰ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	CBT (trout)	δ ¹⁵ N ‰
Std. Deviation 0.04 %CV 0.23 n 6 Mink δ ¹⁵ N ‰ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31		
%CV 0.23 n 6 Mink δ ¹⁵ N ‰ Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31		
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Average 11.51 Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	n	6
Std. Deviation 0.13 %CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	Mink	δ ¹⁵ N ‰
%CV 1.13 n 6 Urea δ ¹⁵ N ‰ average -0.31	Average	11.51
n 6 <u>Urea</u> δ ¹⁵ N ‰ average -0.31	Std. Deviation	0.13
<u>Urea</u> δ ¹⁵ N‰ average -0.31	%CV	1.13
average -0.31	n	6
5	Urea	δ ¹⁵ N ‰
Std. Deviation 0.14	average	-0.31
	Std. Deviation	0.14
%CV 45.16	%CV	45.16
n 6	n	6

Note: %CV = Coefficient of Variation

Table 7:

Summary Statistics for Day 4 Validation - Primary Standards

Primary Standards Statistics	
<u>USGS 40</u>	δ^{15} N ‰
Average	-4.67
Std. Deviation	0.06
%CV	1.28
%Acc	103.32
n	6
Known δ ¹⁵ N _{air N2}	-4.52
<u>USGS 41</u>	δ ¹⁵ N ‰
Average	47.79
Std. Deviation	0.24
%CV	0.50
%Acc	100.46
n	6
Known δ ¹⁵ N _{air N2}	+47.57
IAEA-N-2	δ^{15} N ‰
Average	20.75
Std. Deviation	0.17
%CV	0.82
%Acc	102.22
n	6
Known δ ¹⁵ N _{air N2}	+20.3
KNO3	δ^{15} N ‰
Average	2.91
Std. Deviation	0.15
%CV	5.15
%Acc	61.91
n	6
Known δ ¹⁵ N _{air N2}	+4.7

File Name: N2_022510_Sec_Prim.xlxs

Note: %CV = Coefficient of Variation

% Acc = Accuracy

Table 8:

Summary Statistics for Day 4 Validation – Secondary Standards

File Name:	N2	022510	Sec	Prim.xlxs
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Secondary Standards Statistics	
Caffeine	δ ¹⁵ N ‰
Average	-2.97
Std. Deviation	0.09
%CV	3.03
%Acc	103.48
n	5
Experimentally Determined Value	-2.87
<u>CBT (trout)</u>	δ ¹⁵ N ‰
Average	17.51
Std. Deviation	0.12
%CV	0.69
%Acc	99.32
n	5
Experimentally Determined Value	17.63
Mink	δ ¹⁵ N ‰
Average	11.43
Std. Deviation	0.11
%CV	0.96
%Acc	98.96
n	5
Experimentally Determined Value	11.55
<u>Urea</u>	δ ¹⁵ N ‰
Average	-0.40
Std. Deviation	0.15
%CV	37.50
%Acc	90.91
n	6
Experimentally Determined Value	-0.44

Note: %CV = Coefficient of Variation

%Acc = Accuracy

Table 9:

Analysis Date	Validation Day	Regression Line	\mathbf{R}^2
02/17/2010	Day 1	y = 1.0326x + 1.0426	0.9997
02/18/2010	Day 2	y = 1.0279x + 1.0189	0.9999
02/22/2010	Day 3	y = 1.0293x + 1.0839	0.9999
02/25/2010	Day 4	y = 1.0394x + 1.0926	0.9997

Regression Line Equations used to correct δ^{15} N‰ Instrument Values

3. COMMENTS

Three primary standards, in duplicate (one at the beginning of the analysis and one at the end) were used to generate the regression line. (IAEA-NO3 (KNO3) was not used for the regression as its readback values and statistics were poor.)

The primary standards that were used in the regression line generation were not used in the calculations of the experimentally determined $\delta^{15}N$ ‰ read-back values or the statistics generated for them. Only the additional primary standards (n=3) analyzed in each run were used for this purpose.

An analysis of the $\delta^{15}N$ ‰ value determined for each sample was plotted versus acquisition time. It was determined that there was no temporal bias and as such no drift corrections of determined $\delta^{15}N$ ‰ values were made.

Day 4 Validation (Secondary to Primary Standard experiment) was performed only to evaluate the integrity of the lab (secondary) standards for regression line generation and subsequent sample readbacks. This data was not used in any statistical calculations. (Caffeine, CBT, and Mink were used to generate the regression line.)

Even though acquisition and detection of the CO₂ response was performed, the data was not used.

%Accuracy = {Experimental Value/Known (Established) Value} X 100 %CV = {Standard Deviation/Average Value} X 100

4. DATA RETRIEVAL

The raw data files are stored on the Thermo IRMS instrument computer in the GeoSciences laboratory in the following location: C:\Thermo\Isodat NT\Global\User\ConfloII Interface\Results\N2_Validation\ N2_Val_1_021710\filename.dxf N2_Val_2_021810\filename.dxf N2_Val_3_022210\filename.dxf N2_022510 Sec Prim\filename.dxf

The Excel Worksheets are stored on the Hamilton College network in the following location: "Campus on ESS"(P:)\Instrumentation\Geosciences\Data\Thermo_IRMS\EA\Validation\ Nitrogen\filename.xlsx

5. CONCLUSIONS

This analysis identified samples which could be used for lab (secondary) standards during unknown $\delta^{15}N$ ‰ investigations. This validation also provided $\delta^{15}N$ ‰ values for these lab standards (to be used for regression line generation) along with statistical evaluations of those values. Due to their range of $\delta^{15}N$ ‰ values and sample weight, Caffeine, CBT, and Mink are the recommended secondary standards. The following is a summary of the results:

Secondary Standard	δ^{15} N‰ _{AirN2}	Std. Dev.	%CV	n
Caffeine	-2.87	0.10	3.48	18
CBT	17.63	0.06	0.34	18
Mink	11.55	0.11	0.95	18
CBG*	9.76	0.16	1.64	18
Urea**	-0.44	0.17	38.64	18

Table 10: Secondary Standard Statistics Summary (Three Analysis Days)

* CBG is not recommended due to the weight of sample needed to provide an adequate Nitrogen signal (~ 3.0 mg). Sample weights of greater than 2.5 - 3.0 mg can potentially jam the 100 sample autosampler jeopardizing the entire analysis sequence.

** Urea is not recommended due to its large %CV value of 38.64.

The experimentally determined values and the statistics for the primary standards are given below to assess method accuracy and variability across the 3 days of validation:

Primary Standard	δ^{15} N‰ _{AirN2}	Std. Dev.	%CV	% Acc	n
USGS 40	-4.67	0.07	1.50	103.32	9
USGS 41	47.48	0.18	0.38	99.81	9
IAEA-N-2	20.62	0.15	0.73	101.58	9
IAEA-NO-3 (KNO3)*	3.37	0.24	7.12	71.70	15

* Due to poor readback values, % Accuracy, and %CV statistics, IAEA-NO-3 was not used in the determination of secondary laboratory values in this report.

REFERENCES

Thermo Electron Delta V Advantage Operating Manual Costech Elemental Analyzer Operating Manual

6. FIGURES

Figure 1: δ^{15} N Experimentally Determined Values, Sorted by δ^{15} N (average of three runs)

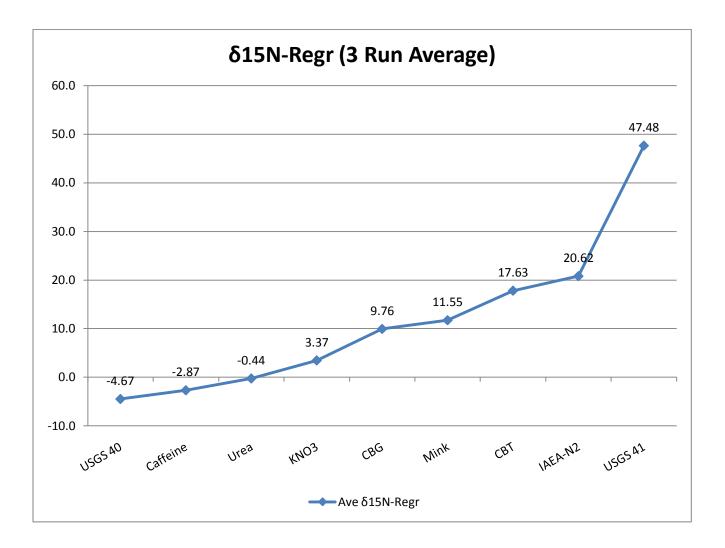


Figure 2: N₂ Autofocus Settings

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Figure 3: δ^{15} N Method File – Instrument Screen

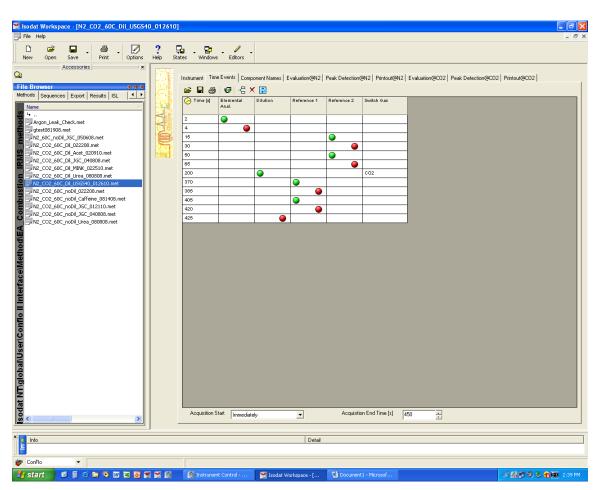


Figure 4: δ^{15} N Method File – Time Events Screen

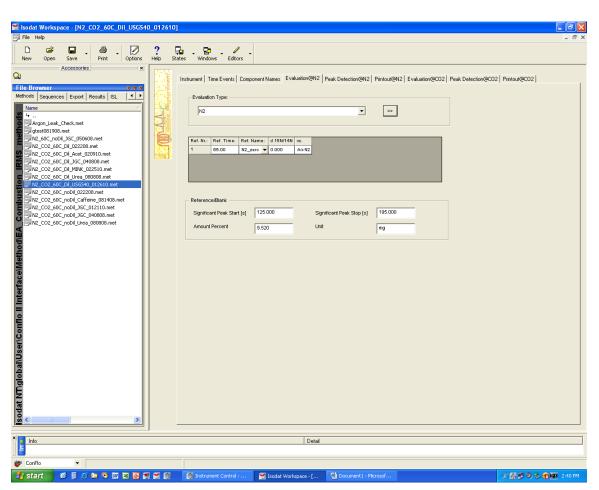


Figure 5: δ^{15} N Method File – Evaluation@N2 Screen

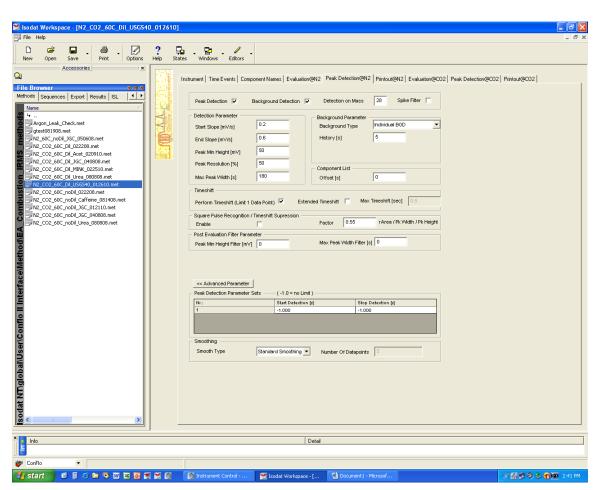


Figure 6: δ^{15} N Method File – Peak Detection@N2 Screen

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100_Autosampler_Sequence.seq	9	✓ 2.875		• 9	1	CBG	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
50_Autosampler_Sequence.seq	10	✓ 0.849		▼ 10	1	Mink	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
AW_Bedrock_Douglas_River_1005 AW_NBP0107_KC8_100709.seq	11	✓ 0.975	Sample	▼ 11	1	CBT	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
AW_0L_100707_101209.seq	12	✓ 0.458	Sample	▼ 12	QC-2	IAEA-N2	EA_Combustion_IRMS_methods\N2_CO2_60C_EA_Combustion_RMS_methods
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AW_OL_100707_101509_B.seq	14	✓ 0.857	Sample	▼ 14	Std Chk-1	KNO3 (Std)	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
Bedrock_Douglas_River.seq	15	✓ 0.900		▼ 15	Std Chk-1	USGS-41	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
Bedrock_Douglas_River_100609.s	16	✓ 0.342	Sample	▼ 16	2	Caffeine	EA_Combustion_IRMS_methodsW2_CO2_60C_Dil_USGS40_012610.met
Bedrock_Douglas_River_123008.s	17	✓ 0.255	Sample	▼ 17	2	Urea	EA_Combustion_IRMS_methodsW2_CO2_60C_Dil_USGS40_012610.met
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MicroTest_012010.seq	20	✓ 0.997	Sample	▼ 20	2	CBT	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
MicroTest_012110.seq	21	✓ 0.304	Sample	▼ 21	3	Caffeine	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
m_bio_spar_070208.seq	22	✓ 0.212	Sample	 22 	3	Urea	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
m_bio_spar_070908.seq	23	✓ 0.440	Sample	 23 	QC-3	IAEA-N2	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
m_lab_helper_dilyana_rebecca_te	24	✓ 0.000	Sample	 24 	Blank-2	Blank	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
m_lab_helper_jen_test_stds.seq N2 012710.seq	25	✓ 0.885	Sample	 25 	Std Chk-2	USGS-40	EA Combustion IRMS methods\N2 CO2 60C Dil USGS40 012610.met
N2_012810.seq	26	✓ 0.759	Sample	▼ 26	Std Chk-2	KNO3 (Std)	EA Combustion IRMS methods\N2 CO2 60C Dil USGS40 012610.met
N2_021710_Val_1.seq	27	✓ 0.835	Sample	▼ 27	Std Chk-2	USGS-41	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
N2_021810_Val_2.seq	28	✓ 3.405	Sample	v 28	3	CBG	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
N2_022210_Val_3.seq	29	✓ 0.959	Sample	▼ 29	3	Mink	EA Combustion IRMS methods/N2 CO2 60C Dil USGS40 012610.met
N2_022510_Sec_Prim.seq	30	✓ 0.973		▼ 30	3	CBT	EA_Combustion_IRMS_methods\N2_CO2_60C_Dil_USGS40_012610.met
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OL_100707_100809.seq	34	✓ 0.852	Sample	▼ 34	4	Mink	EA Combustion IRMS methods/W2 CO2 60C Dil USGS40 012610.met
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Figure 7: δ^{15} N Analysis Sequence File Example

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<pre>✓ co2_c ✓ co2_c ✓ co2_c ✓ co2_c</pre>	022210_Linearity-1.dxf 022210_OnOff-1.dxf 022210_OnOff-2.dxf 022210_OnOff-3.dxf 022210_OnOff-3.dxf 022210_OnOff-4.dxf	4000-	28	■ 26											328.13
	22210_Linearity-1.dxf													_	
culate R	Results						3							288.21	
													<u> </u>	- T	
			d 15N/14	N		_						248.50			
	Mean SqrSum		-0.034								r			Ir	1
	Std.Dev.		0.029							21	18.58			_	
	Max		0.056								4				
	Min		-0.131						168.87		1				
	Regression Slope		-0.021					128.95			4				
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					so		100		150 Time	200 (s)		250		300	
		[N2 In	fos E	rrors Sequence	e Line									
				Rt [5]	Ampl. 28 [mV]	Ampl. 29 [mV]	B⊙D 28 [mV]	BGD 29 [mV]	Area All [Vs]	Amt% [%]	R 29N2/28N2	d 29N2/28N2 [per mil] vs. Air-N2	d 15N/14N [per mil] vs.	d 15N/14N Bik corr	Rps 29N2/28
															•
			1	49.3	509.8036759	372.1629012	22.17872	20.18692	10.061184749494949	0.0000000000	0.007356210	-0.02585	-0.02585		
			1	89.0	801.1354155	584.7161293	21.96656	19.90488	15.808076081464176	0.0000000000	0.007356598	0.02697	0.02697		-
			1 2 3	89.0 129.0	801.1354155 1091.5293724	584.7161293 796.6791255	21.96656 21.80447	19.90488 19.69251	15.808076081464176 21.533645871246186	0.000000000	0.007356598	0.02697 0.05608	0.02697 0.05608	•	-
			1 2 3 4*	89.0 129.0 168.9	801.1354155 1091.5293724 1412.4797034	584.7161293 796.6791255 1030.7796862	21.96656 21.80447 21.67236	19.90488 19.69251 19.55174	15.808076081484176 21.533645871248186 27.856788025354764	0.000000000 0.000000000 0.000000000	0.007356598 0.007356813 0.007356400	0.02697 0.05608 -0.00000	0.02697 0.05608 -0.00000	•	0.00735640
			1 2 3 4* 5	89.0 129.0 168.9 208.6	801.1354155 1091.5293724 1412.4797034 1784.9767363	584.7161293 796.6791255 1030.7796862 1288.0275298	21.96656 21.80447 21.67236 21.56793	19.90488 19.69251 19.55174 19.41392	15.808076081464176 21.533645871246188 27.856788025354764 34.809258230270125	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118	0.02697 0.05608 -0.00000 -0.03830	0.02697 0.05608 -0.00000 -0.03830	• • •	0.00735840
			1 2 3 4* 6 8	89.0 129.0 168.9 208.6 248.5	801.1354155 1091.5293724 1412.4797034 1764.9767363 2167.0269061	584.7161293 796.6791255 1030.7796862 1288.0275298 1581.3084489	21.96656 21.80447 21.67236 21.56793 21.49887	19.90488 19.69251 19.55174 19.41392 19.30753	15.808076081464176 21.533645671246188 27.856788025354764 34.809258230270125 42.766748588808021	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118 0.007356034	0.02697 0.05608 -0.00000 -0.03830 -0.04973	0.02697 0.05608 -0.00000 -0.03830 -0.04973	• • •	- - 0.00735640 -
			1 2 3 4* 5 6 7	89.0 129.0 168.9 208.6 248.5 288.2	801.1354155 1091.5293724 1412.4797034 1764.9767363 2167.0269061 2626.4278877	584.7161293 796.6791255 1030.7798862 1288.0275298 1581.3084469 1916.5040048	21.96656 21.80447 21.67236 21.56793 21.49887 21.44689	19.90488 19.69251 19.55174 19.41392 19.30753 19.25749	15.808076081464176 21.533645671246186 27.856788025354764 34.809256230270125 42.786748588808021 51.752055909188350	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118 0.007356034 0.007355440	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13056	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13058	· · ·	- - 0.00735640 - - -
<			1 2 3 4* 6 8	89.0 129.0 168.9 208.6 248.5 288.2	801.1354155 1091.5293724 1412.4797034 1764.9767363 2167.0269061	584.7161293 796.6791255 1030.7796862 1288.0275298 1581.3084489	21.96656 21.80447 21.67236 21.56793 21.49887	19.90488 19.69251 19.55174 19.41392 19.30753	15.808076081464176 21.533645671246188 27.856788025354764 34.809258230270125 42.766748588808021	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118 0.007356034	0.02697 0.05608 -0.00000 -0.03830 -0.04973	0.02697 0.05608 -0.00000 -0.03830 -0.04973	· · ·	- 0.00735640 - - -
<			1 2 3 4* 5 6 7	89.0 129.0 168.9 208.6 248.5 288.2	801.1354155 1091.5293724 1412.4797034 1764.9767363 2167.0269061 2626.4278877	584.7161293 796.6791255 1030.7798862 1288.0275298 1581.3084469 1916.5040048	21.96656 21.80447 21.67236 21.56793 21.49887 21.44689	19.90488 19.69251 19.65174 19.41392 19.30753 19.25749 19.16983	15.808076081464178 21.533645871246188 27.856798025354764 34.809258230270125 42.766748588908021 51.752055909188350 61.388592273932897	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118 0.007356034 0.007355440	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13056	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13058	- - - - - - - -	0.00735040
<] m			1 2 3 4* 5 6 7	89.0 129.0 168.9 208.6 248.5 288.2	801.1354155 1091.5293724 1412.4797034 1764.9767363 2167.0269061 2626.4278877	584.7161293 796.6791255 1030.7798862 1288.0275298 1581.3084469 1916.5040048	21.96656 21.80447 21.67236 21.56793 21.49887 21.44689	19.90488 19.69251 19.55174 19.41392 19.30753 19.25749	15.808076081464178 21.533645871246188 27.856798025354764 34.809258230270125 42.766748588908021 51.752055909188350 61.388592273932897	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118 0.007356034 0.007355440	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13056	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13058	· · · · ·	0.00735040
Info			1 2 3 4* 5 6 7	89.0 129.0 168.9 208.6 248.5 288.2	801.1354155 1091.5293724 1412.4797034 1764.9767363 2167.0269061 2626.4278877	584.7161293 796.6791255 1030.7798862 1288.0275298 1581.3084469 1916.5040048	21.96656 21.80447 21.67236 21.56793 21.49887 21.44689	19.90488 19.69251 19.65174 19.41392 19.30753 19.25749 19.16983	15.808076081464178 21.533645871246188 27.856798025354764 34.809258230270125 42.766748588908021 51.752055909188350 61.388592273932897	0.000000000 0.000000000 0.000000000 0.000000	0.007356598 0.007356813 0.007356400 0.007356118 0.007356034 0.007355440	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13056	0.02697 0.05608 -0.00000 -0.03830 -0.04973 -0.13058	· · · ·	- 0.00735040 - -

Figure 9: δ^{15} N Linearity Check (Using N₂)

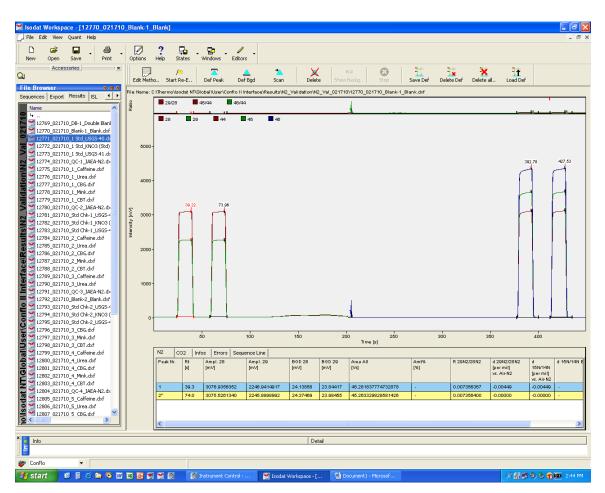


Figure 10: δ^{15} N Data Acquisition File – Blank

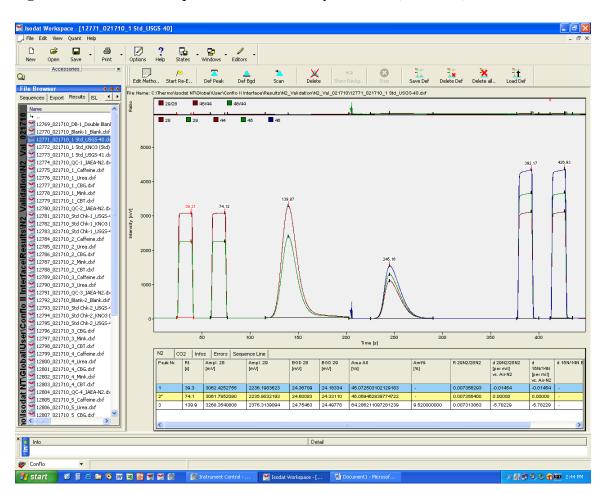


Figure 11: δ^{15} N Data Acquisition File – Primary Standard (USGS 40)

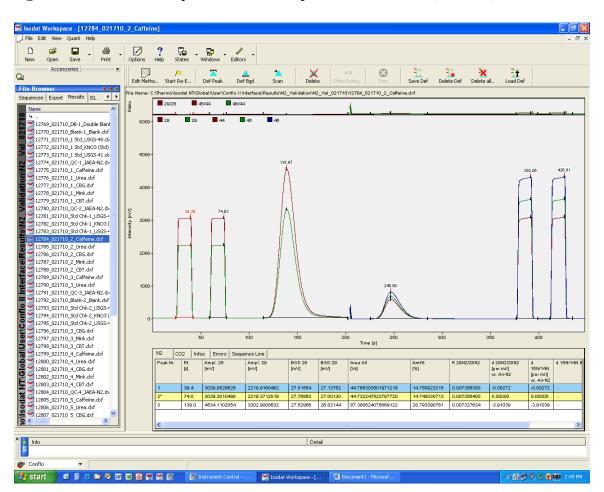


Figure 12: δ^{15} N Data Acquisition File – Proposed Lab Standard (Caffeine)

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lame 🔬	🔽 Data Type					
C+N.wke 0	Sequence Line	Acquisition Message 🔽 Molecule Delta 🔽 Valuated F	Results			
GR-test1.wke 0 GR test.wke 0	Method Part	Result Peak Relement Ratio V Intensity				
N2_and_CO2_wt%_030408.wke 0	Gas Configuration	Raw Ratio	nt			
N2 and CO2 wt% 050808.wke 0		V Molecule Ratio V Atom % V Mass Rele				
N2 and CO2 wt% 052308.wke 0		, indestruction	Y GI K			
N2_wt%_050708.wke 0		Disable All Enable	All			
test021808.wke 0						
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	Preparation	Sequence Information		Method	Sequence Information	
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	Err Information	Error Grid		Area 30	Result Data	
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	EQ Unit - Bank	Sequence Part - Equilibration Unit		d 29N2/28N2	Result Data	
	Pressadjust	Sequence Part - Dual Inlet Device		🔠 d 15N/14N	Result Data	
	Background	Sequence Part - Dual Inlet Device		I 15N/14N Blk corr	Result Data	
	🔷 Port	Sequence Part - Conflo		33 Ampl. 44	Result Data	
	Type	Sequence Part - Conflo	~	Ampl. 45	Result Data	
	Amount Preview	Sequence Part - Conflo		[33] Ampl. 46	Result Data	
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Figure 13: δ^{15} N Data Export File – N2_and_CO2_wt%_052308.wke