# METHOD VALIDATION REPORT

## Secondary (Lab) Standard Validation for the Analysis of $\delta^{18}O$ in Water Samples Using the GasBench and IRMS

**Date: December 18, 2009**

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# Secondary (Lab) Standard Validation for the Analysis of $\delta^{18}O$ in Water Samples Using the GasBench and IRMS

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### SUMMARY

| | | | | | | |
|---|---|---|---|---|---|---|
| International Standards (IAEA Reference Material) | SLAP2 – Standard Light Antarctic Precipitation 2<br>GISP – Greenland Ice Sheet Precipitation<br>VSMOW2– Vienna Standard Mean Ocean Water 2 | | | | | |
| International Standard (Primary Standard) Given Values | **Primary Standard** | | **$\delta^{18}O_{VSMOW/SLAP\ ‰}$** | | | |
| | SLAP2 | | **-55.5** | | | |
| | GISP | | **-24.8** | | | |
| | VSMOW2 | | **0.00** | | | |
| Primary Standard Experimental Values and Statistics | **Primary Standard** | **$\delta^{18}O_{VSMOW/SLAP\ ‰}$** | **S.D.** | **%CV** | **%Acc** | **n** |
| | SLAP2 | **-55.559** | 0.0781 | 0.14 | 100.11 | 9 |
| | GISP | **-24.827** | 0.101 | 0.41 | 100.11 | 9 |
| | VSMOW2 | **-0.042** | 0.0385 | 91.7* | * | 9 |
| | * Value skewed due to zero being the target value. | | | | | |
| Water Lab (Secondary) Standards | 1. Science Center RO: Rm. 1036<br>2. Vostok: Obtained from T. Rayne, GeoSciences Dept., Hamilton College<br>3. Sylvan Beach Tap: B. Wegter (employee Hamilton College) home<br>4. Bottle Distilled: Fisher, Optima LCMS Grade, Lot: 086933<br>5. Well: D. Tewksbury (employee Hamilton College) home<br>6. Science Center Tap: Hamilton College, Rm. 1036<br>7. Deuterium Prepared Lab Standard (see preparation section)<br>8. Millipore RO: Science Center Rm. 2093 | | | | | |
| Lab (Secondary) Standard Experimentally Determined $\delta^{18}O$ Values and Statistics | **Secondary Standard** | **$\delta^{18}O_{VSMOW/SLAP\ ‰}$** | **S.D.** | **%CV** | **n** | |
| | Science Center RO | **-10.217** | 0.0473 | 0.46 | 27 | |
| | Vostok | **-53.448** | 0.0596 | 0.11 | 30 | |
| | Sylvan Beach Tap | **-6.602** | 0.0486 | 0.74 | 30 | |
| | Bottle Distilled | **-7.258** | 0.0470 | 0.65 | 30 | |
| | Well | **-11.366** | 0.0550 | 0.48 | 30 | |
| | Science Center Tap | **-9.652** | 0.0600 | 0.62 | 30 | |
| | Lab Standard | **-10.344** | 0.0571 | 0.55 | 30 | |
| | Millipore RO | **-10.223** | 0.0540 | 0.53 | 27 | |
| Sample Analysis Volume | 200 µL | | | | | |

**SIGNATURE PAGE**

# Secondary (Lab) Standard Validation for the Analysis of $\delta^{18}O$ in Water Samples Using the GasBench and IRMS

Written by:

| | |
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# 1. INTRODUCTION

This report describes the qualification/validation process for Water $\delta^{18}O$ Secondary (Lab) Standards using the automated $CO_2$ equilibration Gas Bench Isotope Ratio Mass Spectrometry technique. Various water samples were analyzed to be evaluated as possible Secondary (Lab) standards. Three international (primary) standards were included in the analyses, they are GISP, SLAP2 and VSMOW2. The goal of the analysis was to identify the laboratory standards which provided acceptable experimental precision and encompassed the $\delta^{18}O$ ranges expected for samples submitted for analysis. The Lab Standards identified in the Summary section of this report fulfilled these requirements.

# 2. EXPERIMENTAL

## 2.1. CHEMICALS AND MATERIALS

Eight water samples were chosen for this secondary (Lab) standard determination validation, as well as the three international (or primary) standards. The eight laboratory standard candidates were as follows:

1. Science Center RO
2. Vostok
3. Sylvan Beach Tap
4. Bottle Distilled
5. Well
6. Science Center Tap
7. Prepared Deuterium Laboratory Standard (50 ppm $D_2O$)
8. Millipore RO

Note: The 50 ppm (v/v) $D_2O$ laboratory standard was prepared as follows:

- ~100 mL of Science Center RO water were first placed into a 1000 mL volumetric flask
- Using a pipette, exactly 50 µL of $D_2O$ ( Acros $D_2O$ 100.0 Atom% D, Lot A020127801) were then placed into the volumetric flask
- Science Center RO water was then added to the flask to the mark
- A stir bar was inserted and the solution mixed for ~ 1 day

Note: This standard was actually prepared for the Deuterium validation but it was decided to analyze this sample knowing that it should give a value exactly as the Science Center RO water that was also analyzed.

The three international standards were as follows:

1. SLAP2
2. GISP
3. VSMOW2

Other than the prepared lab standard, all waters were used neat “as received”.

A 0.3% $CO_2$ in Helium was used as the equilibration gas which allowed for oxygen atom incorporation from the water sample into the $CO_2$ gas introduced to each sample's headspace.

Other materials were as follows:

Capillary Column – Varian PN: CP7551, PLOT Fused Silica, CP-PoraPLOT Q, length - 27.5 meter (including 2.5 m particle trap), (0.32 mm I.D., 0.45 mm O.D., 10 mm film thickness) held at 70ºC.
Exetainer Vials – 12 mL Borosilicate, obtained from LabConco with vial caps and disposable septa.
Valco Sample Loop in GasBench – 100 µL
GasBench Sample Block – set at 30ºC.
He Gas - Grade 5.0 (50 psi tank gauge, 13-14 psi GasBench gauge)
0.3% $CO_2$ Gas (Grade 4.5) in Helium - Grade 4.6, P/N 105-MIXZW300C. (45 - 50 psi tank gauge, adjust to give ~ 125 ml/min flush fill rate, check at vent of FlushFill needle during the FlushFill event.)
$CO_2$ Reference Gas - Grade 4.5 (35 psi tank gauge, 30 – 35 psi GasBench gauge, adjust pressure at GB gauge to give ~ 7 – 8 volts m/z 44 signal, cup 2)
Pipettor – Finnpipette 40 – 200 µL maximum range, S/N J57232 (Calibrated – 12/07)
Pipettor Tips – Eppendorf – "Yellow", capacity up to 200 µL (Fisher # 02-707-500)

## 2.2. INSTRUMENTATION (IRMS, GASBENCH AND PAL)

The IRMS instrument is a Thermo Scientific Delta V Advantage along with a ThermoFinnigan GasBench III and CTC Analytics PAL autosampler system. (The GasBench unit is equipped with a self-contained continuous flow interface.)

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 standard to Secondary standard evaluations and one Secondary to Primary standard evaluation) were performed during the course of the validation. Three of the days consisted of 96 samples and the Secondary to Primary standard evaluation consisted of 83 samples. (It should be noted that $^{3}H$ sample analysis can be performed on samples that have previously been evaluated for $^{18}O$ but not vice versa.)

Nine peaks (consisting of ion current for m/z 44, m/z 45 and m/z 46) of decreasing signal are obtained for each sample (in addition to four reference pulses). The first peak is omitted (due to

potential detector saturation) and the statistics (average, S.D., % accuracy, etc.) are generated on the $\delta^{18}O‰$ values given by the Isodat software on the remaining eight peaks. The final $\delta^{18}O‰$ values and associated statistical parameters given for each water sample were calculated two ways: using the average $\delta^{18}O‰$ value of the eight peaks for each sample (intra) and using each $\delta^{18}O‰$ value for every peak in each sample (inter). This latter method provided a much bigger population of experimental results (eight values per individual sample) than just using one value (average of eight values) per sample. Both statistical treatments of data yielded essentially identical results for each water sample given in the Summary.

**Sample Preparation**

The exetainer sample tubes were cleaned by washing in a soap bath and followed by multiple Science Center RO water rinses. Next, the vials were placed in a RO water bath to soak (as a final rinse) at least overnight. Each vial was then removed from the bath and given an acetone rinse. The vials were then placed into an oven to be baked out. The oven was set at ~ 150°C, and the vials were left in at least overnight. After baking, the vials were wrapped in new, clean aluminum foil for storage.

The sample preparation was as follows:

- Into a clean, dry and labeled exetainer vial, 200 µL of water sample were placed using a pipette. (Sample blanks did not contain the water.)
- A cap with septa was then placed on the exetainer tube to seal it.
- Vials were placed into the GasBench sample block (maintained at ~ 30ºC) and the cover was secured.
- Each sample vial was then flush-filled with 0.3% $CO_2$ in Helium gas before the analysis.
    - Attach the two flush-fill needles to the PAL autosampler.
    - Turn the T-valve so it points away from the GasBench (towards the ConFlo).
    - In Isodat Acquisition, verify instrument configuration is set for GasBench+PAL, click the mouse on the gasbench flush-fill button in the GasBench area, this will purge the 0.3% $CO_2$ in Helium gas flush-fill line. Note: If Instrument Control is open, always close it prior to using Isodat Acquisition.
    - Allow the 0.3% $CO_2$ in Helium gas line to purge for ~ 15 minutes.
    - Use the ***FlushFill_6min_18O.seq*** as a template (in Workspace), create a flush-fill sequence for the appropriate number of samples.
    - Ensure the sequence contains the correct method, e.g., ***Vial_Flush_6min.met.***
    - Ensure the use of an appropriate AS Method, **Internal No 1, (A200S-1) 6 injections of 61 seconds each** (see Figure 2).
    - In Acquisition, start the flush-fill sequence just created. Identify the folder for the data with the date and type of analysis. Note: To minimize potential computer issues, it is recommended to reset the computer before starting any extended analysis sequence.

    - Once started, verify the flush-fill flow rate by placing a flow meter onto the vent tube of the flush-fill needle (check this on both needles!), the flow rate should be ~ 125 mL/min.
- When the Helium flush-fill has been completed, turn the T-valve back 90° to point to the back wall and shut off the 0.3% $CO_2$ gas in Helium at the cylinder.
- Remove both flush-fill needles from the PAL autosampler.
- Let samples remain in heated sample block for a minimum of 18 hours for the $^{18}O$ incorporation/equilibration to occur.
- Attach the sampling needle to the left position on the PAL autosampler syringe holder.
- Open Instrument Control software, check and record the MS pressure.
- Open the GasBench 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 on-off (**CO2_On-Off.met**) and linearity (**CO2_On-Off.met**) system suitability using $CO_2$ as the reference gas. **($\delta^{18}O$ - On-off: std.dev. < 0.08‰, $\delta^{18}O$ - Linearity: regression slope std. dev. < 0.08‰ with increasing $CO_2$ pressure** (see Figures 8 and 9).
- Adjust the $CO_2$ reference gas to give a reference peak (m/z 44, cup 2) signal of between 7000 and 8000 mV (m/z 45 ~ 8500 mV, m/z 46 ~ 10,000 mV).
- Create, identify, and save a new Analysis sequence using the file ***18O_H2O_96_Samples.seq*** as a template (see Figure 7).
- Use ***18O_H2O_100uL_Loop.met*** as the analysis method (see Figures 3 – 6).
- Ensure the correct autosampler method is entered in the sequence, **Internal No. 9 (A200S-9) 11 injections of 59 seconds each** (See Figure 2).
- 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 $CO_2$, $N_2$, $H_2$, 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, capillary column temperature, T-valve position, alignment of syringes, vial location and identification, 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 18O 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". (Depending on the number of samples, the sequence can continue for more than 20 hours.)
- 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 ***GB_18O_Export.wke***, this will put the data into EXCEL format (see 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 $\delta^{18}O‰$ 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 $\delta^{18}O‰$ values.
    - Perform statistical analysis (mean, standard deviation, accuracy, and %CV) on all average $\delta^{18}O‰$ values determined for each sample. This is the intra-statistical analysis.
    - Next, perform the same statistical analysis on all the individual peaks of each sample. This is the inter-statistical analysis.

**Instrument Conditions**

**GasBench**

- Capillary Column Temperature - 70ºC
- Capillary Column Flow Rate – 1.0 ml/min - 1.5 ml/min
- Sample Block Temperature - 30ºC
- Flush-Fill Flow rate - ~125mL/min
- He Pressure (at Tank) – 50 psi
- He pressure (at GasBench) – 13 – 14 psi (flow rate ~ 0.8 ml/min)
- 0.3% $CO_2$ in He pressure (at Tank) - ~45 psi (adjust to give ~125ml/min FlushFill rate)
- $CO_2$ pressure – at Tank – 35 psi
  at GasBench – adjust to 7 – 8 volts m/z 44 signal in cup 2

**PAL**

- Syringe Configuration – 10 µL
- FlushFill method – Internal 1
- Analysis method – Internal 9

**IRMS**

- Electron Energy – 124 eV
- Tune File – e.g.: autofocus_CO2_GB_(Date of last tune)
- ~ High Vacuum (Valve open) – ~5.5e-7mB
- ~High Vacuum (Valve closed) - ~9.5e-8mB
- Instrument configuration – GasBench+PAL
- CO2 reference peak intensity (m/z 44 cup 2) - ~8000 mV
- Method – FlushFill – Vial_Flush_6min.met
  Analysis – 18O_H2O_100uL_Loop.met

## 2.4. WATER STANDARD VALIDATION DATA

The Excel files used for this validation can be found on the Hamilton College network, the path is Campus on ESS
P:\Instrumentation\Geosciences\Data\Thermo_IRMS\GasBench\Water\Oxygen_18\(file names).
The file names and contents are listed below:

1. 081309_18O_Val_1.xlxs – Validation day 1 results
2. 081809_18O_Val_2.xlxs - Validation day 2 results
3. 082109_18O_Val_3.xlxs - Validation day 3 results
4. 18O_082709_Sec_to_Primary.xlxs – Experimentally determined values for Secondary standards used to determine Primary standard values
5. 18O_Validation_Summary.xlxs – Accuracy and precision analysis for all analyses performed during validation

**Table 1:**

**Validation Day 1 Statistics (Primary Standards)**

File Name: 081309_18O_Val_1.xlxs

| **Primary Standards Statistics** | |
|---|---|
| **SLAP** | **$\delta^{18}O$ ‰** |
| **average** | -55.560 |
| **Std. Deviation** | 0.118 |
| **%CV** | 0.21 |
| **%Acc** | 100.11 |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -55.5 |
| | |
| **VSMOW2** | **$\delta^{18}O$ ‰** |
| **average** | 0.00367 |
| **Std. Deviation** | 0.0560 |
| **%CV** | 1528.3* |
| **%Acc** | * |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | 0.00 |
| | |
| **GISP** | **$\delta^{18}O$ ‰** |
| **average** | -24.852 |
| **Std. Deviation** | 0.110 |
| **%CV** | 0.44 |
| **%Acc** | 100.21 |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -24.8 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

* Value skewed due to zero being the target value

**Table 2:**

**Validation Day 1 Statistics (Secondary Standards)**

| **Secondary Standards Statistics** | |
|---|---|
| **Well** | **$\delta^{18}O$ ‰** |
| **average** | -11.38496 |
| **Std. Deviation** | 0.0782 |
| **%CV** | 0.69 |
| **n** | 10 |
| | |
| **Prepared Lab Standard** | **$\delta^{18}O$ ‰** |
| **average** | -10.33679 |
| **Std. Deviation** | 0.0584 |
| **%CV** | 0.56 |
| **n** | 10 |
| | |
| **Millipore RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.22694 |
| **Std. Deviation** | 0.0600 |
| **%CV** | 0.59 |
| **n** | 9 |
| | |
| **Science Center RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.20802 |
| **Std. Deviation** | 0.0527 |
| **%CV** | 0.52 |
| **n** | 9 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

**Table 2: (cont'd.)**

**Validation Day 1 Statistics (Secondary Standards)**

| **Secondary Standards Statistics** | |
|---|---|
| **Science Center Tap** | **$\delta^{18}O$ ‰** |
| **average** | -9.64723 |
| **Std. Deviation** | 0.0675 |
| **%CV** | 0.70 |
| **n** | 10 |
| | |
| **Bottled Distilled** | **$\delta^{18}O$ ‰** |
| **average** | -7.25881 |
| **Std. Deviation** | 0.0598 |
| **%CV** | 0.82 |
| **n** | 10 |
| | |
| **Vostok** | **$\delta^{18}O$ ‰** |
| **average** | -53.47425 |
| **Std. Deviation** | 0.0593 |
| **%CV** | 0.11 |
| **n** | 10 |
| | |
| **Sylvan Beach Tap** | **$\delta^{18}O$ ‰** |
| **average** | -6.58259 |
| **Std. Deviation** | 0.0569 |
| **%CV** | 0.86 |
| **n** | 10 |

Note: %CV = Coefficient of Variation
%Acc = Accuracy

**Table 3:**

**Validation Day 2 Statistics (Primary Standards)**

File Name: 081809_18O_Val_2.xlxs

| **Primary Standards Statistics** | |
|---|---|
| **SLAP** | **$\delta^{18}O$ ‰** |
| **average** | -55.526 |
| **Std. Deviation** | 0.0672 |
| **%CV** | 0.12 |
| **%Acc** | 100.05 |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -55.5 |
| | |
| **VSMOW2** | **$\delta^{18}O$ ‰** |
| **average** | -0.0551 |
| **Std. Deviation** | 0.0618 |
| **%CV** | 112.09* |
| **%Acc** | * |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | 0.00 |
| | |
| **GISP** | **$\delta^{18}O$ ‰** |
| **average** | -24.826 |
| **Std. Deviation** | 0.0565 |
| **%CV** | 0.23 |
| **%Acc** | 100.11 |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -24.8 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

* Value skewed due to zero being the target value

**Table 4:**

**Validation Day 2 Statistics (Secondary Standards)**

File Name: 081809_18O_Val_2.xlxs

| **Secondary Standards Statistics** | |
|---|---|
| **Well** | **$\delta^{18}O$ ‰** |
| **average** | -11.35209 |
| **Std. Deviation** | 0.0392 |
| **%CV** | 0.35 |
| **n** | 10 |
| | |
| **Prepared Lab Standard** | **$\delta^{18}O$ ‰** |
| **average** | -10.35583 |
| **Std. Deviation** | 0.0586 |
| **%CV** | 0.57 |
| **n** | 10 |
| | |
| **Millipore RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.23311 |
| **Std. Deviation** | 0.0413 |
| **%CV** | 0.40 |
| **n** | 9 |
| | |
| **Science Center RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.21444 |
| **Std. Deviation** | 0.0564 |
| **%CV** | 0.55 |
| **n** | 9 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

**Table 4: (cont'd.)**

**Validation Day 2 Statistics (Secondary Standards)**

| **Secondary Standards Statistics** | |
|---|---|
| **Science Center Tap** | **$\delta^{18}O$ ‰** |
| **average** | -9.66312 |
| **Std. Deviation** | 0.0531 |
| **%CV** | 0.55 |
| **n** | 10 |
| | |
| **Bottled Distilled** | **$\delta^{18}O$ ‰** |
| **average** | -7.24647 |
| **Std. Deviation** | 0.0457 |
| **%CV** | 0.63 |
| **n** | 10 |
| | |
| **Vostok** | **$\delta^{18}O$ ‰** |
| **average** | -53.40472 |
| **Std. Deviation** | 0.0735 |
| **%CV** | 0.14 |
| **n** | 10 |
| | |
| **Sylvan Beach Tap** | **$\delta^{18}O$ ‰** |
| **average** | -6.62288 |
| **Std. Deviation** | 0.0546 |
| **%CV** | 0.82 |
| **n** | 10 |

Note: %CV = Coefficient of Variation
%Acc = Accuracy

**Table 5:**

**Validation Day 3 Statistics (Primary Standards)**

File Name: 082109_18O_Val_3.xlxs

| **Primary Standards Statistics** | |
|---|---|
| **SLAP** | **$\delta^{18}O$ ‰** |
| **average** | -55.591 |
| **Std. Deviation** | 0.0515 |
| **%CV** | 0.09 |
| **%Acc** | 100.17 |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -55.5 |
| | |
| **VSMOW2** | **$\delta^{18}O$ ‰** |
| **average** | -0.0753 |
| **Std. Deviation** | 0.0320 |
| **%CV** | 42.55* |
| **%Acc** | * |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | 0.00 |
| | |
| **GISP** | **$\delta^{18}O$ ‰** |
| **average** | -24.802 |
| **Std. Deviation** | 0.105 |
| **%CV** | 0.42 |
| **%Acc** | 100.01 |
| **n** | 3 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -24.8 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

* Value skewed due to zero being the target value

**Table 6:**

**Validation Day 3 Statistics (Secondary Standards)**

File Name: 082109_18O_Val_3.xlxs

| **Secondary Standards Statistics** | |
|---|---|
| **Well** | **$\delta^{18}O$ ‰** |
| **average** | -11.36173 |
| **Std. Deviation** | 0.0476 |
| **%CV** | 0.42 |
| **n** | 10 |
| | |
| **Prepared Lab Standard** | **$\delta^{18}O$ ‰** |
| **average** | -10.33867 |
| **Std. Deviation** | 0.0542 |
| **%CV** | 0.52 |
| **n** | 10 |
| | |
| **Millipore RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.20939 |
| **Std. Deviation** | 0.0606 |
| **%CV** | 0.59 |
| **n** | 9 |
| | |
| **Science Center RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.22948 |
| **Std. Deviation** | 0.0330 |
| **%CV** | 0.32 |
| **n** | 9 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

**Table 6: (cont'd.)**

**Validation Day 3 Statistics (Secondary Standards)**

| **Secondary Standards Statistics** | |
|---|---|
| **Science Center Tap** | **$\delta^{18}O$ ‰** |
| **average** | -9.64643 |
| **Std. Deviation** | 0.0593 |
| **%CV** | 0.61 |
| **n** | 10 |
| | |
| **Bottled Distilled** | **$\delta^{18}O$ ‰** |
| **average** | -7.26981 |
| **Std. Deviation** | 0.0355 |
| **%CV** | 0.49 |
| **n** | 10 |
| | |
| **Vostok** | **$\delta^{18}O$ ‰** |
| **average** | -53.46616 |
| **Std. Deviation** | 0.0459 |
| **%CV** | 0.09 |
| **n** | 10 |
| | |
| **Sylvan Beach Tap** | **$\delta^{18}O$ ‰** |
| **average** | -6.60138 |
| **Std. Deviation** | 0.0344 |
| **%CV** | 0.52 |
| **n** | 10 |

Note: %CV = Coefficient of Variation
%Acc = Accuracy

**Table 7:**

**Validation Day 4 (Secondary-to-Primary) Statistics (Primary Standards)**

File Name: 18O_082709_Sec_to_Primary.xlxs

| **Primary Standards Statistics** | |
|---|---|
| **SLAP** | **$\delta^{18}O$ ‰** |
| **average** | -55.620 |
| **Std. Deviation** | 0.0643 |
| **%CV** | 0.28 |
| **%Acc** | 100.16 |
| **n** | 7 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -55.5 |
| | |
| **VSMOW2** | **$\delta^{18}O$ ‰** |
| **average** | -0.0818 |
| **Std. Deviation** | 0.0570 |
| **%CV** | 69.73* |
| **%Acc** | * |
| **n** | 7 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | 0.00 |
| | |
| **GISP** | **$\delta^{18}O$ ‰** |
| **average** | -24.839 |
| **Std. Deviation** | 0.0698 |
| **%CV** | 0.28 |
| **%Acc** | 100.16 |
| **n** | 7 |
| **Known $\delta^{18}O_{VSMOW/SLAP}$** | -24.8 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

* Value skewed due to zero being the target value

**Table 8:**

**Validation Day 4 (Secondary-to-Primary) Statistics (Secondary Standards)**

File Name: 18O_082709_Sec_to_Primary.xlxs

| **Secondary Standards Statistics** | |
|---|---|
| **Well** | **$\delta^{18}O$ ‰** |
| **average** | -11.415 |
| **Std. Deviation** | 0.0457 |
| **%CV** | 0.40 |
| **%Acc** | 100.43 |
| **n** | 6 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -11.366 |
| **Prepared Lab Standard** | **$\delta^{18}O$ ‰** |
| **average** | -10.346 |
| **Std. Deviation** | 0.0814 |
| **%CV** | 0.79 |
| **%Acc** | 100.01 |
| **n** | 7 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -10.344 |
| **Millipore RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.290 |
| **Std. Deviation** | 0.0441 |
| **%CV** | 0.43 |
| **%Acc** | 100.66 |
| **n** | 7 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -10.223 |
| **Science Center RO** | **$\delta^{18}O$ ‰** |
| **average** | -10.310 |
| **Std. Deviation** | 0.0478 |
| **%CV** | 0.46 |
| **%Acc** | 100.90 |
| **n** | 7 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -10.217 |

Note: %CV = Coefficient of Variation

%Acc = Accuracy

**Table 8: (cont'd.)**

**Validation Day 4 (Secondary-to-Primary) Statistics (Secondary Standards)**

| **Secondary Standards Statistics** | |
|---|---|
| **Science Center Tap** | **$\delta^{18}O$ ‰** |
| **average** | -9.636 |
| **Std. Deviation** | 0.0758 |
| **%CV** | 0.79 |
| **%Acc** | 99.83 |
| **n** | 7 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -9.652 |
| **Bottled Distilled** | **$\delta^{18}O$ ‰** |
| **average** | -7.282 |
| **Std. Deviation** | 0.0659 |
| **%CV** | 0.91 |
| **%Acc** | 100.33 |
| **n** | 7 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -7.258 |
| **Vostok** | **$\delta^{18}O$ ‰** |
| **average** | -53.557 |
| **Std. Deviation** | 0.0456 |
| **%CV** | 0.09 |
| **%Acc** | 100.01 |
| **n** | 6 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -53.448 |
| **Sylvan Beach Tap** | **$\delta^{18}O$ ‰** |
| **average** | -60642 |
| **Std. Deviation** | 0.0501 |
| **%CV** | 0.75 |
| **%Acc** | 100.60 |
| **n** | 6 |
| **Experimentally Determined $\delta^{18}O_{VSMOW/SLAP}$** | -6.602 |

Note: %CV = Coefficient of Variation
%Acc = Accuracy

**Table 9:**

**Regression line equations used to correct $\delta^{18}O‰$ instrument values**

| Analysis Date | Validation Day | Regression Line | $R^2$ |
|---|---|---|---|
| 08/13/2009 | Day 1 | y=0.9978x-1.0040 | 1.00 |
| 08/18/2009 | Day2 | y=0.9964x-1.1421 | 1.00 |
| 08/21/2009 | Day3 | y=0.9969x-1.1180 | 1.00 |
| 08/27/2009 | Day4 | y=0.9983x-1.1356 | 1.00 |

## 3. COMMENTS

Three standards, in duplicate (one at the beginning of the analysis and one at the end) were used to generate the regression line.

The Primary Standards that were used in the regression line generation were not used in the calculations of the experimentally determined $\delta^{18}O‰$ 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 $\delta^{18}O‰$ 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 $\delta^{18}O‰$ values were made.

$\delta^{18}O‰$ values given in the above Tables originate from the "intra" values determined in the Excel spreadsheets since the "intra" and "inter" values were essentially identical.

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. (Sylvan Beach Tap, Vostok and the well water sample were used to generate the regression line.)

%Accuracy = Experimental Value/Known (Established) 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\Gas Bench\Results\O18_Analysis Folder\
18O_Val_081309\filename.dxf
18O_Val_081809\filename.dxf
18O_Val_082109\filename.dxf
18O_082709_Sec_Primary\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\ GasBench\Water\Oxygen_18\Validation Data\filename.xlsx and Campus on "ESS"(P:) \Instrumentation\Geosciences\Data\Thermo_IRMS\GasBench\Water\Oxygen_18\Analysis Worksheets\filename.xlsx.

## 5. CONCLUSIONS

This analysis identified water samples which could be used for Lab (Secondary) Standards during unknown $\delta^{18}O‰$ investigations. This validation also provided $\delta^{18}O‰$ values for these Lab Standards (to be used for regression line generation) along with statistical evaluations of those values. The following is a summary of the results:

### Table 10: $\delta^{18}O$ Values and Statistical Analysis (Secondary Standards)

| Water Sample | $\delta^{18}O_{VSMOW/SLAP}‰$ | Std. Dev. | %CV | n |
|---|---|---|---|---|
| | | | | |
| Science Center RO | **-10.217** | 0.0473 | 0.46 | 27 |
| Vostok | **-53.448** | 0.0596 | 0.11 | 30 |
| Sylvan Beach Tap | **-6.602** | 0.0486 | 0.74 | 30 |
| Distilled | **-7.258** | 0.0470 | 0.65 | 30 |
| Well | **-11.366** | 0.0550 | 0.48 | 30 |
| Science Center Tap | **-9.652** | 0.0600 | 0.62 | 30 |
| Prepared Lab Standard | **-10.344** | 0.0571 | 0.55 | 30 |
| Millipore RO | **-10.223** | 0.0540 | 0.53 | 27 |

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: $\delta^{18}O$ Values and Statistical Analysis (Primary Standards)

| Primary Standard | $\delta^{18}O_{VSMOW/SLAP}‰$ | Std. Dev. | %CV | % Acc | n |
|---|---|---|---|---|---|
| | | | | | |
| SLAP2 | **-55.559** | 0.0781 | 0.14 | 100.11 | 9 |
| GISP | **-24.827** | 0.101 | 0.41 | 100.11 | 9 |
| VSMOW2 | **-0.042** | 0.0385 | 91.7* | * | 9 |

* Value skewed due to zero being the target value

## 6. REFERENCES

Thermo Electron Delta V Advantage Operating Manual
Finnigan GasBench II Operating Manual

## 7. FIGURES

**Figure 1:**

**$\delta^{18}O$ Experimentally Determined Values, Sorted by $\delta^{18}O$ (average of three runs)**

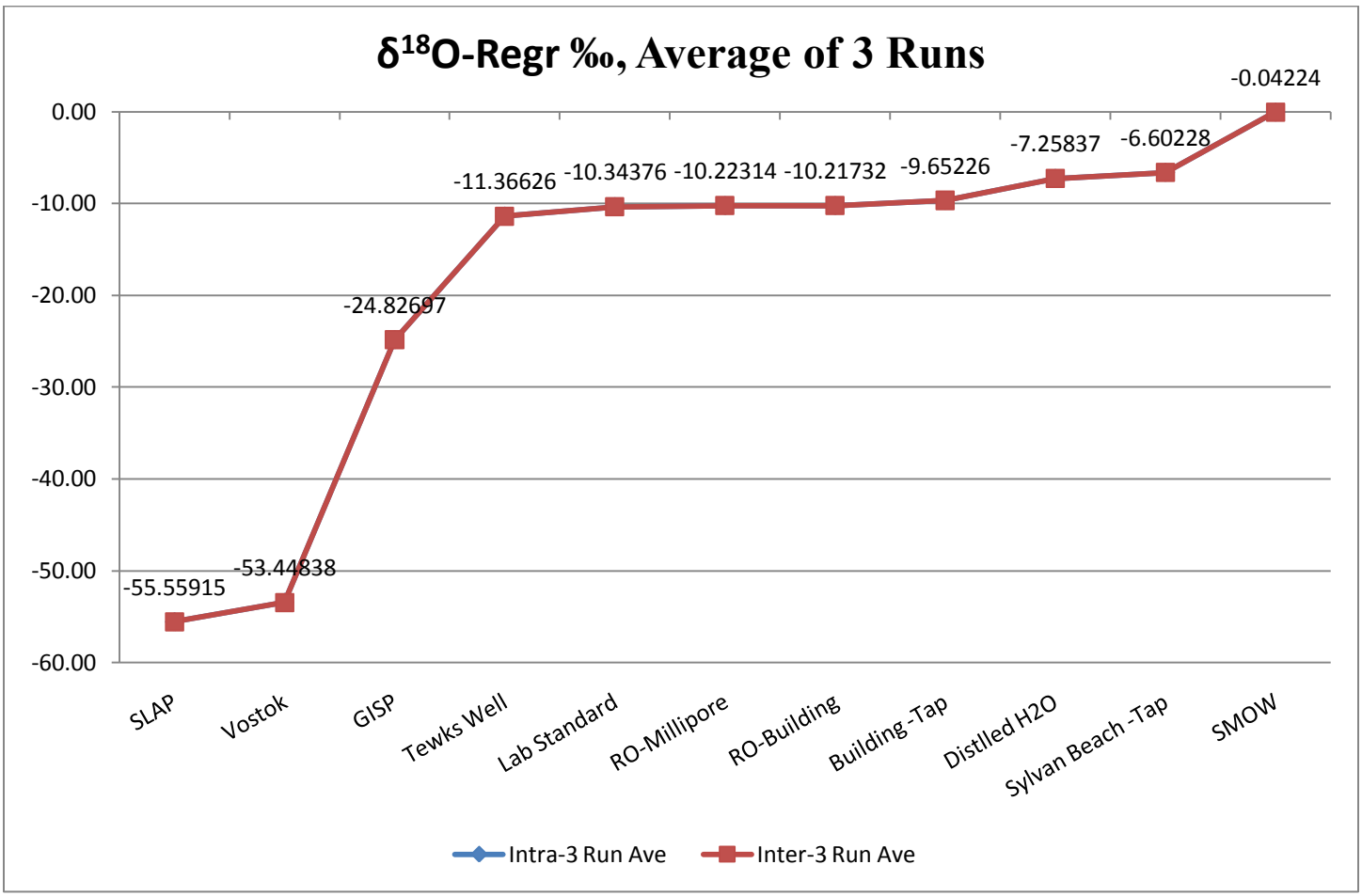


**Figure 2:**

PAL Autosampler Methods Used for $\delta^{18}O$ Analysis and FlushFill

| **Internal No. 1 (A200S-1) (FlushFill)** | |
|---|---|
| Cycle | GC-Inj |
| Syringe | 10 µL |
| Sample Volume | 10.0 µL |
| Air Volume | 0 µL |
| Pre Cln Slv1 | 0 |
| Pre Cln Slv2 | 0 |
| Pre Cln Spl | 0 |
| Fill Volume | 0 nL |
| Fill Speed | 5.0 µL /s |
| Fill Strokes | 6 |
| Pullup Del | 61 |
| Inject to | Flush |
| Inject Speed | 50 µL /s |
| Pre Inj Del | 0 ms |
| Pst Inj Del | 0 ms |
| Pst Cln Slv1 | 0 |
| Pst Cln Slv2 | 0 |

| **Internal No. 9 (A200S-9) (Analysis)** | |
|---|---|
| Cycle | GC-Inj |
| Syringe | 10 µL |
| Sample Volume | 10.0 µL |
| Air Volume | 0 µL |
| Pre Cln Slv1 | 0 |
| Pre Cln Slv2 | 0 |
| Pre Cln Spl | 0 |
| Fill Volume | 0 nL |
| Fill Speed | 5.0 µL /s |
| Fill Strokes | 11 |
| Pullup Del | 59 s |
| Inject to | Flush |
| Inject Speed | 50 µL /s |
| Pre Inj Del | 0 ms |
| Pst Inj Del | 0 ms |
| Pst Cln Slv1 | 0 |
| Pst Cln Slv2 | 0 |

**Figure 3**: Method File – Instrument Screen

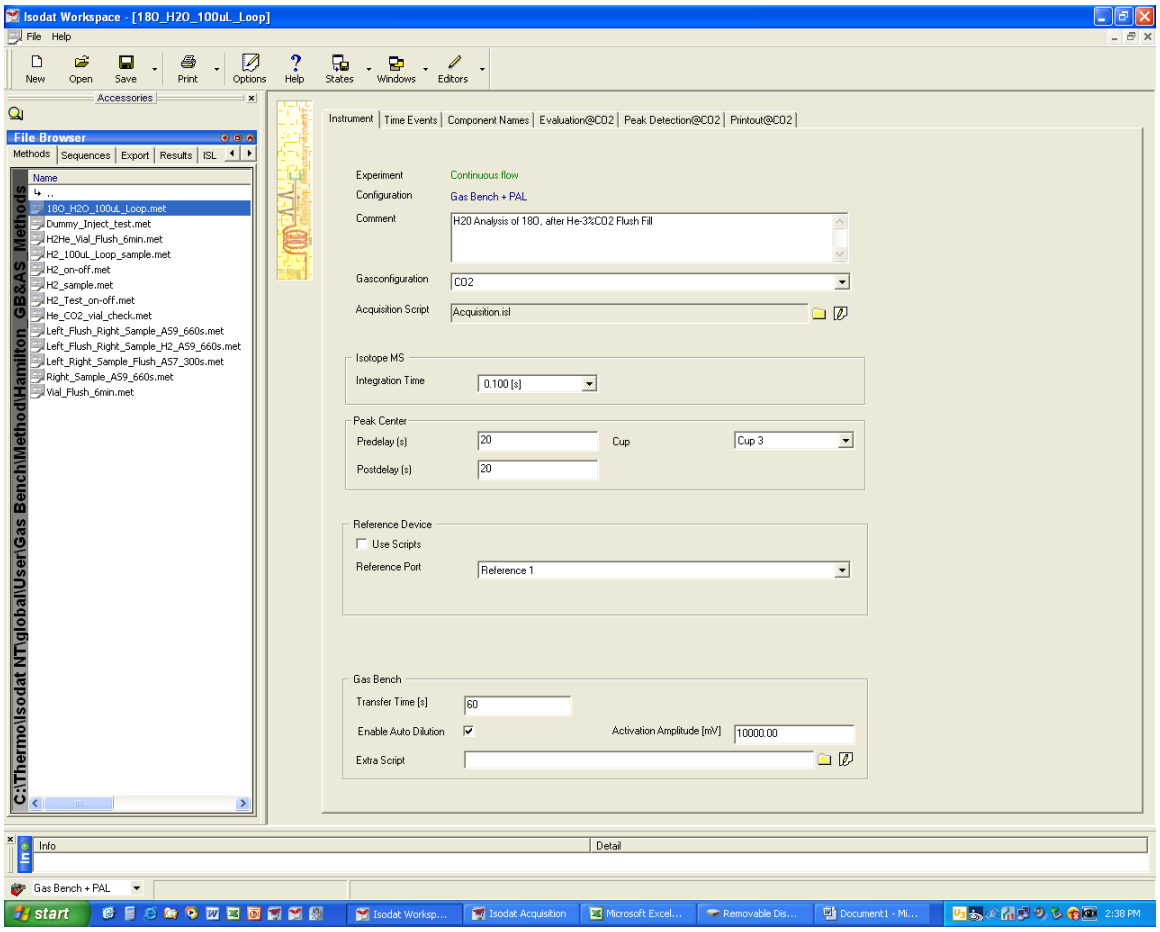


**Figure 4:** Method File – Time Events Screen

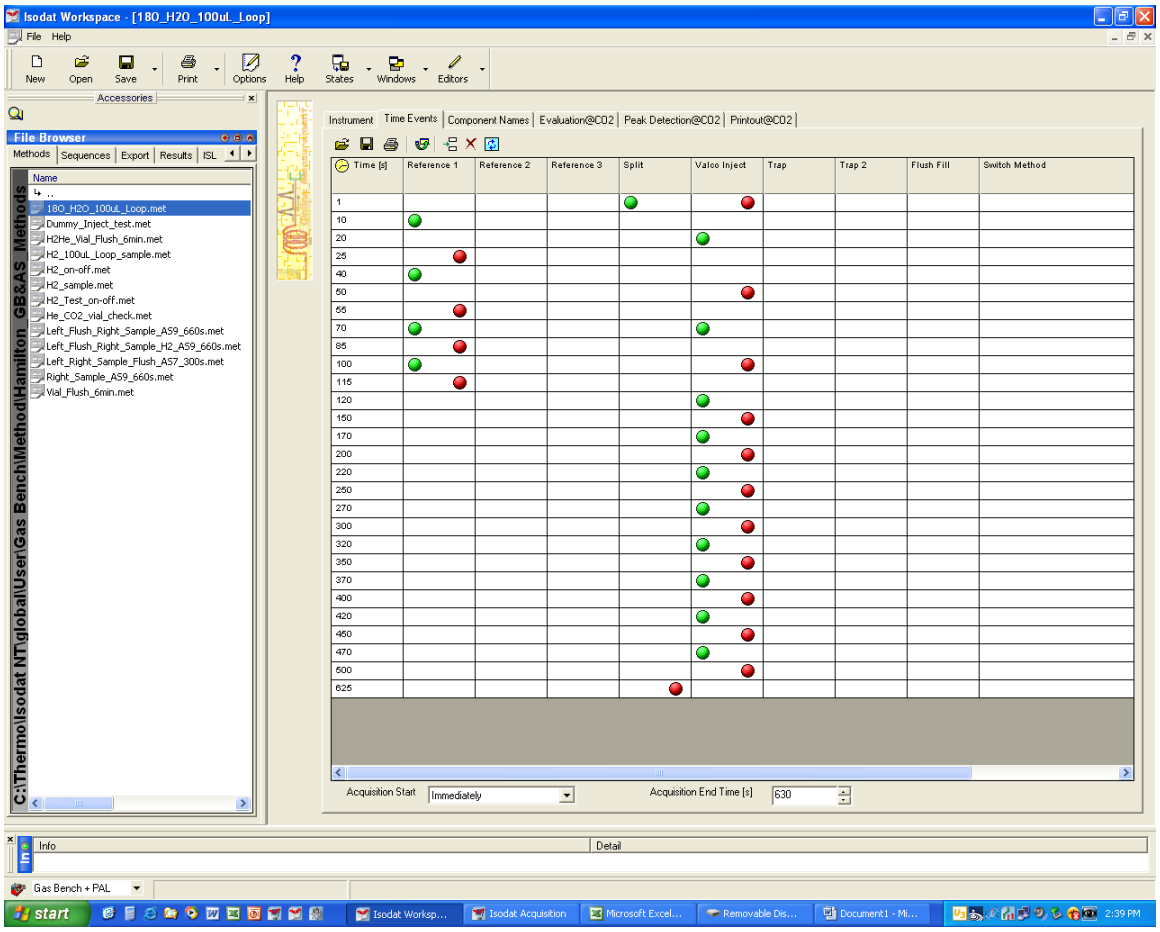


**Figure 5:** Method File – Evaluation@CO2 Screen

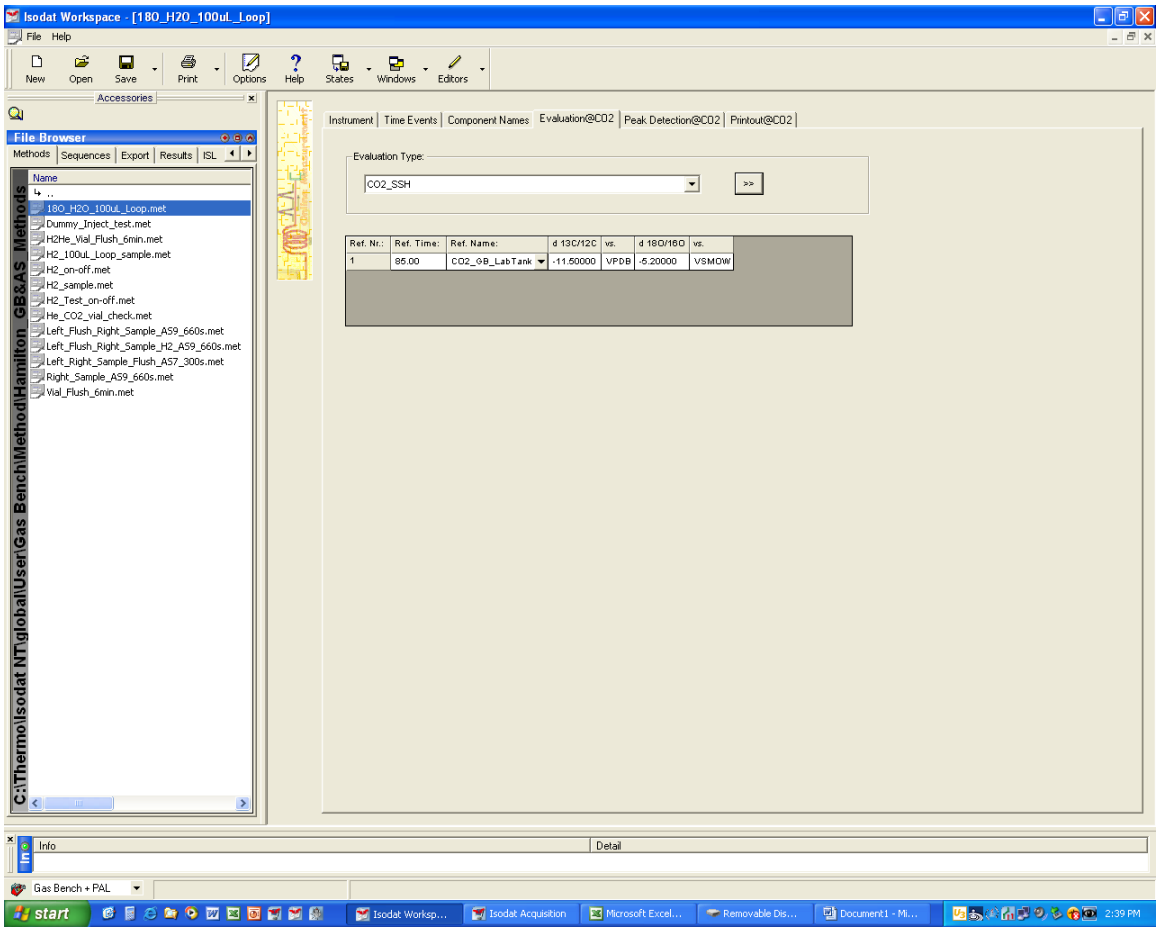


**Figure 6:** Method File – Peak Detection@CO2 Screen

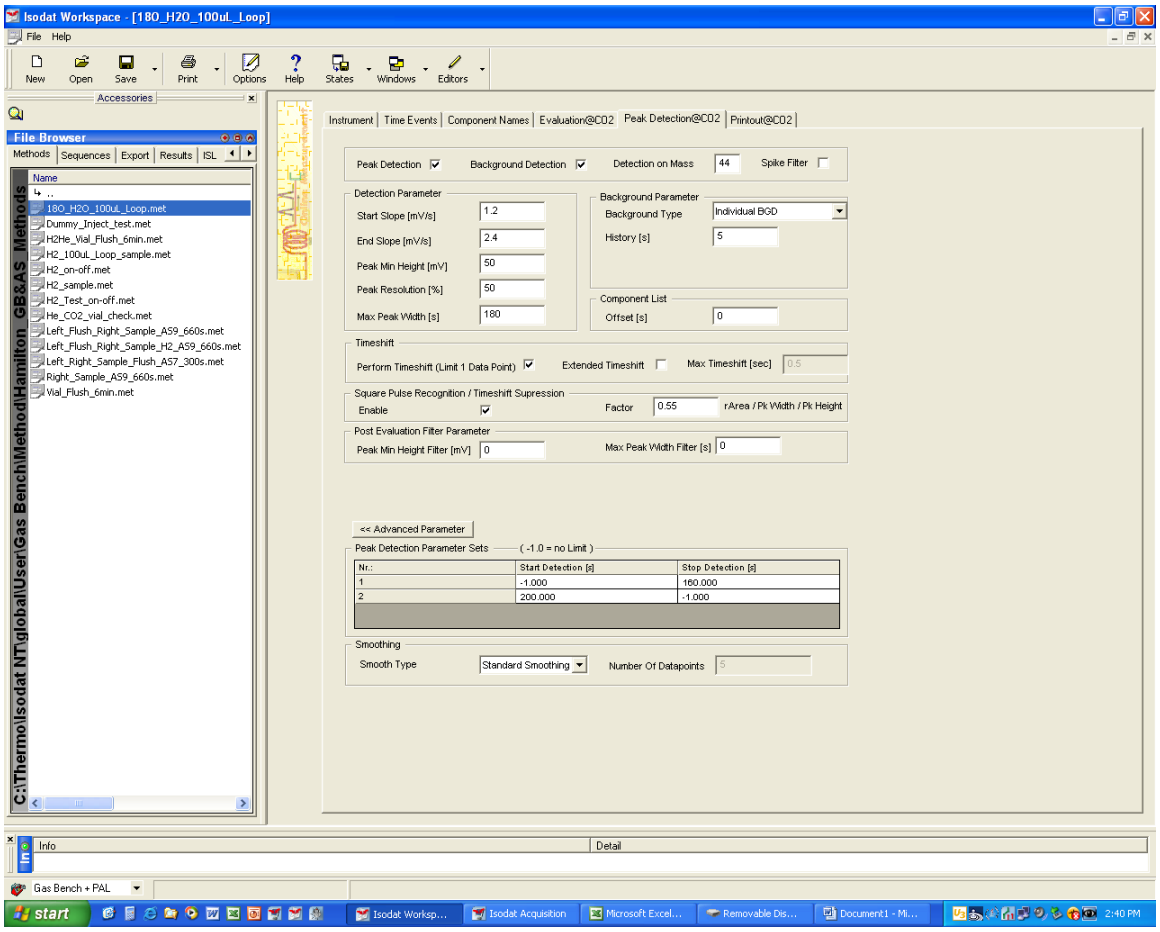


**Figure 7:** Example of Water $\delta^{18}O$ Sequence File.

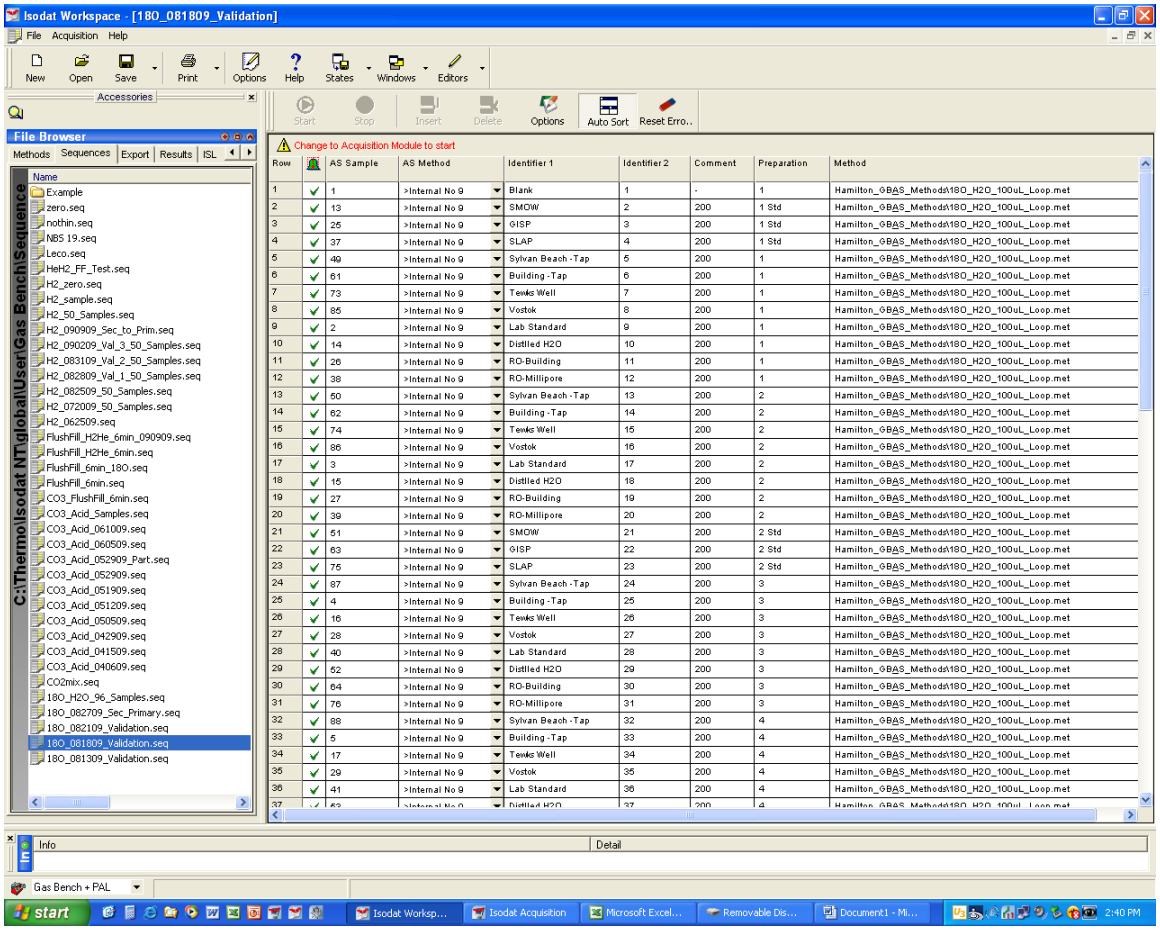


**Figure 8:** $^{18}O$ On-Off Check (Using $CO_2$)

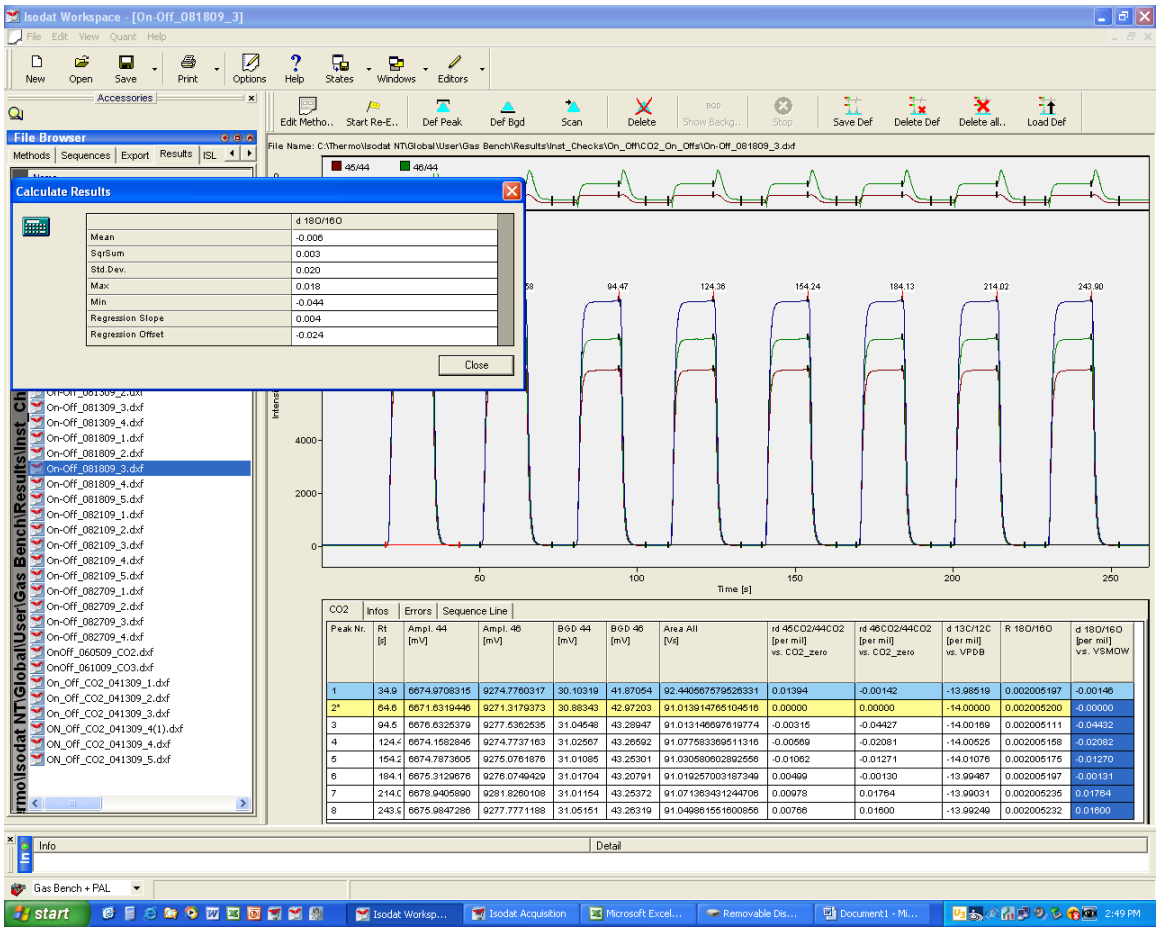


**Figure 9:** $^{18}O$ Linearity Check (Using $CO_2$)

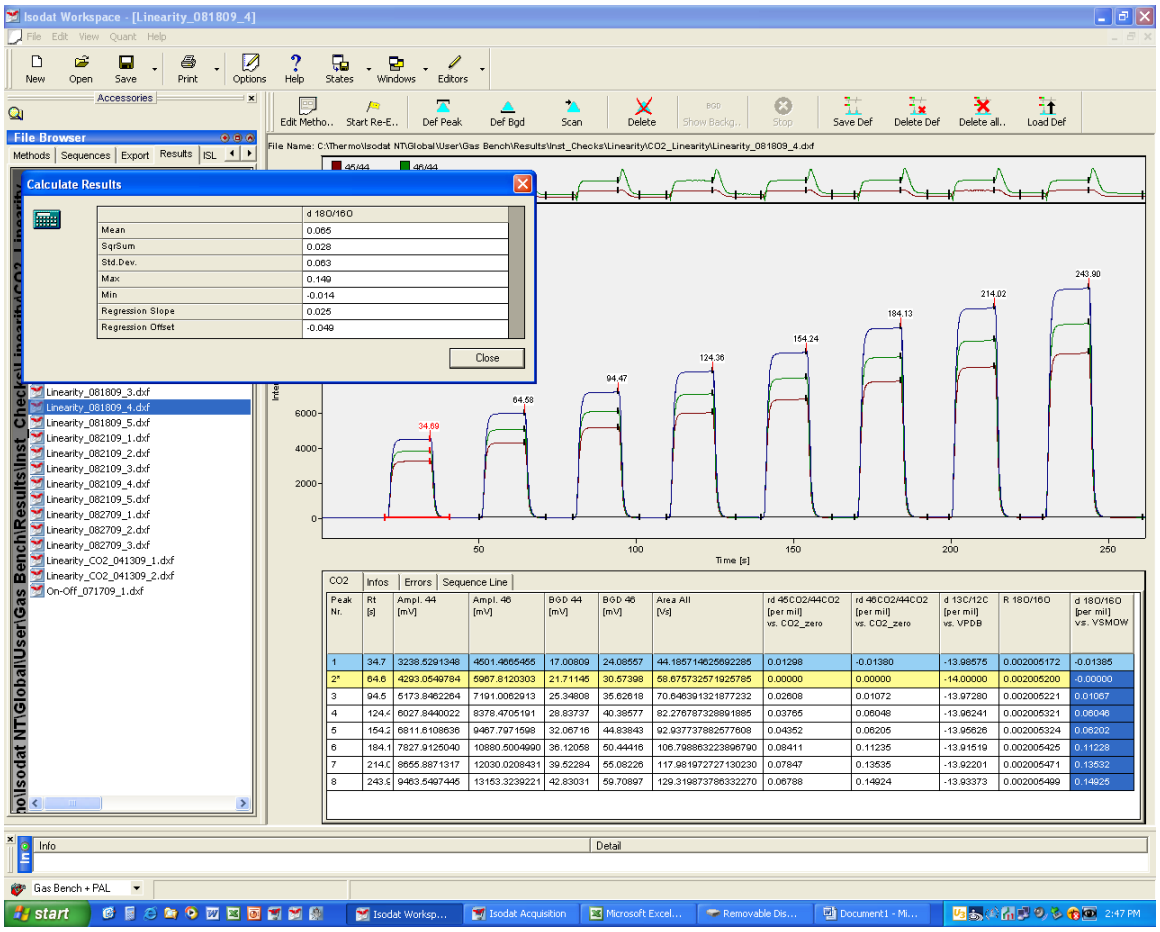
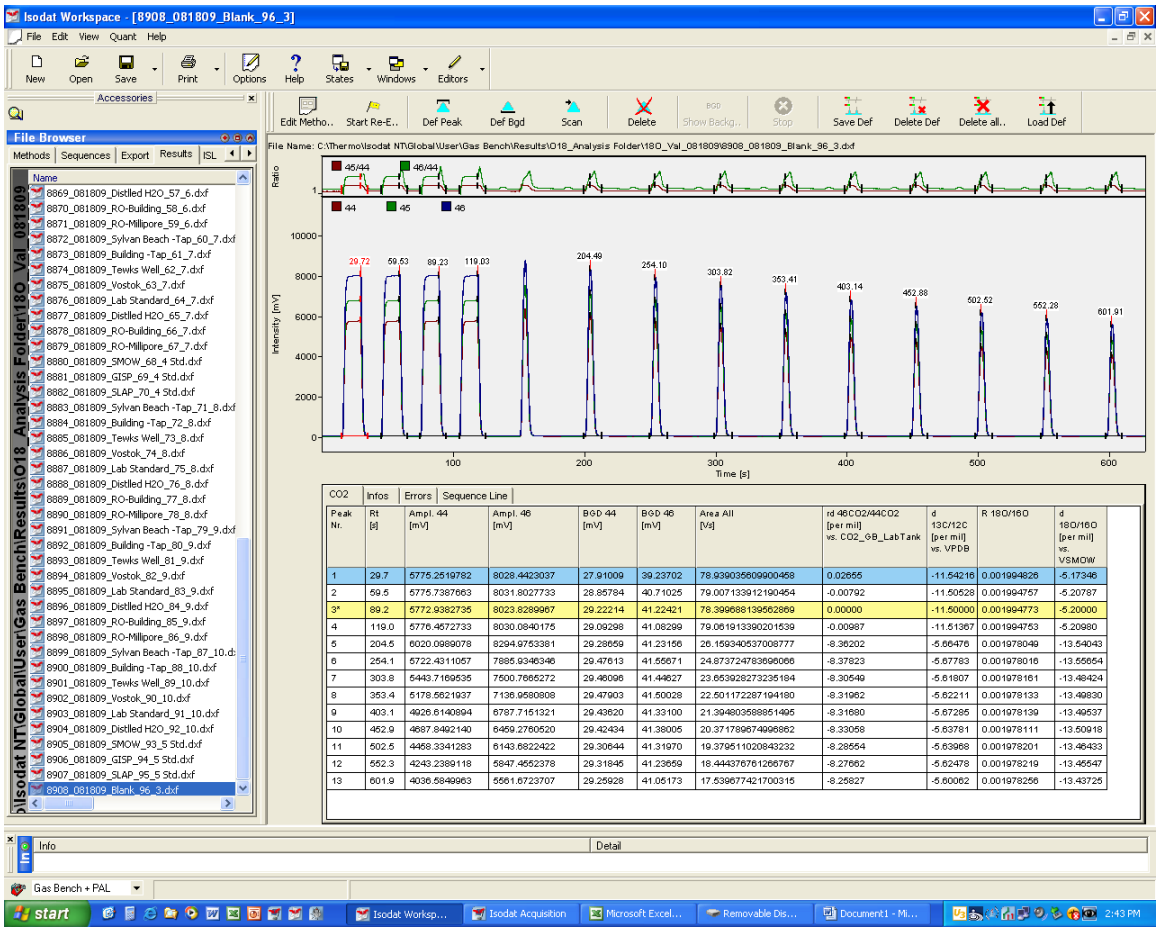


**Figure 10:** $^{18}O$ Data Acquisition File - Blank




| Peak Nr. | Rt [s] | Ampl. 44 [mV] | Ampl. 46 [mV] | BGD 44 [mV] | BGD 46 [mV] | Area All [Vs] | rd 46CO2/44CO2 [per mil] vs. CO2_GB_LabTank | d 13C/12C [per mil] vs. VPDB | R 18O/16O | d 18O/16O [per mil] vs. VSMOW |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 29.7 | 5775.2519782 | 8028.4423037 | 27.91009 | 39.23702 | 78.93903560900458 | 0.02655 | -11.54216 | 0.001994826 | -5.17346 |
| 2 | 59.5 | 5775.7387663 | 8031.8027733 | 28.85784 | 40.71025 | 79.007139121904 54 | -0.00792 | -11.50528 | 0.001994757 | -5.20787 |
| 3* | 89.2 | 5772.9382735 | 8023.8289967 | 29.22214 | 41.22421 | 78.39968813956 2869 | 0.00000 | -11.50000 | 0.001994773 | -5.20000 |
| 4 | 119.0 | 5776.4572733 | 8030.0840175 | 29.09298 | 41.08299 | 79.061913390201539 | -0.00987 | -11.51367 | 0.001994753 | -5.20980 |
| 5 | 204.5 | 6020.0989078 | 8294.9753381 | 29.28659 | 41.23156 | 26.159340537008777 | -8.36202 | -5.66476 | 0.001978049 | -13.54043 |
| 6 | 254.1 | 5722.4311057 | 7885.9346346 | 29.47613 | 41.55671 | 24.873724783696066 | -8.37823 | -5.67783 | 0.001978016 | -13.55654 |
| 7 | 303.8 | 5443.7169535 | 7500.7665272 | 29.46096 | 41.44627 | 23.653928273235184 | -8.30549 | -5.61807 | 0.001978161 | -13.48424 |
| 8 | 353.4 | 5178.5621937 | 7136.9580808 | 29.47903 | 41.50028 | 22.501172287194180 | -8.31962 | -5.62211 | 0.001978133 | -13.49830 |
| 9 | 403.1 | 4926.6140894 | 6787.7151321 | 29.43620 | 41.33100 | 21.394803588851495 | -8.31680 | -5.67285 | 0.001978139 | -13.49537 |
| 10 | 452.9 | 4687.8492140 | 6459.2760520 | 29.42434 | 41.38005 | 20.371789674996862 | -8.33058 | -5.63781 | 0.001978111 | -13.50918 |
| 11 | 502.5 | 4458.3341283 | 6143.6822422 | 29.30644 | 41.31970 | 19.37951102084323 2 | -8.28554 | -5.63968 | 0.001978201 | -13.46433 |
| 12 | 552.3 | 4243.2389118 | 5847.4552378 | 29.31845 | 41.23659 | 18.444376761266767 | -8.27662 | -5.62478 | 0.001978219 | -13.45547 |
| 13 | 601.9 | 4036.5849963 | 5561.6723707 | 29.25928 | 41.05173 | 17.539677421700315 | -8.25827 | -5.60062 | 0.001978256 | -13.43725 |

**Figure 11:** $^{18}O$ Data Acquisition File – Primary Standard (GISP)

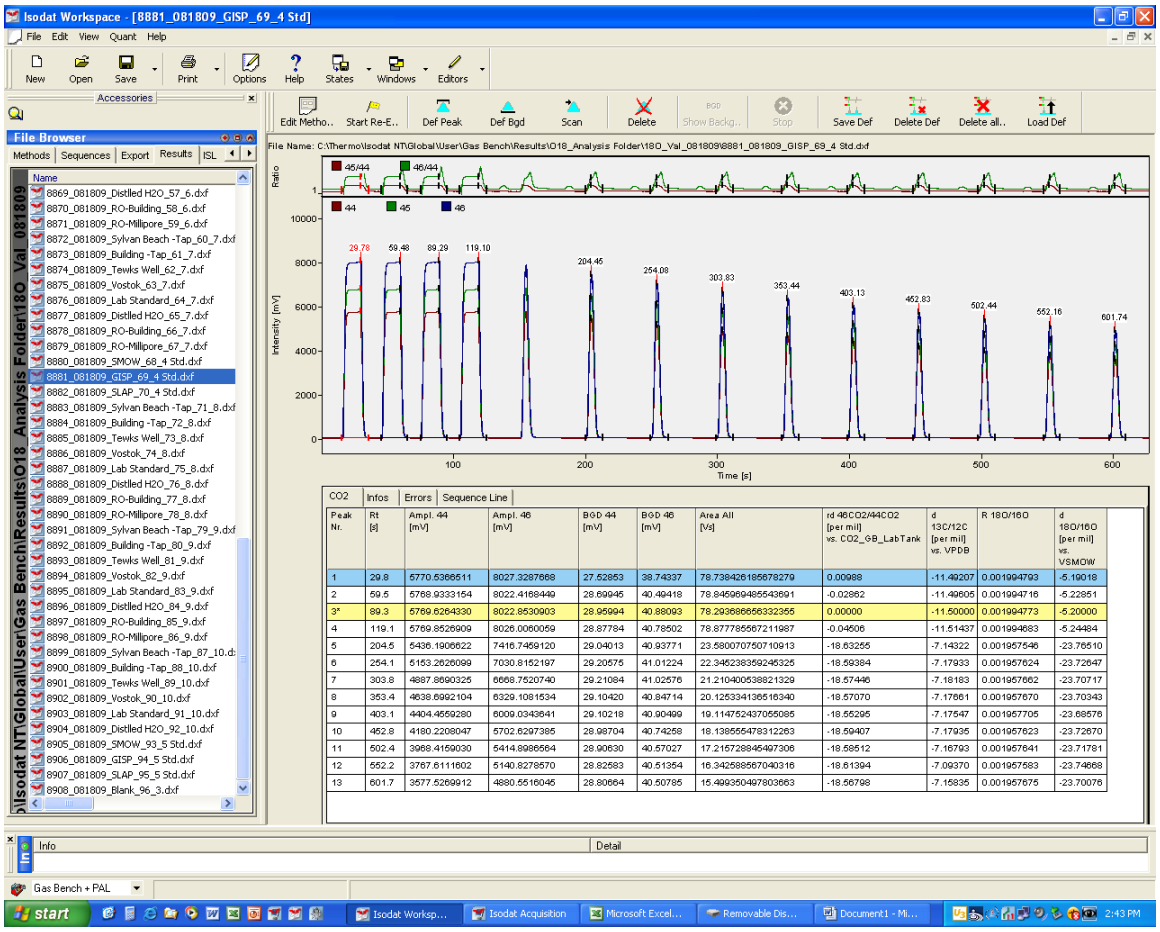


**Figure 12:** $^{18}O$ Data Acquisition File – Sample (Vostok Water)

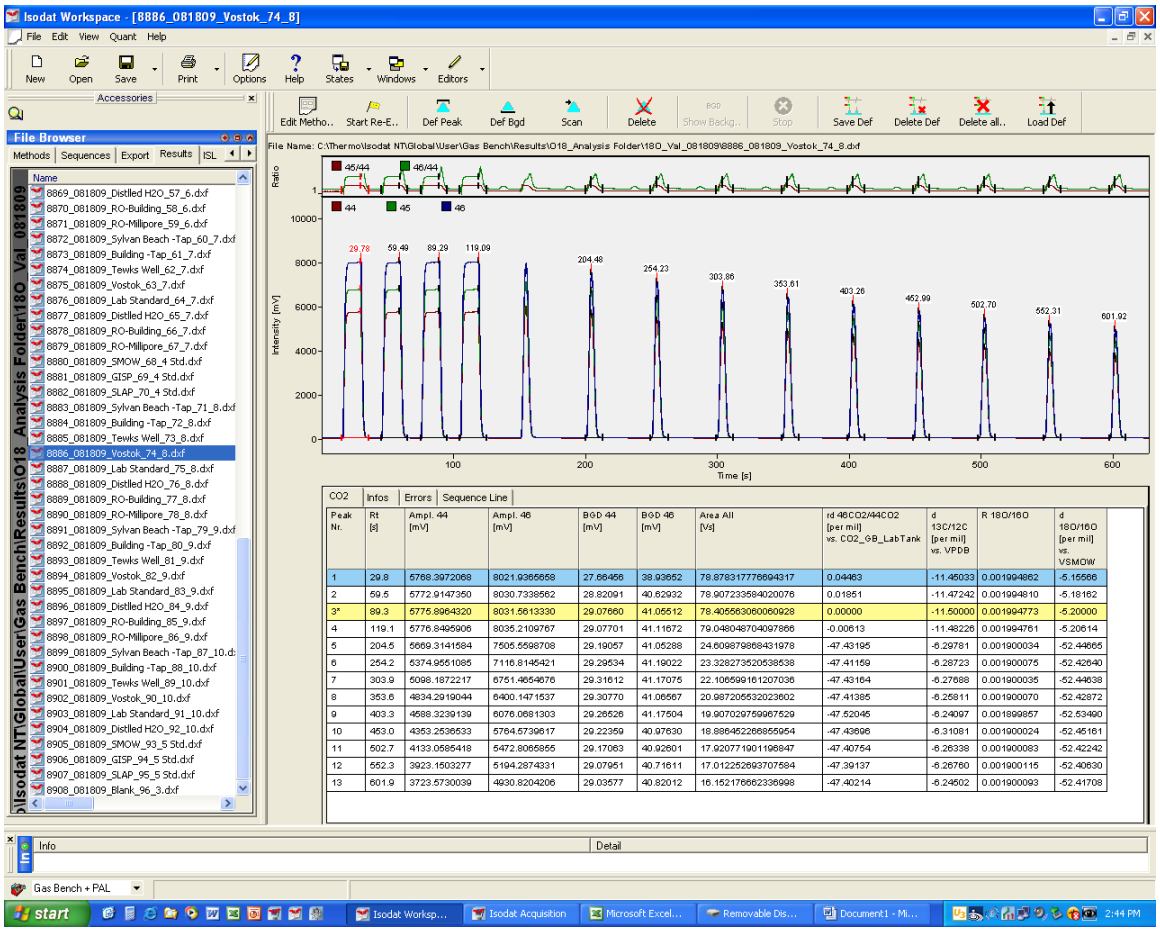


**Figure 13:** $^{18}O$ Export File – GB_18O_Export