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)	USGS40, L-Glutamic acid USGS41, L-Glutamic acid IAEA-N-2, Ammonium Sulfate $(NH_4)_2SO_4$ IAEA-NO-3, Potassium Nitrate (KNO_3)					
Primary Standard Absolute Values	**Primary Standard**		**$\delta^{15}N‰_{airN2}$**			
	USGS40		**-4.52**			
	USGS41		**+47.57**			
	IAEA-N-2		**+20.3**			
	IAEA-NO-3		**+4.7**			
Primary Standard Experimental Values and Statistics	**Primary Standard**	**$\delta^{15}N‰_{airN2}$**	**S.D.**	**%CV**	**%Acc**	**n**
	USGS40	**-4.67**	0.07	1.50	103.32	9
	USGS41	**+47.48**	0.18	0.38	99.81	9
	IAEA-N-2	**+20.62**	0.15	0.73	101.58	9
	IAEA-NO-3*	**+3.37**	0.24	7.12	71.70	15
	*Standard not used due to inconsistent readback values and statistics					
Secondary (Laboratory) Standards	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%					
Lab (Secondary) Standard Experimentally Determined $\delta^{15}N$ Values and Statistics	**Secondary Standard**	**$\delta^{15}N‰_{airN2}$**	**S.D.**	**%CV**	**n**	
	Caffeine	**-2.87**	0.10	3.48	18	
	CBG**	**9.76**	0.16	1.64	18	
	CBT	**+17.63**	0.06	0.34	18	
	Mink	**+11.55**	0.11	0.95	18	
	Urea***	**-0.44**	0.17	38.64	18	

Standard Target Weights and Approximate m/z 28 Intensities	**Standard**	**Target Weight (mg)**	**~ m/z 28 Intensity (mV)**
	USGS 40	0.7 – 0.9	3500
	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

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

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1. INTRODUCTION

This report describes the qualification/validation process for $\delta^{15}N$ 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 (KNO_3) 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 $\delta^{15}N$ 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 δ15N = -4.52‰)
2. USGS41, L-Glutamic acid (Certificate of Analysis δ15N = +47.57‰)
3. IAEA-N-2, Ammonium Sulfate (IAEA Website δ15N = +20.3‰)
4. IAEA-NO-3, Potassium Nitrate* (IAEA Website δ15N = +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 CO_2 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 (H_2O) 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 N_2 using Autofocus_N2_(Date) file in Instrument Control. Turn on the N_2 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
    - 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 N_2 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 N_2 pressure (see Figures 8 and 9).
- Adjust the N_2 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 CO_2, N_2, 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.
    - After the reference peaks have been removed, sort on the sample ID.
    - Create a calibration curve for $\delta^{15}N$‰ using the primary standards, plot the known values vs. the IRMS determined values.
    - Plot the trend line, the equation of the trend line is the regression formula used to determine the corrected $\delta^{15}N$ ‰ values.

- 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 - 60ºC
- 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
- N_2 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. $\delta^{15}N$ 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_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

File Name: N2_Val_1_021710.xlxs

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

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
n	6
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
Urea	**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

File Name: N2_Val_2_021810.xlxs

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

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 ‰**
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

File Name: N2_Val_3_022210.xlxs

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

Note: %CV = Coefficient of Variation

%Acc = Accuracy

Table 6:

Summary Statistics for Day 3 Validation – Secondary Standards

File Name: N2_Val_3_022210.xlxs

Secondary Standards Statistics	
Caffeine	**$\delta^{15}N$ ‰**
Average	-2.77
Std. Deviation	0.14
%CV	5.05
n	6
CBG (cabbage)	**$\delta^{15}N$ ‰**
Average	9.66
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	1.13
n	6
Urea	**$\delta^{15}N$ ‰**
average	-0.31
Std. Deviation	0.14
%CV	45.16
n	6

Note: %CV = Coefficient of Variation

Table 7:

Summary Statistics for Day 4 Validation - Primary Standards

File Name: N2_022510_Sec_Prim.xlxs

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

Note: %CV = Coefficient of Variation

%Acc = Accuracy

Table 8:

Summary Statistics for Day 4 Validation – Secondary Standards

File Name: N2_022510_Sec_Prim.xlxs

Secondary Standards Statistics	
Caffeine	**$\delta^{15}N$ ‰**
Average	-2.97
Std. Deviation	0.09
%CV	3.03
%Acc	103.48
n	5
Experimentally Determined Value	-2.87
CBT (trout)	**$\delta^{15}N$ ‰**
Average	17.51
Std. Deviation	0.12
%CV	0.69
%Acc	99.32
n	5
Experimentally Determined Value	17.63
Mink	**$\delta^{15}N$ ‰**
Average	11.43
Std. Deviation	0.11
%CV	0.96
%Acc	98.96
n	5
Experimentally Determined Value	11.55
Urea	**$\delta^{15}N$ ‰**
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:

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

Analysis Date	Validation Day	Regression Line	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

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 δ^{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 δ^{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 δ^{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 read-backs. 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 CO_2 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:

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

Secondary Standard	$\delta^{15}N‰_{AirN2}$	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

* 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:

Table 11: Primary Standard Statistics Summary (Three Analysis Days)

Primary Standard	$\delta^{15}N‰_{AirN2}$	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: $\delta^{15}N$ Experimentally Determined Values, Sorted by $\delta^{15}N$ (average of three runs)

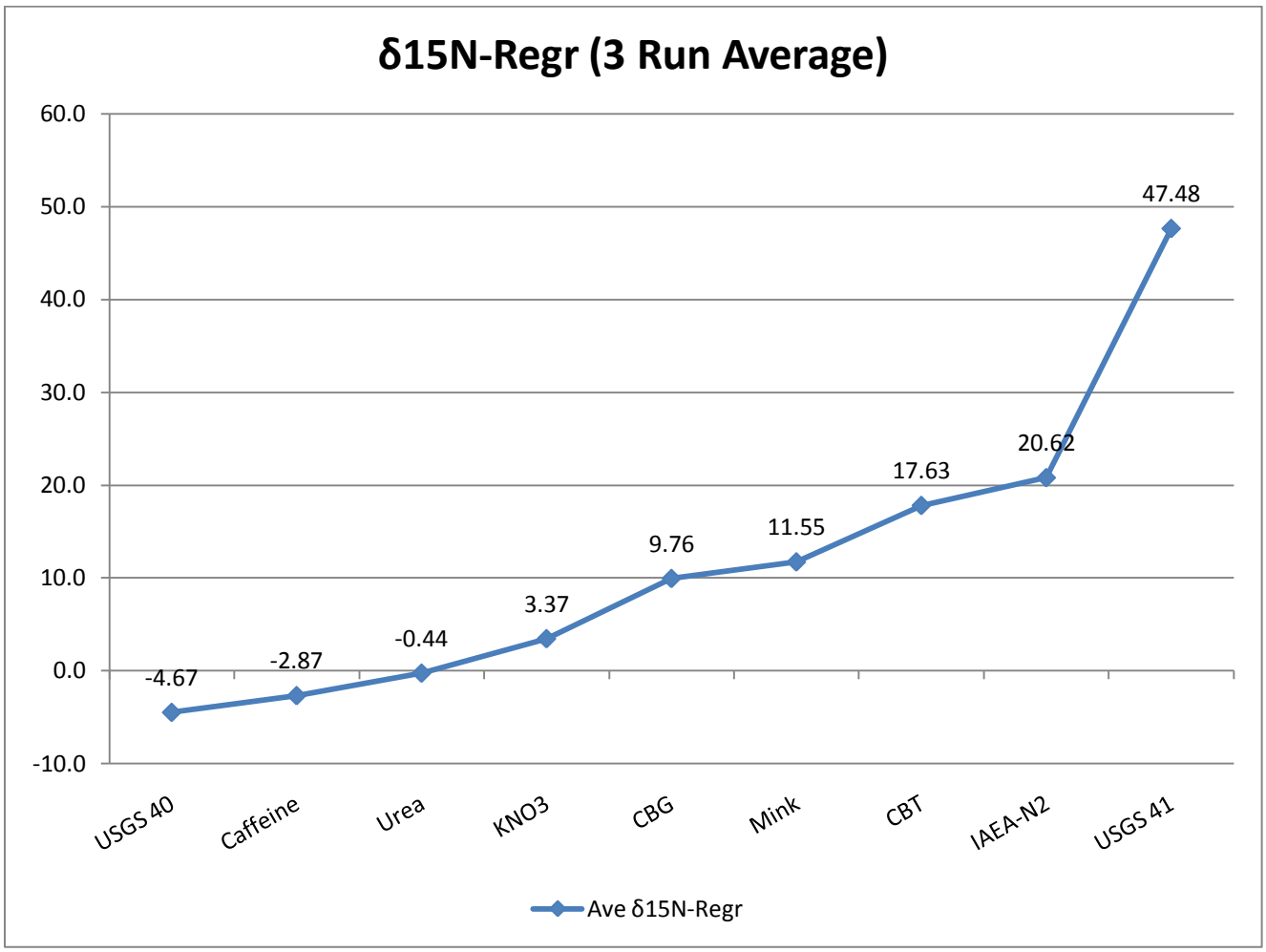

Figure 2: N_2 Autofocus Settings

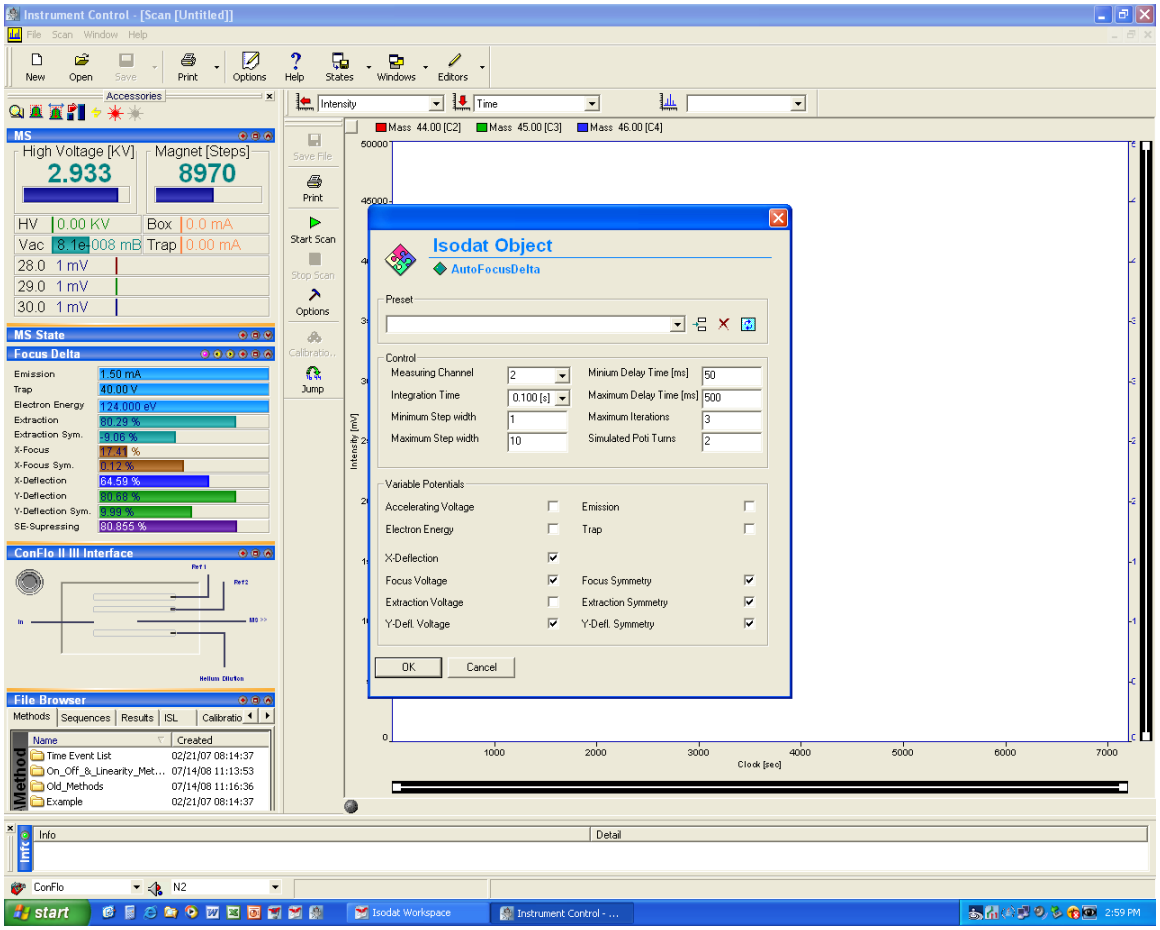

Figure 3: $\delta^{15}N$ Method File – Instrument Screen

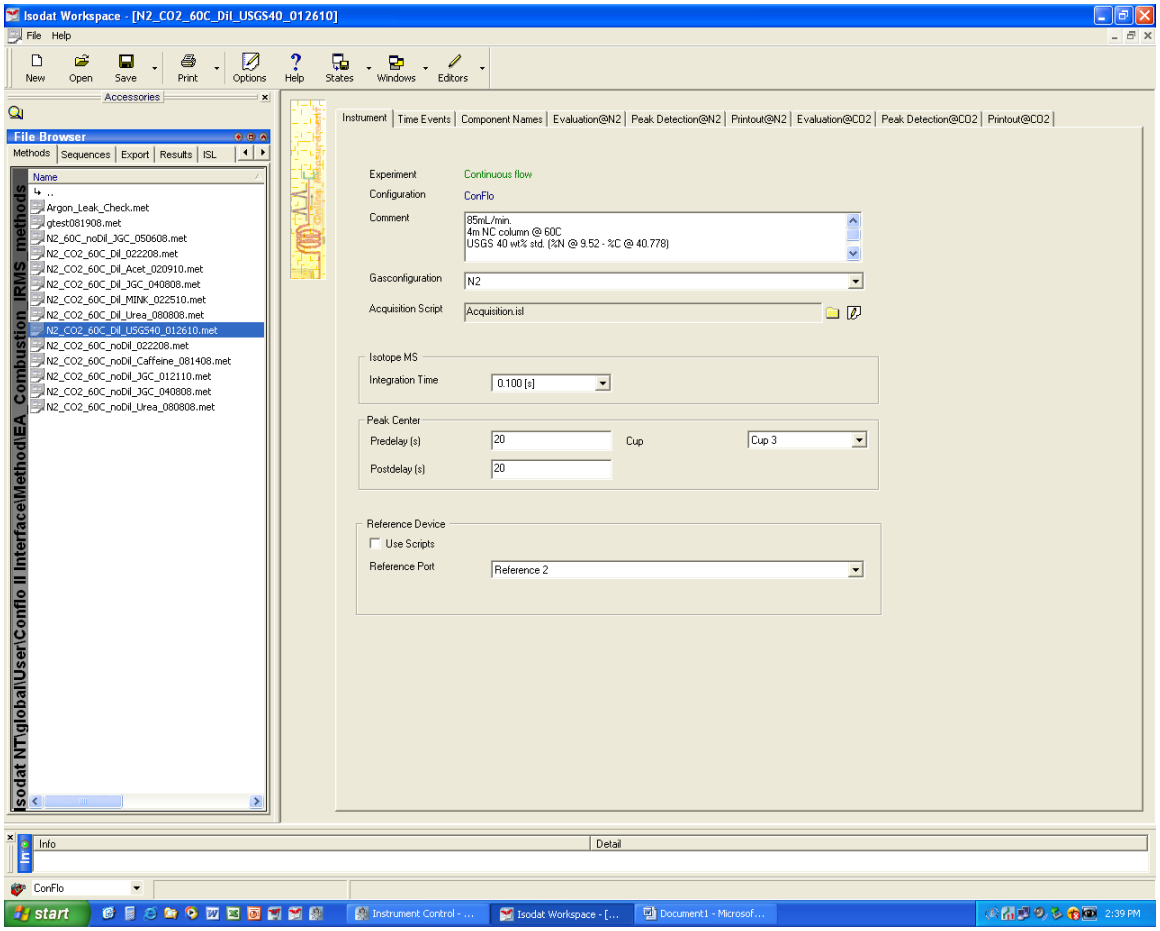

Figure 4: $\delta^{15}N$ Method File – Time Events Screen

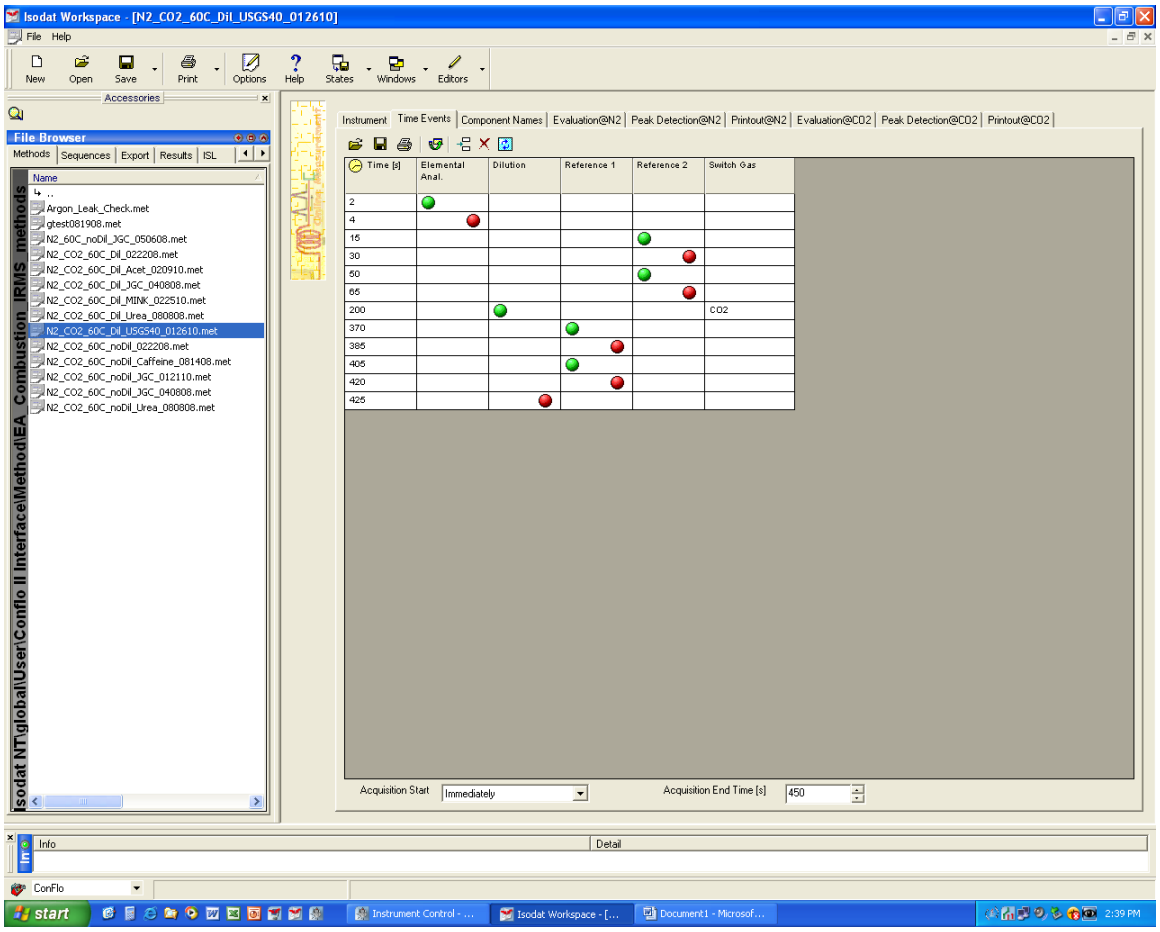

Figure 5: $\delta^{15}N$ Method File – Evaluation@N2 Screen

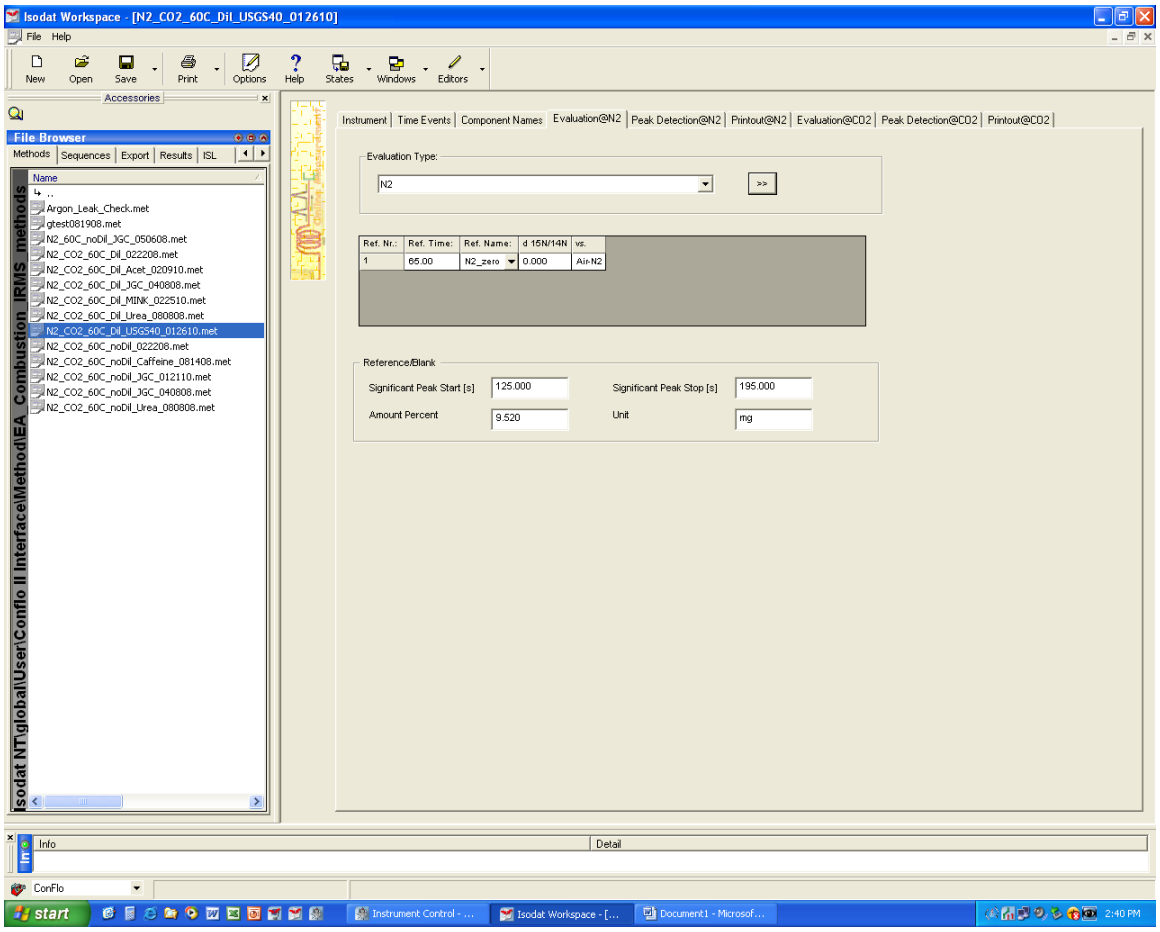

Figure 6: $\delta^{15}N$ Method File – Peak Detection@N2 Screen

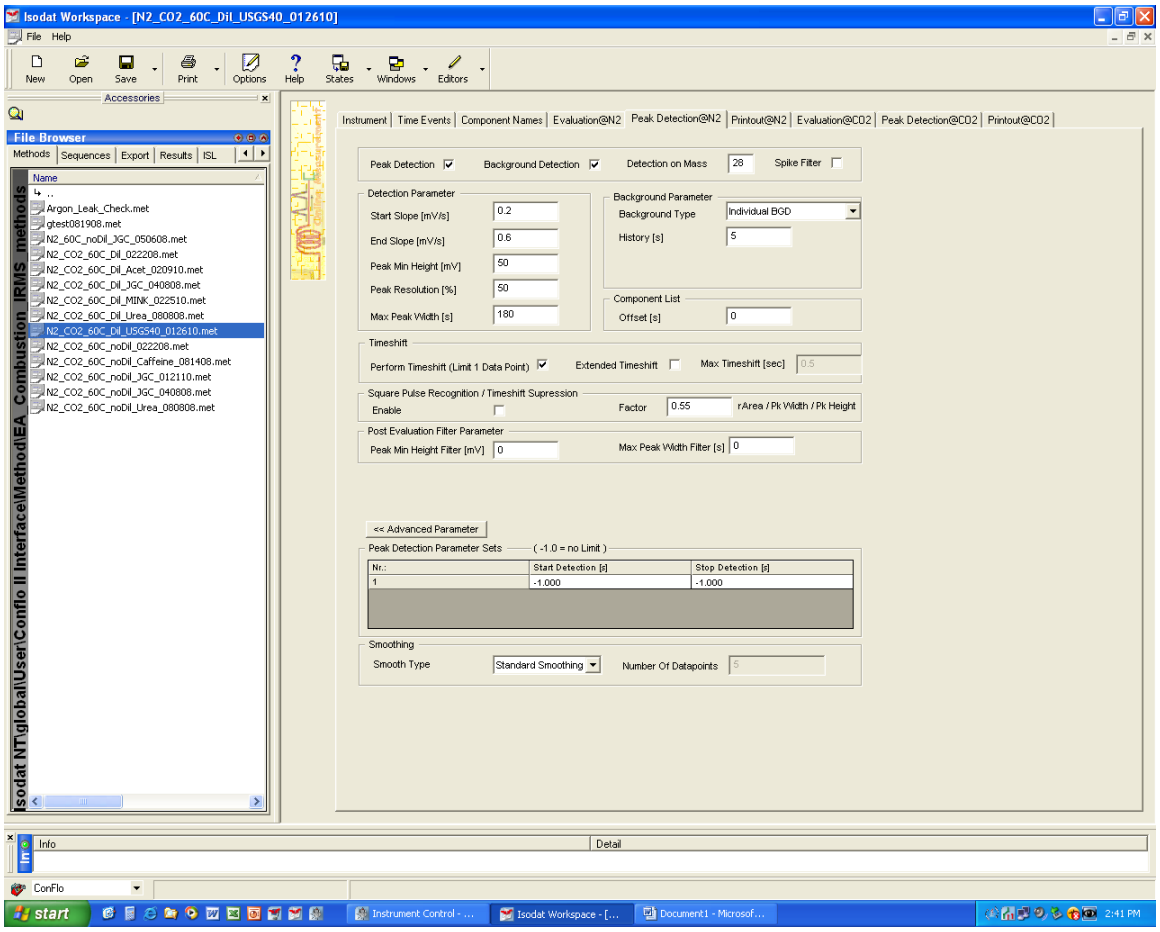

Figure 7: $\delta^{15}N$ Analysis Sequence File Example

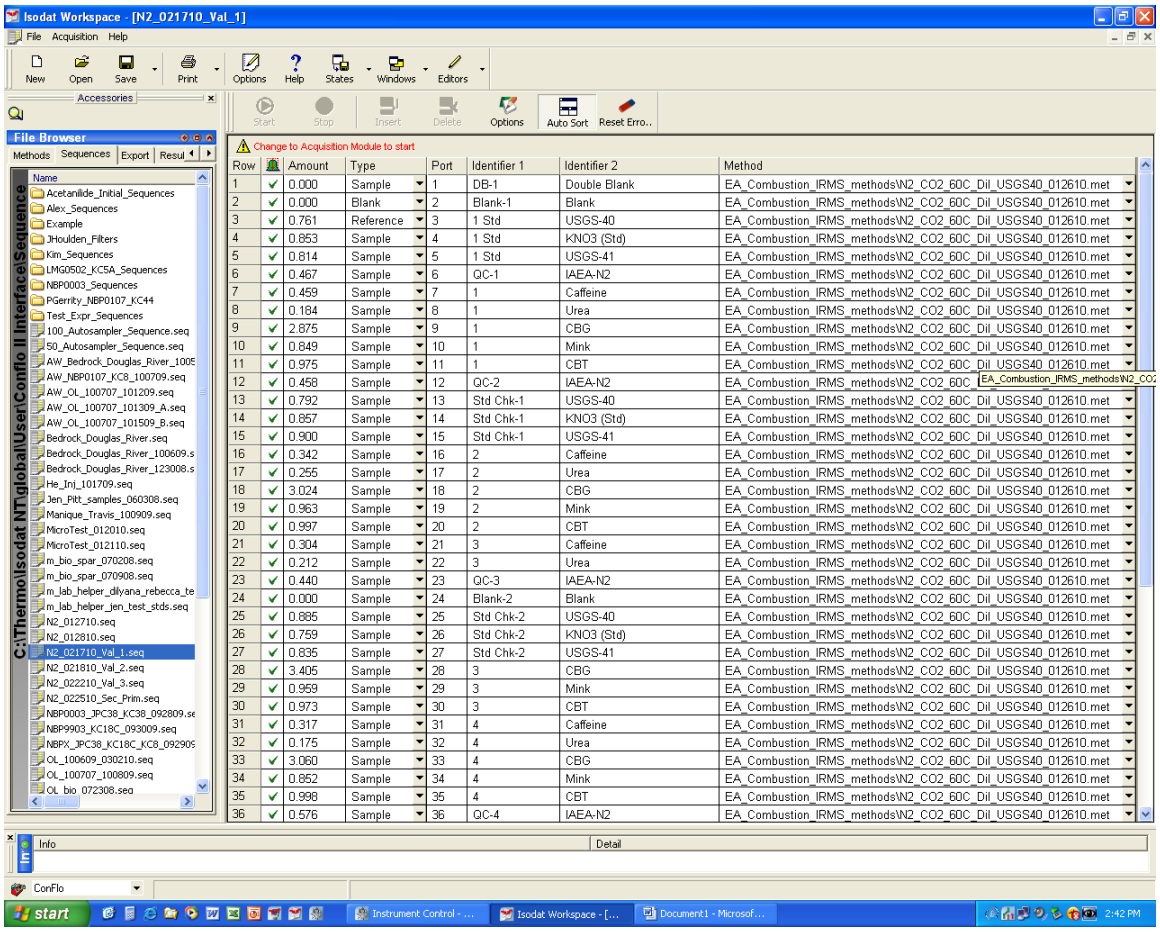

Figure 8: $\delta^{15}N$ On-Off Check (Using N_2)

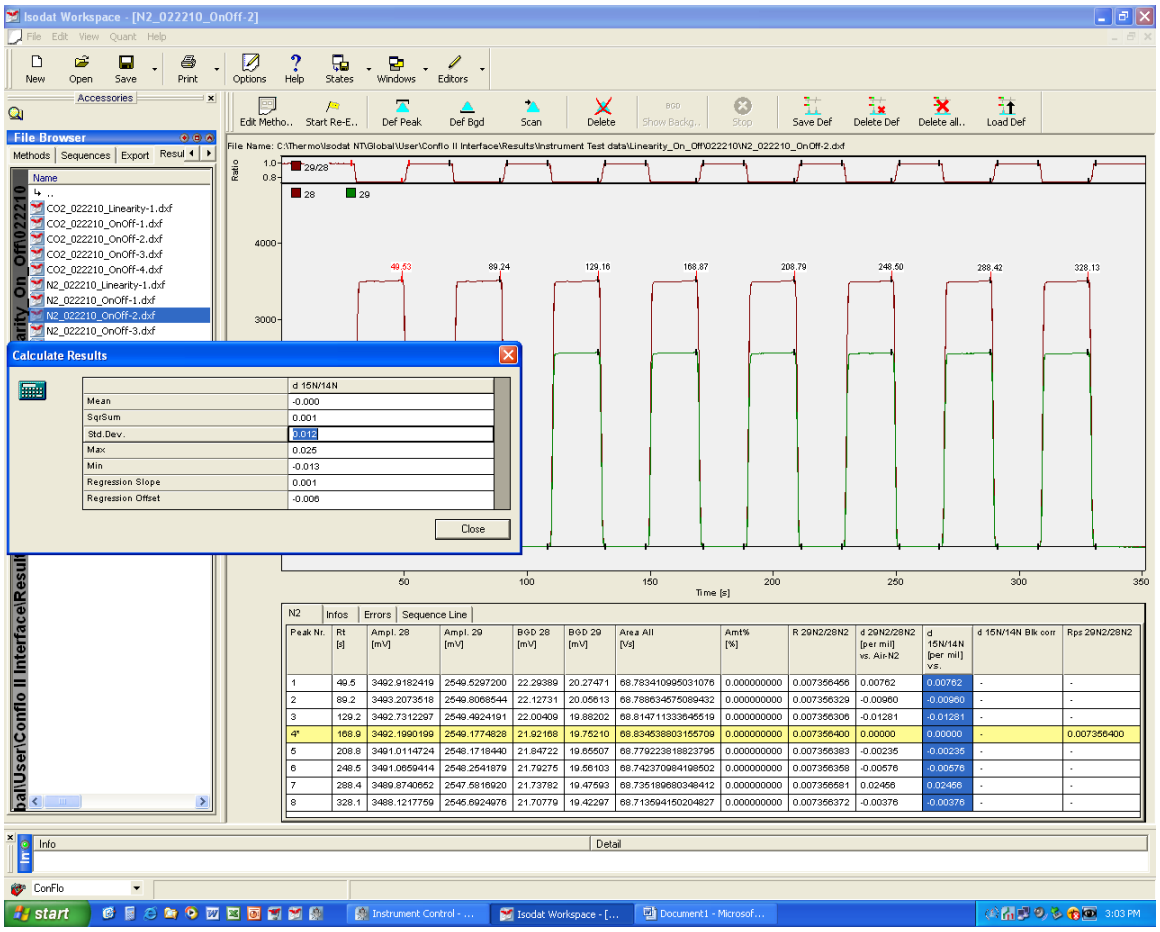

Figure 9: $\delta^{15}N$ Linearity Check (Using N_2)

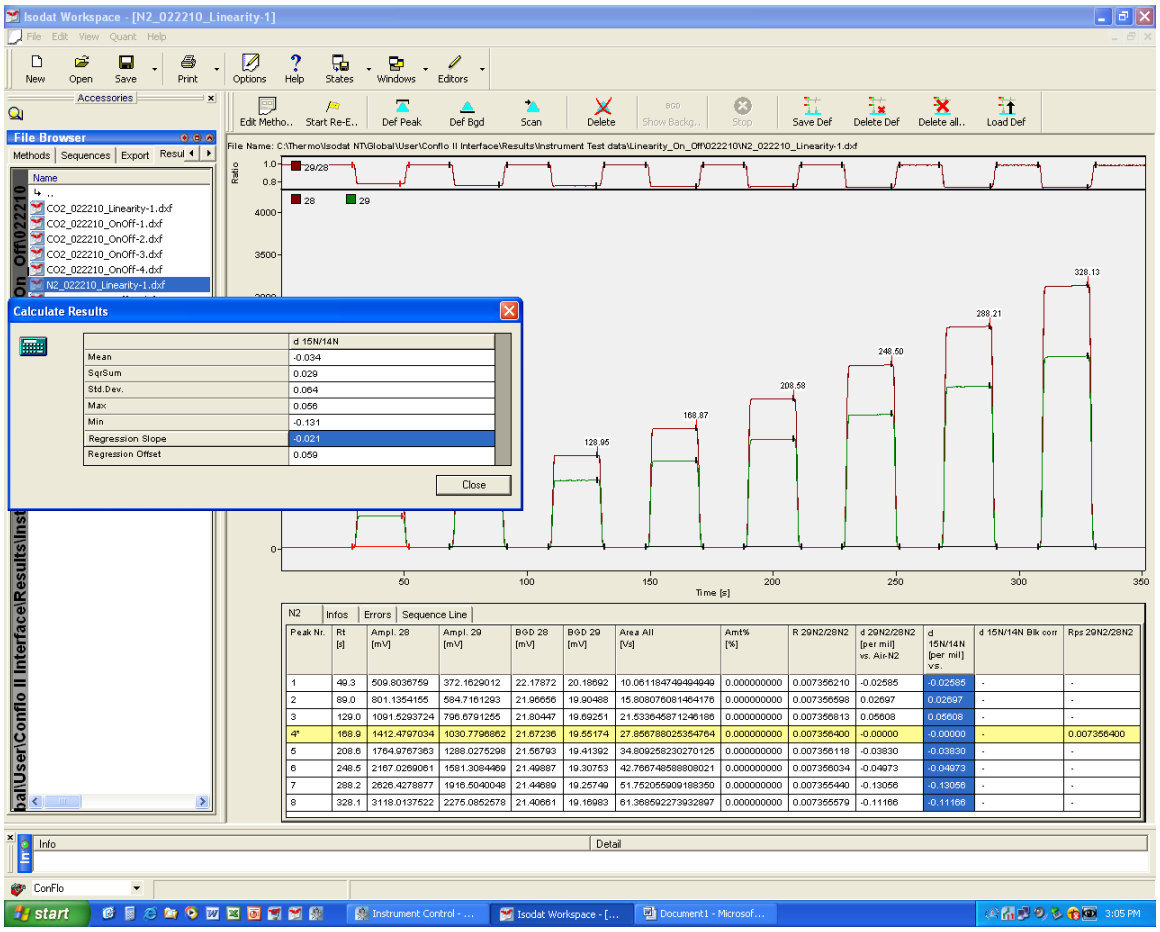

Figure 10: $\delta^{15}N$ Data Acquisition File – Blank

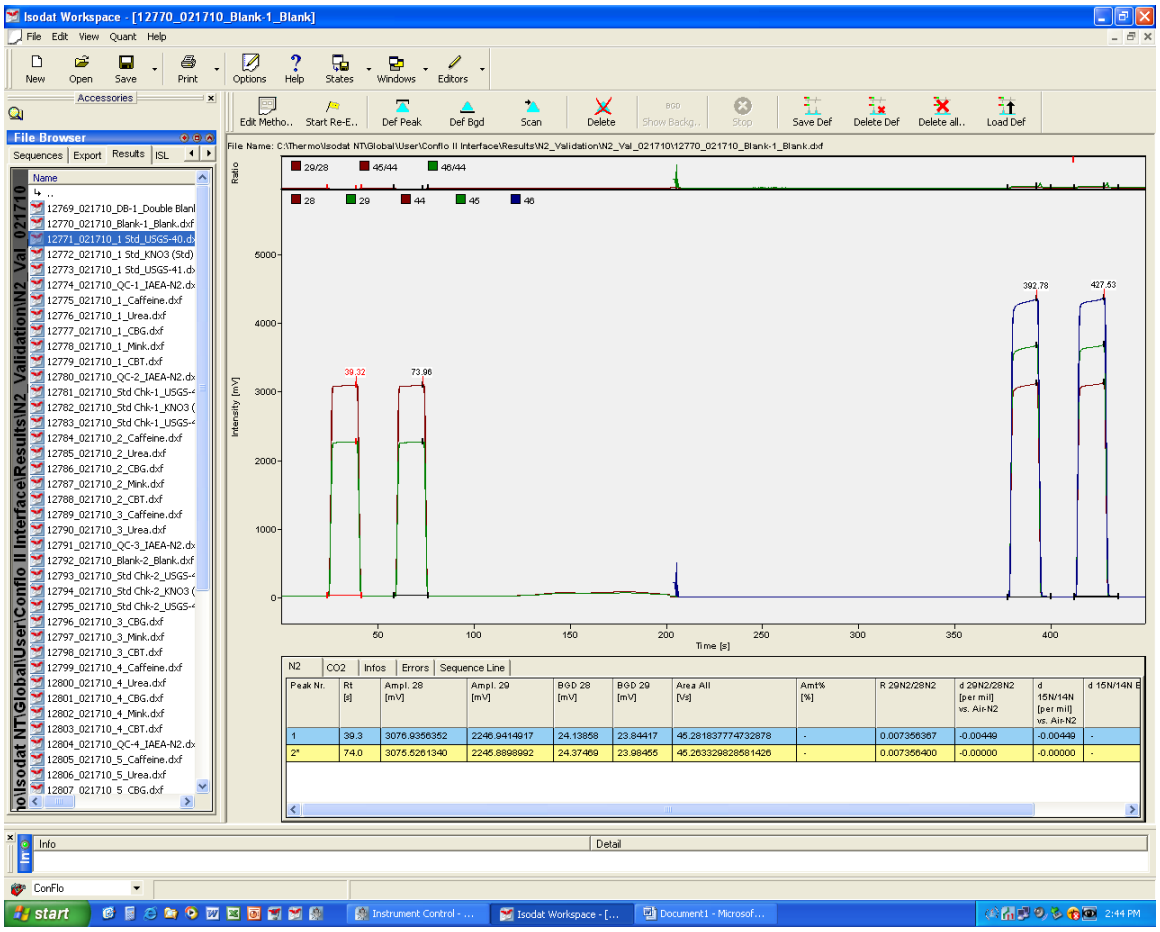

Figure 11: $\delta^{15}N$ Data Acquisition File – Primary Standard (USGS 40)

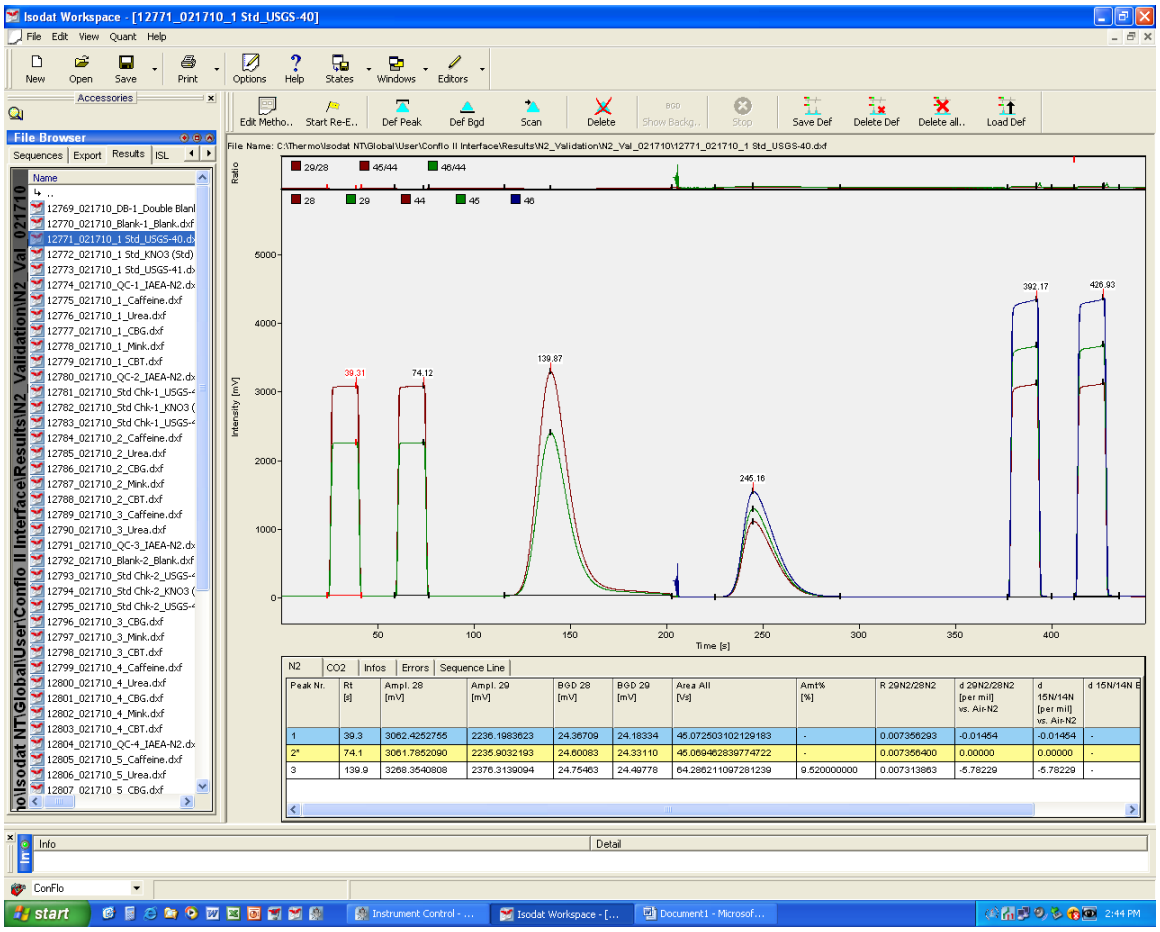

Figure 12: $\delta^{15}N$ Data Acquisition File – Proposed Lab Standard (Caffeine)

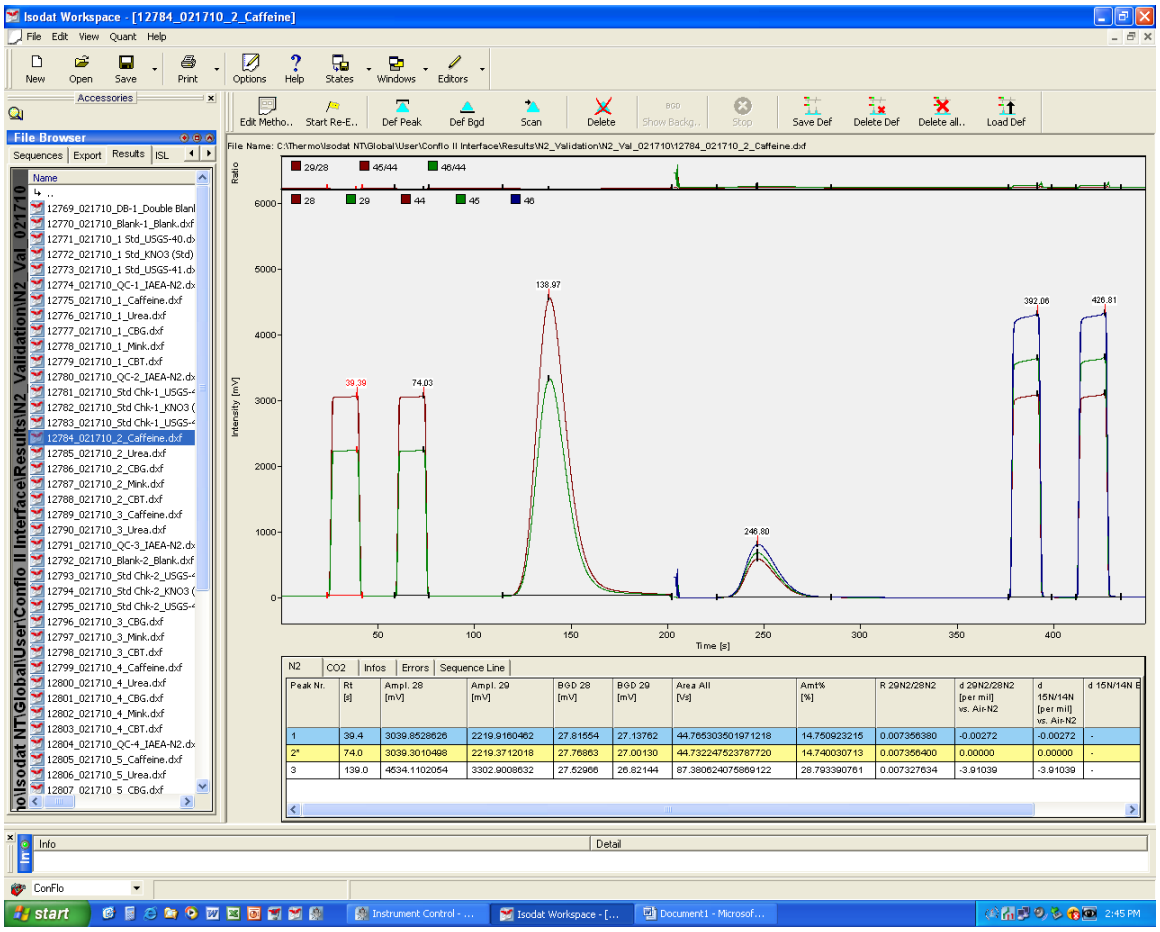

Figure 13: $\delta^{15}N$ Data Export File – N2_and_CO2_wt%_052308.wke